The effects of biochar-manure on crop yield, greenhouse gases, and organic matter stability in an Orthic Gray Luvisol

by

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Abstract

Cattle production contributes to the largest portion of livestock agricultural greenhouse gas (GHG) emissions in Canada, mainly in the form of methane (CH_4). Therefore, there is interest in the modification of livestock diet composition to try and reduce the emission of CH_4, resulting in a corresponding change in manure properties. One such modification includes using biochar, a stable carbon (C) C-rich and porous compound from thermal alteration (pyrolysis) of organic material (OM) with little or no oxygen, resulting in biochar-manure. Many report overall positive impacts on crop growth (Calvelo Pereira et al. 2014; Gomez et al. 2014; Steinbeiss et al. 2009), decreased GHG emissions (Bruun et al. 2011; Feng et al. 2012; Jeffery et al. 2016), and C sequestration (Certini 2005; Chen et al. 2020; Du et al. 2017) with biochar application due to changes in soil properties (chemical, physical, and biological) through direct and indirect impacts. Yet, information concerning the agronomic performance and long-term stability potential of biochar-manure under temperate field conditions is scarce.

In Chapter 2, a laboratory incubation investigated the effects of biochar-manure, regular manure, and biochar by itself to a Gray Luvisol soil with loamy sand texture in a controlled environment. A 64-day incubation was used to study C and N mineralization by measuring CO_2-C fluxes and inorganic N (nitrate (NO_3-N) and ammonium (NH_4-N)) concentrations. In Chapters 3-5, various biochar manure, regular manure, and biochar alone treatments were studied in a field trial at the Breton Research Station to examine the effect of these amendments on crop growth, fertility, GHG emissions, and C sequestration. Treatments included plots a control, (CT), biochar at 5 and 10 Mg ha^{-1} (BC5 and BC10), stockpiled manure (RM) at 100 kg total N ha^{-1}, stockpiled biochar-manure (BM) (manure from cattle with biochar added at 2% of diet dry matter) at 100 kg total N ha^{-1}, and v) BC and RM (BC + RM) or BM (BC + BM) at the

aforementioned rates. Fields were planted with wheat (*Triticum aestivum*) in June 2020 and canola (*Brassica napus*) in May 2021, using a field plot seeder and harvested in September of 2020 and 2021. Soil samples were collected after amendment, pre-seeding, and post-harvest each year and analyzed for microbial biomass, microbial functioning, cations and anion concentrations, OM stability, C/N, EC, and pH. Atmospheric and soil temperature and moisture data was collected throughout the study.

Chapter 2, the lab incubation, results showed that BM+BC lowered ($P < 0.05$) C mineralization relative to RM+BC, with no statistical differences in N mineralization. Chapter 3 showed that BM+BC10 had greater ($P < 0.05$ in wheat) grain and protein yield, as well as amino acid utilization in treatments with biochar, likely related to N use efficiency. Chapter 4, showed that BM+BC5 (133.0 kg ha^{-1}) did not significantly impact anthropogenic emissions of N_2O and CH_4, while still improving grain productivity and protein content compared to BM alone, while BM+BC10 limited CH_4 oxidation due to microbial community changes in the rumen. Finally, Chapter 5 findings revealed that BM+BC10 resulted in the highest ($P > 0.05$) organic C (OC) in aggregated (29.4 g kg^{-1}), and non-aggregated mineral-associated organic matter (MAOM; 35.5 g kg^{-1}). Analysis by PCA/FTIR showed higher absorbance, and hence greater long-term stability, with BM+BC10 at 2930 cm^{-1} and 1650 cm^{-1} (recalcitrant aliphatic and aromatics) than RM+BC10.

Results from the four experiments demonstrate that various amendments and their interaction significantly ($P = 0.05$) affected soil properties, so further research is needed to investigate the effect of cattle fed various amounts of biochar and other soil types. Treatments were compared to the control to determine percentage of increase in GHG emissions and C stability using data from chapter 4 and 5, where RM+BC10 and BM+BC10 emitted 7% more

GHG, on average; but also sequestered 43% more, compared to the control. In conclusion, combinations of BM and BC are promising means to increase C storage in soil.

Preface

This dissertation is an original work conducted by Tien Luo Ying Weber. Chapter 2 is published as T.L. Weber, C.M. Romero, and M.D. MacKenzie. "Biochar–manure changes soil carbon mineralization in a Gray Luvisol used for agricultural production" *Canadian Journal of Soil Science,* https://doi.org/10.1139/cjss-2020-0157. Chapters 3-5 are being prepared for submission to a peer-reviewed journals. I am responsible for writing Chapters 1 and 6, and for data collection, analysis, and manuscript writing for Chapters 2-5. M.D. MacKenzie was the supervisory author and was involved in research concept formulation and edited manuscripts.

Dedication

Once again, to my parents and to everyone who studies soils.

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Fig. 5.5. PCA biplot showing the influence of biochar (a) treatments and (b) fractions on wavelength and C and N as assessed by DRIFT spectroscopy. Treatments: CT, control; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}

Fig. 5.6. PCA biplot showing the influence of biochar and manure (a) treatments and (b) fractions on wavelength and C and N as assessed by DRIFT spectroscopy. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}

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List of Abbreviations

Abbreviation	Meaning
AAFC	Agriculture and Agri-Food Canada
Al	Aluminum
ANOVA	Analysis of variance
BC	Biochar
BC10	Biochar at 10 Mg ha^{-1}
BC5	Biochar 5 Mg ha^{-1}
BM	Biochar manure
C	Carbon
C/N	carbon to nitrogen ratio
Ca	Calcium
CEC	Cation exchange capacity
CH_4	Methane
CLPP	Community Level Physiological Profiles
CO_2	Carbon dioxide
CO_2-eq	Carbon dioxide equivilance
CT	Control
DM	Dry matter
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
DRIFT	Diffuse Reflectance Infrared Fourier Transform Spectroscopy
DSC	Differential scanning calorimetry
EB	Enhanced biochar
EC	Electrical conductivity
ECD	electron capture detector
EF_{area}	Area-based emission factor/cumulative GHG emissions
EF_{yield}	yield-based emission factor/yield emission intensity
EGA	Evolved gas analysis
FID	flame ionization detector
FTIR	Fourier transform infrared spectroscopy
GWP	Global warming potential
H	Hydrogen
H_2	Dihydrogen
HCl	Hydrochloric acid
HI	Harvest index
HSD	Honest significance difference
HTT	Highest treatment temperature

ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IPCC	Intergovernmental Panel on Climate Change
K	Potassium
K2SO4	Potassium sulphate
LCA	Life cycle analysis
LeRDC	Lethbridge Research and Development Centre
MAOM	Mineral-associated organic matter
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MRPP	Multi-response permutation procedure
MT	Megaton
N	Nitrogen
N_2	Dinitrogen
Na	Sodium
NH_4-N	Ammonium
NIR	Near-infrared spectroscop
NMR	Nuclear magnetic resonance
NMS	Non-metric multidimensional scaling
NO	Nitric oxide
NO_2^-	Nitrite
NO_3-	Nitrate
NUE	Nitrogen use efficiency
O_2	Oxygen
OC	Organic carbon
OM	Organic matter
P	Phosphorus
POM	Particulate organic matter
RM	Regular stockpiled manure
SO_4^{2-}	Sulphate
SOC	Soil organic carbon
SOM	Soil organic matter
STA	Simultaneous thermal analysis
TC	Total carbon
TCD	thermal conductivity detector
TGA	thermogravimetric analysis
TN	Total nitrogen
WFPS	Water filled pore space
WHC	Water holding capacity

Chapter 1. Introduction

1.1 Biochar Introduction

Natural wildfires change many of soil's biological, physical, and chemical components, such as nutrient availability, microbial activity, and pH; yet, the influence on soil properties remains often overlooked (Zackrisson et al. 1996). Pyrogenic carbon (C), is the result of thermal alteration (pyrolysis) of organic material (OM) with little or no oxygen, resulting in a stable, C-rich, aromatic (Table 1.1), and porous compound (Gomez et al. 2014; Lehmann and Kleber 2015; Pereira et al. 2014). Pyrogenic carbon produced as an energy carrier is termed charcoal, and charcoal specifically produced for application to soil is termed biochar (Lehmann and Joseph 2015). The heterogeneous structure of biochar can hold hydrophilic, hydrophobic, acidic, and basic properties (Atkinson et al. 2010; Mohammed and Tak 2018). Its physical characteristics depend on the type of biomass, the pyrolysis system, and the pre-and post-handling of the substance (Lehmann and Joseph 2009). For example, as highest treatment temperature (HTT) increases, the structure changes from a disordered amorphous mass to a turbostratically arranged graphite at 1500 °C and above (Lehmann and Joseph 2009).

By understanding the characteristics and properties of biochars, there is a chance to understand biochar's potential positive impact on agricultural production, microbes, C cycling, and climate change (Brown et al. 2006). Anthropogenic applications of pyrogenic C can deliver its properties to soil, including, but not limited to, changes in pH (ion mobility/liming), microbial communities (new habitats through increased surface area/porosity (Table 1.1)), OM stability (H/C ratio), electrical conductivity (EC), cation exchange capacity (CEC), and nutrient and water adsorption (Atkinson et al. 2010; Blanco-Canqui 2017; Mohammed and Tak 2018). Continued, long-term traditional cultivation of soils for agriculture can deplete soil OM and permanently remove critical nutrients from soils if restorative measures are not taken (Li et al. 2018), calling for biochar research.

A well-documented anthropogenic use of biochar to improve soil function occurred in Central Amazonia soils called *Terra Preta de Indio*, Portuguese for *black earth* (Karer et al. 2013). From 2,500 to 500 years B.P., *Terra Preta* soils have high OM and fertility despite the high decomposition rates caused by the tropical climate of the region (Lehmann et al. 2003; Weil and Brady 2008). Both natural and anthropogenic sources of pyrogenic carbon were crucial components in *Terra Preta* (Karer et al. 2013; Pereira et al. 2014), which was 25% richer in microbial communities than adjacent forest soils (Atkinson et al. 2010). Despite the high fertility and microbial biomass in *Terra Preta* soils, there were low C respiration rates compared to adjacent native tropical soils due to increases in microbial efficiency (Table 1.1) (Atkinson et al. 2010; El-Naggar et al. 2015). Thanks to *Terra Preta*, scientists continue to investigate biochar's potential to improve soil quality (the capacity to function physically, chemically, and biologically) including here at the Breton Research Station (Fig. 1.2).

1.2 Biochar and livestock

The addition of biochar directly to soil poses potential air and water pollution risks due to its light, fine, particulate nature (Pereira et al. 2014). To alleviate this concern, biochar has become a viable additive in the livestock industry, such as adding biochar to livestock bedding material and manure (Joseph et al. 2015a; Schmidt et al. 2019). Since 2010, new research has been dedicated to finding different ways to enrich the biochar consumed by animals (Mohammed and Tak 2018). One example is enhanced biochar (EB) production (Table 1.1), which has undergone a post-pyrolysis treatment with mineral salts or weak acids to be safely added to feedstock (Tamayao et al. 2021; Terry et al. 2020).

Once biochar enters the rumen, abiotic and biotic chemical reactions in acidic and alkaline environments may change biochar functional groups (Joseph et al. 2015a). However, results show that biochar does not significantly change as it passes through the rumen of cattle (Joseph et al. 2015a; Romero et al. 2021a). Romero et al. (2021a) found the manure from cattle-fed biochar at 2% feed (dry wt) had a 5.7% higher TC than the control and changes in the manure ^{13}C nuclear magnetic resonance (NMR) spectra were limited to the aromatic-C region, indicating the presence of biochar in manure. Research on the effects of this biochar-manure as an amendment, specifically in temperate climates; however, is lacking.

With the addition of biochar to silage, Calvelo et al. (2014) and Schmidt et al. (2019) reported a reduction in toxins, pesticides, acid formation, and higher quality of lactic bacteria in livestock. In addition, Mohammed & Tak (2018) noted improvements in digestion, blood values, feed use efficiency, livestock weight gain, meat quality, and enteric methane emissions. Conversely, a recent study by Tamayao et al. (2021) found that biochar fed in a barley silage-based diet did not affect diversity or richness of bacteria in an artificial rumen system, suggesting that the indigestible nature of biochar may override positive changes in nutrient availability.

These positive effects with cattle nutrition come partially from biochar's potential gas sorption capacity (Joseph et al. 2015a; Lehmann and Joseph 2015; Schmidt et al. 2019). Other positive effects come from biochar’s redox potential that can serve as an electron mediator/shuttle, enhancing long-distance electron exchange and feed efficiency (Schmidt et al. 2019). Terry et al. (2020) found that adding 1-2% biochar to cattle feed (dry wt) was unsuccessful in promoting growth, but did not see any adverse effects on health. Furthermore, Tamayao et al. (2021) found no changes in rumen nutrient disappearance, pH, volatile fatty acids, total gas production, and fermentation with biochar application at 2%. Therefore, the addition of biochar to cattle feed does not harm the animals and can improve soil health (Table 1.1) as a manure amendment and reduce loss of biochar during application. The overarching research objectives of this thesis were to examine: i) how cattle fed biochar would affect the resulting manure properties, ii) how soil properties are influenced when it is applied as an organic amendment; and iii) if these changes can lead to long-term sustainable agricultural practices, in terms of reduced GHG and increased C sequestration in Western Canada.

Application of biochar-manure may stabilize manure OM and alter C and N cycling in agricultural fields (Kammann et al. 2017). Romero et al. (2021a) found that biochar increases manure OM recalcitrance and its overall C sequestration potential. However, yet its effect on OM mineralization is mostly unknown; therefore, the specific objective of Chapter 2 to understand how biochar, biochar-manure, and manure combinations in an incubation would change soil C and N mineralization. It was hypothesized that i) that biochar would retain some of its properties once excreted by feedlot cattle and ii) limit microbial activity and associated soil C and N mineralization.

1.3 Biochar, biogeochemistry, and crop production

Agricultural land is necessary worldwide, and as such, the rapid depletion of soil organic matter from croplands is a concern (Paustian et al. 2019; Pokharel and Chang 2019). Many sustainable agricultural practices are being researched to combat this, such as transitioning from inorganic fertilizers to biochar and other organic amendments (El-Naggar et al. 2015; Güereña et al. 2013). To better understand the potential of biochar, biochar-manure, and manure applications on soil health (a metaphor for soil function; Table 1.1), this thesis focus was wheat and canola production, a crop typical of Western Canadian Prairies (McCallum and DePauw 2008). Wheat is a fundamental grain produced around the world (Terman et al. 1969), and extensive research has been conducted to find ways to improve wheat production and enhancing protein concentration (Carew et al. 2009), but none yet with biochar-manure.

Nitrogen availability is crucial as the grain yield and harvest index for wheat peaks around 125 kg N ha^{-1} (Iqbal et al. 2012). Biochar is typically characterized by a high C/N ratio, meaning that N immobilization outweigh mineralization, potentially limiting crop growth (Pokharel and Chang 2019). Additionally, with most N in biochar tied up in the C matrix, this N is often unavailable to plants, requiring additional fertilizers for crop growth (Galinato et al. 2011; Lentz et al. 2014; Pokharel and Chang 2019). One benefit to this is that although these nutrients are unavailable to plants, they are no longer subject to leaching (Güereña et al. 2013; Pokharel and Chang 2019). Biochar nutrients that are available to plants, such as extractable P and K, are often quickly immobilized or utilized by plants within the first year (Karer et al. 2013). It is important to examine if application of biochar-manure will hinder, or help, productivity in various years.

Lehmann et al. (2015) and Steinbeiss et al. (2009) reported an overall positive impact on crop growth with biochar application rates >10 Mg ha^{-1}. Several authors have also found insignificant changes in wheat yield (Alburquerque et al. 2013; Chan et al. 2007; Karer et al. 2013; Liu et al. 2014), while others reported negative crop responses (Galinato et al. 2011). Even with biochar rates of up to 72 Mg ha^{-1}, Hood-Nowotny et al. (2018) found no significant effects on crop yield. However, Shadzad et al. (2018) saw a 15-23% increase in aboveground biomass, 16% increase in grain yield, and 5-10% increase in wheat protein with 10 Mg ha^{-1} of poultry-waste biochar application, but no significant effects of equal applications of green-waste biochar. In situations where there were no significant changes to the current crop, positive effects, such as reduced N leaching, could help future harvests (Güereña et al. 2013). Different rates of biochar application are of interest and need further research, given that high amounts of biochar are required to see significant positive agricultural effects (Shahzad et al. 2018).

Most of the positive effects from biochar addition come from indirect effects such as nutrient retention, increased water-holding capacity, decreased compaction, and enriched soil biological properties (Alburquerque et al. 2013; El-Naggar et al. 2015; Hood-Nowotny et al. 2018; Karer et al. 2013). These effects needed additional exploration and were an essential part of understanding biochar-manure correlations between crop production and soil properties. Biochar can moderate soil temperatures through reduced bulk density and reflectance, and impacts on soil temperature and moisture during the sowing-to-emergence period in spring are the most important (McGill et al. 1986; Terman et al. 1969; Vaccari et al. 2011), as biochar can increase water holding capacity, directly benefiting crops and improving fertilizer efficiency (Hood-Nowotny et al. 2018). The physicochemical properties of biochar effectively adsorb NH_4^+-N and reduce NO_3^--N leaching, enhancing plant nutrient uptake (Pokharel and Chang 2019; Shahzad et al. 2018). For example, Terman et al. (1969) found wheat yield-protein content increased with increasing NO_3^--N retained by biochar and Güereña et al. (2013) suggests that repeated applications of biochar could be promising for future crop production.

Biochar has been found to increase pH, organic C content, exchangeable Na, K, and Ca, and decreases Al toxicity (Chan et al. 2007; Güereña et al. 2013). Because wheat's optimal pH is 6-7, biochar liming properties will not be as crucial at the Breton Research Station that already have an acidic to neutral pH (Galinato et al. 2011), but require more evaluation given the impact of pH on other soil functions. Temperate soils do not show changes as significant as tropical soils due to their inherent fertility, high CEC, and neutral pH (Hood-Nowotny et al. 2018; Karer et al. 2013) though there are limited studies in these regions. Breton's clay-dominated soil may still benefit from biochar additions through reduced soil compaction, enhanced root growth, and increased N retention (Chan et al. 2007). Furthermore, in a biochar-feed experiment, Joseph et al. (2015a) found that biochar adsorbed a range of nutrients in the rumen, and these nutrients are likely to be retained when applied to the soil.

The availability and type of substrates adsorbed onto biochar can lead to colonization by different microbial communities, but can also decrease microbial biomass, altering soil fertility (Dempster et al. 2012). For example, Abujabhah et al. (2016) note that biochar application increased bacterial utilization of amino acids and decreased carboxylic acid use, but these effects varied between soil types. This relationship between SOM pools and microbial biomass changes leaves much to be explored, such as how specific microbial communities may change with biochar application (Li et al. 2018). In short, given the recalcitrant nature of biochar and its high cost of production, this research will focus on the addition of biochar combined with other agricultural practices (cover crops, etc.) and amendments (inorganic and organic fertilizers) for viable crop growth (Romero et al. 2021b).

The specific objective of Chapter 3 was to better understand soil function and crop production after amendment with biochar, manure, and biochar-fed manure in agricultural systems in Western Canada. It was hypothesized that i) biochar (BC) and manure additions would increase microbial biomass due to their favourable soil environment and new C source, and ii) RM will result in the greatest yield due to the manure's labile OM, followed by BM due to additional nutrients from the cattle's rumen, and highest protein content in RM, followed by BM, due to the low N in BM and its ability to adsorb water and nutrients.

1.4 Greenhouse gases

Globally, the last three decades have been warmer than any preceding decade, highlighting the human impact on climate change (IPCC 2014). The heating of the earth's surface from this energy balance is called the greenhouse gas effect, where GHGs (primarily CO_2, N_2O, and CH_4) absorb infrared radiation emitted from the surface and reradiate it back to the earth (Manabe 2019). Methane (CH_4) has 28x the greenhouse effect compared to CO_2, and nitrous oxide (N_2O) has 265x the potential of CO_2 (IPCC 2014), and as such, global warming potential (GWP) is used to equate the contribution to global warming from each gas relative to CO_2 (Guenet et al. 2021). Agriculture contributes 14% in a global 100-year GWP calculation (IPCC 2014) and a growing human population of 7.5 billion will require even further farming (Kammann et al. 2017). There is a possibility to reduce emissions by up to 4500–6000 Mt CO_2-eq yr^{-1} in agricultural operations by 2030 if using sustainable strategies (Vaccari et al. 2011) and thus, the overarching goal of this thesis is to see if biochar-manure can be a part of these approaches.

The addition of organic fertilizers causes a priming effect (Table 1.1), releasing CO_2 (Watzinger et al. 2014). This release of CO_2 can be mitigated through sustainable agricultural practices, such as C sequestration in biochar (Zimmerman et al. 2011). However, increasing SOC may influence N_2O emissions, potentially offset the climate change benefit from increased C storage (Guenet et al. 2021). Biochar addition increases pH in acidic soils, which typically enhance microbial biomass and activity (Dempster et al. 2012; Gomez et al. 2014; Pokharel and

Chang 2019; Watzinger et al. 2014). Despite this, Dempster et al. (2012) reported a decrease in microbial biomass C, resulting in a decreased OM decomposition and N mineralization. This connection between C and N cycling calls for an investigation of both (Guenet et al. 2021).

The addition of biochar usually increases CO_2 emissions compared to unamended soil due to stimulation of co-mineralization of the more labile components of biochar, improved aerobic conditions, and increased microbial colonization (Ameloot et al. 2013; Troy et al. 2013; Zimmerman et al. 2011). Biomass changes are closely related to desiccation and remoistening cycles (McGill et al. 1986), which may be altered by biochar additions. Biochar decreases bulk density, potentially increasing soil thermal conductivity and water retention, which may be advantageous in warm, dry climates (Zhang et al. 2013). Zhang et al. (2013) discovered biochar moderates soil temperatures by reducing soil temperature fluctuations on both daily and seasonal scales. However, Anders et al. (2013) found no biomass changes with biochar additions in temperate soils, but rather a change in microbial community structure due to nutrient availability changes. Conflicting results call for further investigation of soil microbial and chemical/physical property correlations in Western Canada.

Biochar can also offset CO_2 emissions and sequester C in the soil (Abagandura et al. 2019; Spokas and Reicosky 2009). Negative priming (decrease in mineralization of native SOC following the addition of OM) has been attributed to dioxin, furan, phenol and polyaromatic hydrocarbon toxicity from biochar on microorganisms, lowering their activity and respiration (Dodor et al. 2018). Biochar can adsorb (enrichment or depletion of a chemical species at an interface) CO_2 directly in its pores, decreasing C mineralization rates, plant root growth, and CO_2 emissions from root respiration (Abagandura et al. 2019; Calvet 1989; Lehmann and Joseph 2015). Other possible explanations for decreased CO_2 emissions include increased C use efficiency within plants or soil microbes (Kammann et al. 2012). However, increases in plant growth from biochar additions will increase CO_2 emissions from greater photosynthesis rates (Kammann et al. 2012). If the biochar being applied has enough labile C, it can produce extracellular enzymes that degrade SOC through co-metabolism (Dodor et al. 2018).

Compared to biochar, manure C mineralization is much more complete and rapid, with 44-54% of manure C being utilized in the first 28 days compared to <1% of biochar C (Troy et al. 2013). However, biochar co-applied with manure usually lowers CO_2 emissions compared to the sum of CO_2 emissions in biochar or manure alone (Dodor et al. 2018). Some explanations for the decline in CO_2 emissions include protection of organic substrates from manure additions and chemisorption of evolved CO_2 on the biochar surface (Dodor et al. 2018). There is a need to fill the knowledge gap of biochar, biochar-manure, and co-applications for CO_2 mitigation with correlations to biotic and abiotic factors (Liu et al. 2016).

Nitrous oxide is a relatively stable GHG that contributes to stratospheric ozone destruction and climate change (Chapuis-Lardy et al. 2007). Nitrification is the oxidation of NH_4^+ to nitrite (NO_2^-) or nitrate (NO_3^-), occurring primarily between water-filled pore space (WFPS) of 35-60% by autotrophic microorganisms (Bremner 1997; Lin et al. 2017) and is dependent on low pH, water content, phosphate availability, and NO_2^- toxicity (Ameloot et al. 2013; Bremner 1997). Denitrification, the reduction of nitrogen oxides ($NO_3^- \rightarrow NO_2^- \rightarrow$ nitric oxide (NO) $\rightarrow N_2O \rightarrow$ dinitrogen (N_2)) through heterotrophic bacteria (Bergaust et al. 2010; Bremner 1997; Chapuis-Lardy et al. 2007), is dependent on pH, temperature, NO_3^- concentration, O_2 concentration, C substrates, OM and DOC availability, and water content of the soil (Ameloot et al. 2013; Bergaust et al. 2010; Bremner 1997; Troy et al. 2013). Aerobic and anaerobic microsites can develop in the same aggregate and nitrification can occur during anaerobic conditions (Ball 2013).

Biochar's ability to reduce N_2O emissions is linked to environmental conditions and soil properties (Kammann et al. 2012; Romero et al. 2021b). Because denitrification requires anaerobic conditions (WFPS >80%), biochar's porous nature increases O_2 content through increased overall soil porosity and gas diffusion (Lehmann and Joseph 2009). WFPS is vital in field applications, as up to 60% of N_2O release can be emitted during spring thaw (Lin et al. 2017; Russenes et al. 2016; Thilakarathna et al. 2021), and the proper application of amendments can decrease NO_3^- leaching, lower N_2O emissions, optimize plant N use, and increase crop yield (Hernandez-Ramirez et al. 2011; Roman-Perez and Hernandez-Ramirez 2020). For example, while increased rainfall usually increases N_2O fluxes due to anaerobic conditions, they may also restrict N_2O diffusion and lead to N_2O consumption before emission (Chapuis-Lardy et al. 2007). Atmospheric temperature can also play a significant role in N_2O emissions (Lin et al. 2017), as Keeney et al. (1979) found that denitrification increased with rising temperatures due to increased microbial activity up to 75°C.

Furthermore, soil texture and structure, such as bulk density and clay content, are primary contributors to N_2O emissions in the spring. Biochar's macropores and mesopores may enhance soil-draining properties and increase O_2 content in soil (Cimò et al. 2014; Liu et al. 2017). Biochar application can cause short-term immobilization of N due to adsorption NH_4^+/NO_3^- ions and N_2O due to biochar's surface charges and pore spaces (Abujabhah et al. 2016; Bruun et al. 2011). However, other factors, such as previous crops, tillage methods, etc., overshadow the impact of soil structure during other seasons of the growing season (Ball 2013). We aim to fill the knowledge gap of environmental conditions on biochar-manure treatment emissions in a correlation analysis.

Biochar co-applied with manure can minimize yield reductions from biochar and maximize net N mineralization from manure (Lentz et al. 2014). In a study by Lentz et al. (2014), when biochar was applied alone, there was decreased N mineralization, and when manure was applied alone, there was increased N mineralization (Lentz et al. 2014). However, when biochar was added to manured soil, net N mineralization increased, and NH_4/NO_3 decreased as the mineralized NH_4 became bound to the biochar's pores (Lentz et al. 2014).

Biochar can alter the composition of soil bacterial populations, increasing the rate of N_2 fixation and denitrification while decreasing nitrification rates of NH_4 to NO_2 (Lentz et al. 2014; Troy et al. 2013). Since biochar is usually alkaline, it can increase soil pH (Liu et al. 2014; Singh et al. 2010; Vaccari et al. 2011) and activity of the N_2O-reductase enzyme that catalyzes the last step of denitrification and lowers the N_2O:N_2 ratio (Liu et al. 2014; Singh et al. 2010). Biochar's change in soil pH facilitates both nitrification and denitrification (Liu et al. 2014; Liu et al. 2017). Russenes et al. (2016) suggested that introducing OC from different amendment sources may form a positive relationship between C-substrate and N_2O fluxes depending on the difference in OC availability of the amendment. Overall, the trend has been fewer N_2O fluxes with increasing biochar rates (Aguilar-Chávez et al. 2012; Kammann et al. 2017). In conclusion, a correlation analysis will aid in the the knowledge gap of various abiotic and biotic factors involved in N_2O production from biochar-manure.

Methane emissions have increased 150% since the 1750s worldwide, with expanding rice agricultural farms, ruminant animals, thawing permafrost, and wetlands as the primary sources (Kammann et al. 2017). Soil can be a CH_4 source from methanogenic archaea during methanogenesis in water-logged anoxic soils (Jeffery et al. 2016). Soil can also serve as a CH_4 sink through oxidation during methanotrophy by obligate and facultative aerobic bacteria (Jeffery et al. 2016). Because both methanogenesis and methanotrophy can co-occur in micro-sites, soil becomes a sink or source depending on the ratio of methanogens to methanotrophs (Feng et al. 2012; Jeffery et al. 2016). For example, Feng et al. (2012) found that biochar increased methanogenic bacteria, despite an overall reduction of CH_4 emissions.

Biochar's effect on CH_4 emissions has been poorly investigated compared to CO_2 or N_2O, (Jeffery et al. 2016). Spokas and Reicosky (2009) compared 16 different pyrolysis methods and feedstock materials and found that all treatments decreased or did not change rates of CH_4 oxidation. Therefore, there is a knowledge gap in the understanding of biochar-manure and its underlying mechanisms in CH_4 emissions. There are many crucial components to CH_4 emission, with one component being gas diffusivity (Ball 2013). Teepe et al. (2004) found that CH_4 consumption decreased >100% in a silty clay loam compared to 77% in a silty soil due to greater changes in macroporosity between soil textures (Kiani et al. 2020). Abagandura et al. (2019) also noted greater CH_4 source activity in finer-textured soils when applied with biochar. Soil moisture is another fundamental factor to CH_4 emissions, changing soil aeration, redox potential, and pH (Spokas and Reicosky 2009; Yu et al. 2013). A 84-day incubation study by Yu et al. (2013) found that at 35-60% WFPS, CH_4 emissions were generally reduced with biochar additions.

Biochar can directly adsorb CH_4 and other gases into its pores and serve as labile C substrates in anoxic environments (Jeffery et al. 2016; Romero et al. 2021b). NO_3^- and sulphate (SO_4^{2-})-reducing bacteria (from NO_3^-- and SO_4^{2-} additions in biochar) can use methanogenic substrates more efficiently than methanogens, reducing CH_4 emissions (Yu et al. 2013). As such, biochar additions can change the microbial biomass carbon to microbial biomass nitrogen (MBC to MBN), where higher MBC:MBN represents a fungus-dominated community (more recalcitrant C pool and lower CH_4 emissions) and a lower ratio reflects a bacterium-dominated community (Yu et al. 2013). The thesis will explore microbial biomass and communities to fill these knowledge gaps with biochar-manure.

Biochar has been found to strongly interact with N fertilizer applications <120 kg h^{-1}, causing a decrease in CH_4 emissions (Jeffery et al. 2016); however, there is still a knowledge gap on the relationship with organic amendments. Potential NO_3^- reduction from N applications can produce NO, NO_2^-, N_2O, which are toxic for methanogens and result in lower CH_4 emissions (Yu et al. 2013). However, Abagandura et al. (2019) found no significant difference when co-applying manure and biochar. Agricultural soils are minor sinks for CH_4, so there are often no significant differences when applying manure and/or biochar to these systems (Abagandura et al. 2019).

Furthermore, biochar was not found to affect enteric CH_4 emissions in the rumen, suggesting there may not be a difference in CH_4 emissions between biochar-manure and regular manure upon amendment to soil (Calvelo Pereira et al. 2014). Cattle-fed biochar retains nutrients absorbed in the stomach and potentially stimulates microbial respiration in the corresponding manure (Joseph et al. 2015a). Because the C content in the biochar-enhanced manure is minimal compared to biochar, the emission reduction potential of biochar-manure may not be significant and needs to be examined.

The specific objective of Chapter 4 was to understand the GHG reduction potential of biochar-manure, biochar, and manure applications in field operations through analysis of environmental conditions and soil parameters. It was hypothesized that i) GHG emissions would be greatest in soil amended with RM, then BM (due to the retained biochar in BM) and lowest in

BC and ii) greater biochar applications increase O_2 content and may adsorb CO_2, CH_4, and N_2O compared to manure applications, resulting in lower denitrification and methanogenesis rates.

1.5 Organic Matter Stability

As human populations increase worldwide, conventional cultivation methods for crop production such as fertilizers and tillage can reduce SOM (Cambardella and Elliott 1992). The ability to mitigate the loss of OM has gained traction in research, as OM plays an important role in the global C cycle amongst biological, chemical, and physical processes in soil (Fernández et al. 2011; Post and Kwon 2000). Soil organic matter stability is linked to OM quality (Table 1.1). With 1500–2000 Pg of C, soil serves as the third-largest global C pool (Fernández et al. 2011; Post and Kwon 2000). As such, Lehmann and Kleber (2015) advocate for a better understanding of the biological, physical and chemical transformations of SOM.

Biochar's high resistance to biological and chemical decomposition means it is a promising sink for C sequestration (Chen et al. 2020; Vaccari et al. 2011). Biochar's amorphous structures and turbostratic crystallites have high physical recalcitrance due to their unordered structure (Lehmann and Joseph 2009). Furthermore, biochar OM can reside in aggregates rather than free OM due to its negatively charged surfaces (Lehmann and Joseph 2009). This structure promotes further aggregation (Table 1.1) and organo-mineral protection of OM from microbial activity by creating a nucleus of biological activity (Aslam et al. 2014; Lehmann and Joseph 2009). Nonetheless, Plaza et al. (2016) found biochar promotes C stabilization by forming organo-mineral complexes outside aggregates that are chemically unaltered and unprotected when co-applied with organic fertilizers. There is not only a knowledge gap in application of biochar-manure stability, but also the combination of biochar and manure applications on the aggregation potential of biochar.

Abiotic oxidation can change the crystal structure of biochar, which forms polycarboxylic compounds, facilitating microbial metabolization and biochar degradation (Lehmann and Joseph 2009). de la Rosa et al. (2018) found changes to aryl structures and degradation of N-heterocyclics in biochar after two months of incubation. Afterwards, microbial breakdown of biochar into smaller pieces further facilitated its degradation (Lehmann and Joseph 2009). Aged biochar enhances exchanges with SOM, soil minerals, nutrients, and contaminants (de la Rosa et al. 2018). A field study by de la Rosa et al. (2018) found a decrease in SOC during the first year, and the C remained statistically unaltered during the second year in some biochars, attributable to the initial mineralization of labile C. We aim to fill the knowledge gap of environmental conditions on biochar-manure soil stability in a correlation analysis throughout the study.

To investigate OM stability, samples can be fractionated (Table 1.1). Fractions include particulate organic matter (POM) >53 µm with a mean residence time (Table 1.1) of <10 years and mineral associated organic matter (MAOM) <53 µm with a mean residence time of decades to centuries (Guan et al. 2019; Lavallee et al. 2020; Lehmann and Kleber 2015; Plaza et al. 2016). POM is comprised of undecomposed or partially decomposed plant products, and MAOM is comprised of more decomposed and plant/microbial products (and a lower C/N ratio) stabilized through organo-mineral bonding (Christensen 2001; Cotrufo et al. 2019; Guan et al. 2019; Lavallee et al. 2020). Aggregation fraction (Table 1.1) will further help understand the overall recalcitrance of biochar-manure and co-applications.

Finally, organic matter stability is also analyzed using simultaneous thermal analysis (STA), which combines thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), measuring the changes of mass and energy, respectively (Pansu and Gautheyrou 2006). TGA (% or mg) evaluates the decomposition of organic substances based on temperature and time, and these slopes can provide information on the soil's thermal stability (Merino et al. 2015; Pansu and Gautheyrou 2006). In conclusion, thermal analysis techniques are of growing interest in the soil science community, especially when coupled with microbial dynamics such as respiration rates or other soil properties such as C/N ratios (Fernández et al. 2012; Grisi et al. 1998; Peltre et al. 2013; Plante et al. 2009).

The specific objective of Chapter 5 was to understand the changes that biochar, biochar-manure, and manure can have on C sequestration potential and stability. It was hypothesized i) biochar and biochar-manure would result in higher OM stabilization, as seen in DRIFT, due to its recalcitrant amorphous structures and turbostratic crystallites protecting it from microbial activity and abiotic oxidation and ii) biochar will promote aggregate stability, with higher C/N ratios and aggregated fractions in biochar and biochar-manure treatments.

Table 1.1. Key definitions and descriptions used in this thesis.

Term	Definition	Reference
Aromaticity	Spatial and electronic structure of cyclic molecular systems which provide for their enhanced thermodynamic stability and tendency to retain the structural type during chemical transformations.	(Lehmann and Joseph 2015)
Aggregation	Soil colloidal particles adhere together depending on net attractive forces amongst them. Organic matter (and biochar) often increases soil aggregation.	(Aslam et al. 2014)
Soil health	The state of self-regulation, sustainability, resilience, and lack of stress symptoms in a soil as an ecosystem of living organisms that supports the growth of plants.	(Weil and Brady 2008)
Soil quality	Capacity of soil to function physically, chemically, and biologically, within natural or managed ecosystems boundaries to maximize provisions and regulatory ecosystem services.	
Organic matter stability/lability	Organic matter's resistance to decomposition.	(Peltre et al. 2013)
Organic matter quality	The set of properties that determine how easily OC in the soil can be mineralized.	(Fernández et al. 2011)
Organic matter mineralization	Conversion of an element from an organic form to an inorganic state because of microbial decomposition.	(Weil and Brady 2008)
Nitrification	The oxidation of NH_4^+ to nitrite (NO_2^-) or nitrate (NO_3^-), occurring primarily between water-filled pore space of 35-60% by autotrophic microorganisms.	(Bremner 1997; Lin et al. 2017)
Denitrification	The reduction of nitrogen oxides ($NO_3^- \rightarrow NO_2^- \rightarrow$ nitric oxide (NO) $\rightarrow N_2O \rightarrow$ dinitrogen (N_2)) through heterotrophic bacteria.	
Pyrogenic carbon	Carbonaceous materials (polycarondensed aromatic moieties) produced by heating organic material at igh temperature under low oxygen.	(Lehmann and Joseph 2015)
Charcoal	Pyrogenic carbon produced as an energy carrier (cooking, heating, etc.).	
Biochar	Pyrogenic carbon specifically produced for application to soil as part of agronomic or environmental management.	
Soil fractionation	Soil dispersion, followed by density, size, or other methods of separation to isolate pools of SOM based on their size and degree of organo-mineral interaction.	(Moni et al. 2012)

Particle size fractionation	Decomposition of SOM should be a function of particle size as decay induces fragmentation.	
Aggregate fractionation	The physical protection of the SOM is major control of soil structure, which can be teased out through separation of aggregate presence or lack therefore of.	
Wet aggregate stability	Wet aggregate stability (extent to which soil aggregates resist falling apart when wetted) is a sensitive indicator of soil structural stability and resilience. Generally increases with biochar additions, but mainly in sandy soils.	(Blanco-Canqui 2017)
Mean residence time	Inverse of the decay rate and average time that biochar is present in soil.	(Lehmann and Joseph 2015)
Soil absorption	The enrichment (positive adsorption) or the depletion (negative adsorption) of one or more chemical species at an interface.	(Calvet 1989)
Direct absorption of CO_2	Biochar has been shown to directly sorb gases into its pores.	(Lehmann and Joseph 2015)
Soil porosity	Biochar can increase soil porosity (volume that is taken up by the pore space) by (i) reducing soil bulk density, (ii) increasing soil aggregation, (iii) interacting with mineral soil particles, and (iv) reducing soil packing.	(Blanco-Canqui 2017)
Microbial efficiency	CO_2 release per unit of soil carbon. Often increased with biochar additions.	(Atkinson et al. 2010; El-Naggar et al. 2015).
Priming	Increase or decrease in mineralization of native OM due to addition of a new OM (such as biochar or manure).	(Lehmann and Joseph 2015; Watzinger et al. 2014)
Enhanced Biochar	Pyrolyzed organic matter heated (350–650 °C) under oxygen-limited conditions that has also undergone a patented post-pyrolysis treatment.	(Terry et al. 2020)

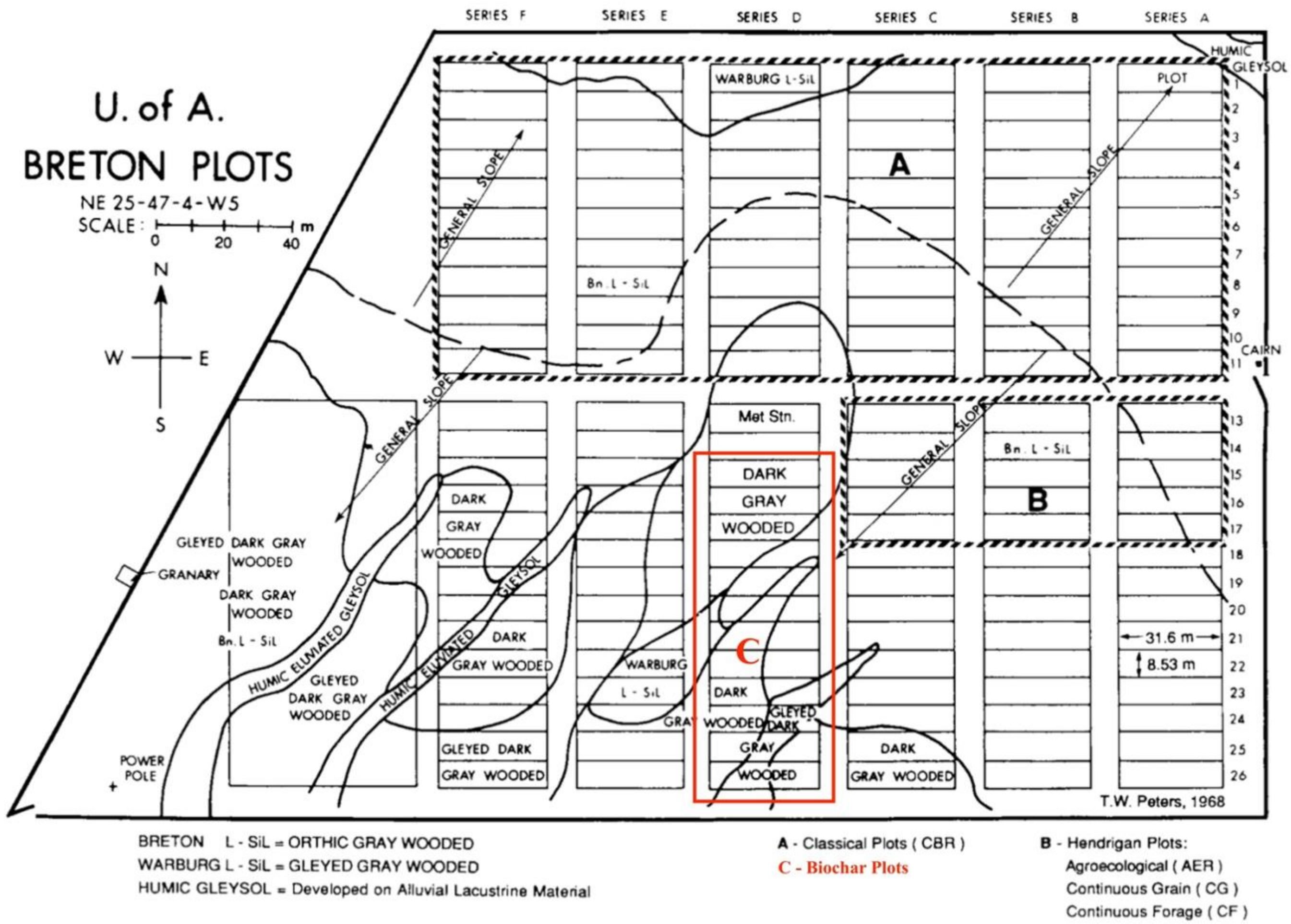

Figure 1.1. Location of the Biochar plots (C) at the Breton Research Station (Wani et al. 1994).

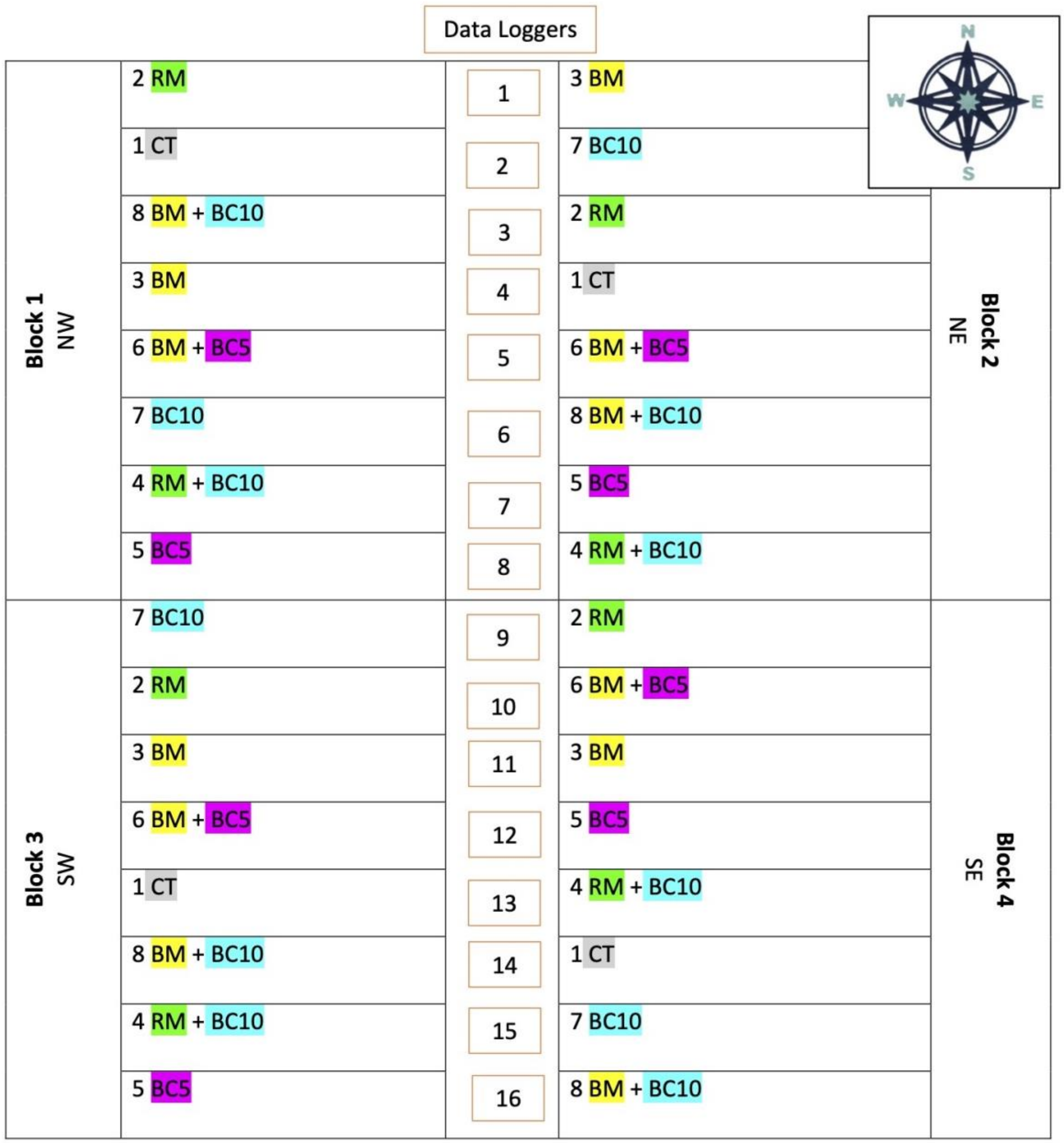

Figure. 1.2. Breton Research Station, biochar plot experimental setup

Chapter 2: Biochar-manure changes soil C mineralization in a Gray Luvisol used for agricultural production

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2.1 Abstract

Biochar is a source of stable organic matter being explored as a manure additive. A 64-day incubation experiment was conducted to quantify the short-term effect of manure (RM), biochar-manure (BM), raw biochar (BC), RM + BC, and BM + BC amendment on soil carbon (C) and nitrogen (N) mineralization. Manure increased CO_2-C emissions, with the highest cumulative CO_2-C emissions being observed for RM + BC. Treatments with BM minimized soil C mineralization, indicating manure-C stabilization. By contrast, neither RM nor BM affected soil N mineralization. Applying BM might benefit soil C sequestration by lowering CO_2-C emissions over the long-term.

2.2 Introduction

Biochar is a promising avenue for carbon (C) sequestration in temperate soils of prairie eco-regions. Biochar is a form of pyrogenic-C produced by O_2 limited thermal decomposition of organic matter (OM) at temperatures < 700 °C. Amending soil with biochar has been shown to transform organic carbon, alter mineralization rates, and mitigate agricultural greenhouse gas (GHG) emissions. Some processes that are important it this role include increasing cation exchange capacity, pH, and water retention as well as altering microbial activities and communities (Alburquerque et al. 2013; El-Naggar et al. 2015; Hood-Nowotny et al. 2018; Karer et al. 2013). In short, biochar stabilizes soil OM, thereby resulting in higher soil C benefits relative to more labile amendments such as manure and compost (Preston and Schmidt 2006; Whitman et al. 2015).

Recently, findings that biochar use in animal feeding could lower enteric methane (CH_4) emissions have expanded the prospect of its utilization in modern-day farming (Whitman et al. 2015). Similarly, biochar-enriched manure may also stabilize manure OM, emitting less ammonia (NH_3), nitrous oxide (N_2O) and CH_4 once applied to croplands (Kammann et al. 2017). Romero et al. (2021a) demonstrated that biochar increases manure OM recalcitrance and its overall C sequestration potential, yet its effect on manure OM mineralization is mostly unknown.

Pyrogenic-C represents up to 20-65% of total soil C in prairie soils (Preston and Schmidt 2006). Nevertheless, in cropped sites across central Canada, farming has removed pyrogenic-C by suppressing wildfires, thus altering its cycling in surface soil layers. Restoring pyrogenic-C levels, mainly through biochar additions, could increase crop production, particularly in Gray Luvisols, soils that are often difficult to farm due to their inherent low pH and poor nutrient availability. The objective of this work was to determine the effect of manure amendment, in the presence or absence of biochar, on soil C and N mineralization over a 64-day incubation period. Due to pyrogenic-C's recalcitrant nature, it was hypothesized that biochar would retain some of its properties once excreted by feedlot cattle (Romero et al. 2021a), thereby limiting microbial activity and associated soil C and N mineralization (Whitman et al. 2015).

2.3 Methods

2.3.1 Experimental design and treatments

Solid manure was retrieved from a 235-day feeding trial conducted at the Lethbridge Research and Development Centre (LeRDC) of Agriculture and Agri-Food Canada (AAFC) near Lethbridge, AB, Canada. Briefly, eighty yearling beef steers were fed a typical Canadian feedlot backgrounding diet consisting of 60% barley silage, 35% barley grain and 5% mineral supplement (Terry et al. 2020). Treatments consisted of adding southern yellow pine (*Pinus echinata*) biochar (National Carbon, Oakdale, MN) at 0% (regular manure, RM) and 2% (biochar-manure, BM), of diet dry matter. Manure samples were collected in January 2019 and sent to the University of Alberta Campus in Edmonton, AB, Canada, for incubation and analysis. The biochar had a pH of 7.3, an electrical conductivity of 317 mS cm^{-1}, a H/C ratio of 0.29, and 733 g kg^{-1} and 2 g kg^{-1} of total C and N, respectively.

Surface soil (0-10 cm) was collected from the Breton Research Station (53°07′N, 114°28′W) near Breton, AB, Canada. In 2009; the plots produced oats, grass, and wheat with no fertilization. For 2010-2011, oats and barley were harvested, followed by a fallow period. From 2013-2015, barley-canola rotations were fertilized with 80 kg N ha^{-1} urea in 2015. Wheat-barley-barley were grown from 2016-2018 with an application of 50 kg N ha^{-1} urea in 2017 and 2018.

The soil, classified as a loamy sand Gray Luvisol (pH 6.3), was amended in four replications with (i) regular stockpiled manure (RM) at 160 Mg ha^{-1}, (ii) biochar-manure (BM) at 160 Mg ha^{-1}, (iii) raw biochar (BC) at 10 Mg ha^{-1}, (iv) a combination of (ii) and (iii) (BM + BC), (v) combination of (i) and (iii) (RM + BC) and (vi) a non-amended control (soil without amendments) (CT). The rate of manure application was selected to mimic field applications for barley forage production in Alberta.

2.3.2 Incubation and analyses

After collection, the soil was air-dried at room temperature, passed through a 2-mm sieve to remove plant litter, homogenized and stored at room temperature (22 °C) for 30 days. Samples were incubated in a Forma Diurnal Growth Chamber-Model 3740 (Thermo Fisher Scientific, Waltham, MA) at 25 °C. Two identical sets of 200 g (dry-weight basis) of air-dried soil were weighed and placed into 500 mL Mason jars. One set was kept undisturbed for carbon dioxide (CO_2) gas measurement. The second set was also kept undisturbed, but small cores were removed for inorganic N measurements at the same sampling intervals as for CO_2. Water holding capacity (WHC) was determined using a pressure plate analysis at field capacity (-0.33 MPa). Both sets were pre-incubated at 60% WHC for seven days to avoid the initial flush of respiration after soil disturbance. Immediately after pre-incubation, manure/biochar amendments were applied to the soil. Jars were loosely capped, and caps were removed weekly for 10 min to ensure adequate aeration over the incubation period, during which samples were weighed and water added to maintain the 60% WHC condition.

CO_2-C fluxes and inorganic N, nitrate (NO_3-N) and ammonium (NH_4-N), concentrations were measured every three days for the first week, once a week for the following two weeks, and then biweekly for the remainder of the study (64 days). CO_2-C fluxes were quantified using a LiCor LI-8100 Soil Gas Flux System and multiplexor (LI-COR, Lincoln, NE) plumbed for flask measurements. Soil was extracted with a 2 M KCl solution at a 1:5 (w:v) soil:extract ratio, shaken (250 rpm, 1 h) and then filtered using a Whatman No. 42 filter paper. NO_3-N and NH_4-N were determined via colorimetry using a Thermo Gallery Plus Autoanalyzer (Thermo Fisher Scientific, Waltham, MA).

Sub-samples of soil and manure/biochar amendments were dried at 105 °C for 48 hours, ball-milled (<0.15 mm) and stored in 20 mL scintillation vials. Total C (TC) and N (TN) were analyzed by dry combustion using a Thermo Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA). Pyrogenic-C was measured using the benzene polycarboxylic acid (BPCA) method following Wiedemeier et al. (2016). Soil pH was measured in a 1:5 soil:water slurry, after samples were shaken for 1 h, vacuum filtered and allowed to settle for 30 min.

2.3.3 Statistical Analysis

All statistical calculations were performed using R v. 1.1 (R Core Team, 2020). To predict soil C and N mineralization dynamics, the data was fit to a first-order reaction,

$$Am = Ao(1 - e^{-kt}) \quad (1)$$

where Am is the cumulative amount of soil C (Cm) or N (Nm) mineralized, Ao represents the labile pool of C(Co) or N (No), t is time, and k is the rate of mineralization constant of C (Kc) or N (Kn) (Riffaldi et al. 1996). First order kinetics was found to best describe mineralization from multiple authors (Ellert and Bettany 1988; Menichetti et al. 2019; Murwira et al. 1990; Riffaldi et al. 1996). Reasons included the ability to account for initial flush of easily mineralization OM (Ellert and Bettany 1988), more accurate represent older, more stable SOC (biochar) (Menichetti et al. 2019), and best fit the chosen 60% WHC (Murwira et al. 1990). The efforts to fit other equations, such as second order, were found to be unnecessary (Menichetti et al. 2019) and not account for various (biochar and manure combinations) amendment mineralization rates (Riffaldi et al. 1996).

A one-way analysis of variance (ANOVA) was used to analyze differences between manure/biochar amendments and soil properties, as well as the effect of manure/biochar treatments on soil C and N mineralization first-order kinetic curves. Assumptions of normal distribution and equal variance were confirmed using Shapiro and Bartlett/Levene tests on residuals, respectively. Means were compared using Tukey's honest significance difference (HSD), where differences were detected at $P < 0.05$.

2.4 Results & Discussion

2.4.1 Soil and manure characteristics

Manure pH ranged from 6.9 to 7.1, whereas the soil had a pH of 6.3. On average, manure contained 29% and 35% more TC and TN than soil, respectively (data not shown). Manure TN was unchanged ($P > 0.05$) by dietary treatment, averaging 18.6 and 20.2 g kg^{-1} for RM and BM, respectively. By contrast, BM contained 11% more TC than RM. This response was likely associated with a higher ($P < 0.05$) pool of pyrogenic-C within BM (6.3 m g^{-1}) relative to BM (2.0 m g^{-1}) (data not shown). Inorganic N of cattle feedlot manure is around 40% of TN (Eghball et al. 2002), so RM had approximately 7.51 g kg^{-1} and BM had approximately 8.11 g kg^{-1}. The CT available N was limited, only 14.5% of TN, and therefore 0.01 g kg^{-1}.

2.4.2 Carbon mineralization

Carbon mineralization (Cm; 528 mg CO_2-C kg^{-1}) was increased ($P < 0.001$) with RM + BC relative to the other amendments (> 354 mg CO_2-C kg^{-1}) (Table 2.1), while Co (577 mg CO_2-C kg^{-1}) was only augmented ($P < 0.001$) with RM + BC compared to BM (397 mg CO_2-C kg^{-1}). Mixing RM with BC stimulated a priming effect on C mineralization of soil OM, while BM alone and BC alone did not (Fig. 2.1a), supporting the hypothesis that biochar and manure mixtures would have a lower soil C mineralization potential relative to manure-only treatments. Cm was reduced in BM (217 mg CO_2-C kg^{-1}) compared to RM (340 mg CO_2-C kg^{-1}) but was similar to CT (215 mg CO_2-C kg^{-1}) (Table 2.1). The reaction rate coefficient (Kc) was increased with RM + BC, BM and BM + BC relative to CT and BC treatments (Table 2.1).

Apparently, there was a synergistic effect between RM and BC, considering that RM- and BC-only resulted in lower Kc values (Table 2.1). Lentz et al. (2014) found that applying manure with biochar improves the ability of heterotrophs to degrade biochar-C, agreeing with the findings of greater soil C mineralization under RM + BC ($P < 0.05$). Adding biochar to manure may also improve aeration, further increasing microbial activity over labile-C (Whitman et al. 2015). Our observation supports this as cumulative CO_2-C emissions in RM + BC did not plateau as quickly as with the other treatments (Fig. 2.1a).

Interestingly, BM + BC and RM + BC exhibited distinct CO_2-C emission patterns (Fig. 2.1a), despite soil C mineralization rates being similar between RM and BM treatments (Table 2.1). Romero et al. (2021a) demonstrated that biochar passes through the gastrointestinal tract of feedlot cattle mostly unaltered. Based on this observation, it is speculated that manure OM in BM is as labile as in RM, given that manure-C does not interact with biochar-C (Romero et al. 2021a). However, biochar-manure is expected to be more aromatic than RM when considering the whole OM mixture (Romero et al. 2021a). The latter supports the findings that adding biochar to BM does not increase CO_2-C emissions as much as adding biochar to RM (Table 2.1).

2.4.3 Nitrogen mineralization

Nm and No were not affected (P = 0.130) by amendment type, even though Kn was increased (P < 0.05) with BM + BC relative to CT and BC treatments (Table 2.1). Joseph et al. (2015) demonstrated that biochar becomes enriched by organic-N within the rumen, potentially explaining higher manure TN contents in BM relative to RM (data not shown). Biochar may also stabilize N *via* sorptive reactions, limiting manure-N availability while prompting excess nutrient mining within BM + BC (Whitman et al. 2015). Amending soil with BM + BC increased NO_3-N + NH_4-N availability, in agreement with Lentz et al. (2014) who found that co-applying manure with biochar maximizes net N mineralization. RM and RM + BC had closer soil N mineralization rates than BM and BM + BC (Table 2.1); cattle-ingested biochar was presumably more reactive than its raw counterpart (Joseph et al. 2015a).

2.5 Conclusions

Application of manure, biochar and biochar-manure impacted soil C mineralization but did not affect soil N mineralization in the studied Gray Luvisol. Cumulative CO_2-C emissions were higher with RM + BC than BM + BC and adding BC to RM or BM increased soil C and N mineralization rates. Our results indicate that BC and BM amendment might benefit soil C sequestration by lowering CO_2-C emissions over time without limiting soil N availability. Further research calls for whole-farm studies to validate the cascaded use of BM amendment in agroecosystems. Probing BM properties at a larger scale, utilizing different biochar types, is critical to identify BM types that maximize soil C benefits in western Canada.

Table 2.1. Respired soil C and mineralized soil N first-order kinetic parameters to amendment types over a 64-day incubation period and their corresponding P-values (means ± SE) (n = 3). Amendments: BC, biochar (10 Mg ha^{-1}); RM, manure from feedlot cattle on a control diet (160 Mg ha^{-1}); CT, control (no amendments); BM, manure from feedlot cattle on a control diet with the addition of BC at 2% of diet dry matter (160 Mg ha^{-1}).

Amendment	Cm (mg CO_2-C kg^{-1})	Co (mg CO_2-C kg^{-1})	Kc (mg CO_2-C d^{-1})	Nm (mg N kg^{-1})	No (mg N kg^{-1})	Kn (mg N d^{-1})
CT	215 ± 20^{d}	397 ± 63^{ab}	18.3 ± 1.9^{c}	141 ± 10^{a}	267 ± 10^{a}	0.012 ± 0.001^{b}
BC	260 ± 3^{cd}	533 ± 65^{ab}	16.0 ± 2.8^{c}	143 ± 4^{a}	276 ± 2^{a}	0.012 ± 0.001^{b}
BM	217 ± 18^{bc}	397 ± 20^{b}	54.9 ±1.5^{a}	169 ± 9^{a}	229 ± 9^{a}	0.021 ± 0.001^{ab}
RM	340 ± 8^{bc}	403 ± 9^{ab}	41.8 ±1.2^{b}	155 ± 6^{a}	246 ± 6^{a}	0.017 ± 0.003^{ab}
BM + BC	354 ± 22^{b}	406 ± 31^{ab}	47.9 ± 4.6^{ab}	146 ± 2^{a}	190 ± 2^{a}	0.023 ± 0.002^{a}
RM + BC	528 ± 25^{a}	577 ± 28^{a}	55.6 ± 1.5^{a}	156 ± 10^{a}	247 ± 10^{a}	0.017 ± 0.003^{ab}
***P*-Value**	<0.001	<0.001	<0.001	0.130	0.130	<0.050

Note: Means followed by a common letter within a column are not significantly different (P < 0.05).
Abbreviations: Cm, cumulative amount of soil C mineralized; Co, labile pool of C; Kc, rate of mineralization constant of C; Kn, rate of mineralization constant of N; Nm, cumulative amount of soil N mineralized; No, labile pool of N; t, time.

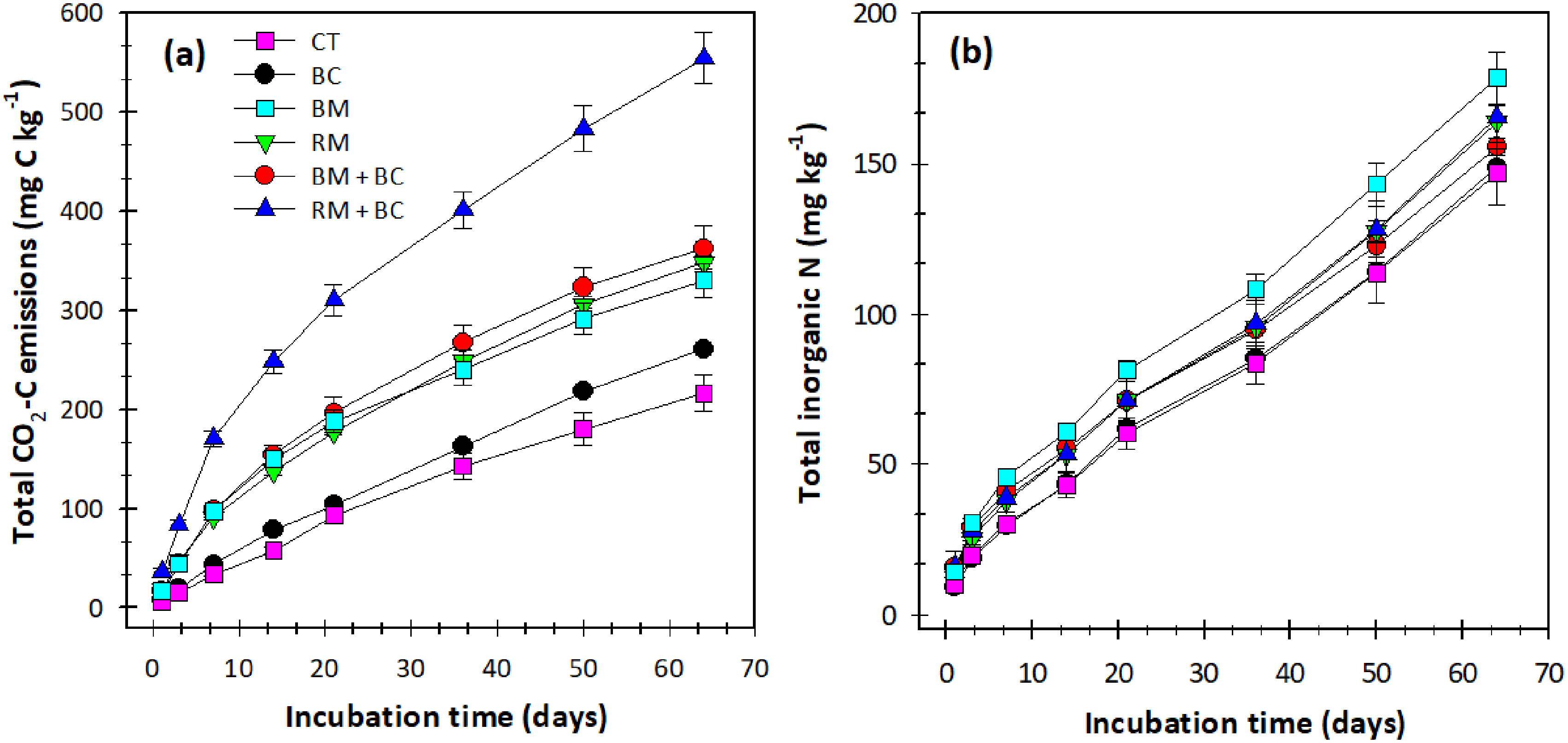

Figure. 2.1. Respired soil C (a) and mineralized soil N (b) as affected by manure/biochar treatments. Amendments: BC, biochar (10 Mg ha^{-1}); RM, manure from feedlot cattle on a control diet (160 Mg ha^{-1}); CT, control (no amendments); BM, manure from feedlot cattle on a control diet with the addition of BC at 2% of diet dry matter (160 Mg ha^{-1}). Vertical bars indicate standard errors of the means (n = 3).

Chapter 3: Biochar-manure impacts wheat and canola grain productivity, dry matter partitioning and protein content in Western Canada

3.1 Abstract

Amending soil with manure from cattle fed biochar (biochar-manure) is a potential best management practice to improve plant nutrition in the circular economy. Yet, information concerning the agronomic performance of biochar-manure under temperate field conditions is scarce. A two-year study on a Gray Luvisol was conducted to determine the effect of biochar-manure for crop growth of spring wheat (*Triticum aestivum* L.), followed by canola (*Brassica napus* L.), soil fertility and microbial function. Treatments included; i) no amendments (CT), ii) biochar at 5 and 10 Mg ha^{-1} (BC5 and BC10), iii) regular stockpiled manure (RM) at 100 kg total N ha^{-1}, iv) stockpiled biochar-manure (BM) at 100 kg total N ha^{-1}, and v) BC and RM (BC+RM) or BM (BC+BM) at the aforementioned rates. During the wheat growing season in 2020, which had high precipitation, grain yield was 2.4 times greater in BM+BC10 than in BM alone (1416 vs. 579 kg ha^{-1}, $P < 0.001$), highlighting synergistic effects of biochar-manure together with biochar application on agronomic performance. Conversely, lower precipitation and warmer temperatures in the canola growing season in 2021 hampered any statistical differences among treatments. Exchangeable N increased by nearly 10x from Fall 2020 to Fall 2021, possibly rom retained straw. No significant ($P > 0.05$) correlations were found between P and S and wheat or canola yields. While soil microbial biomass did not change by the end of the experiment, shifts toward amino acid utilization with biochar additions in both crops, potentially influenced crop growth and nitrogen use efficiency. In summary, biochar-manure + biochar at 10 Mg ha^{-1} performed best in this study, in the first cropping season with cold and rainy conditions.

3.2 Introduction

A growing world population is increasing food demand (Zhao et al. 2020). Wheat is one of the most important crops produced worldwide (Terman et al. 1969), and extensive research has been conducted to improve protein content, days to maturity, straw strength, and disease resistance (Carew et al. 2009). Biochar, a product of thermal alteration (pyrolysis) of OM with little or no oxygen, produces a stable carbon-rich and porous compound that has showed potential to improve wheat production (Gomez et al. 2014; Lehmann and Kleber 2015; Pereira et al. 2014). Lehmann and Joseph (2015) and Steinbeiss et al. (2009) reported an overall positive impact on crop growth with biochar application rates >10 Mg ha^{-1} because the porous nature of biochar can adsorb ammonium (NH_4^+-N) and reduce nitrate (NO_3^--N) leaching, enhancing plant nutrient uptake (Pokharel and Chang 2019; Shahzad et al. 2018). For example, Terman et al. (1969) reported higher wheat yield and protein content with increased NO_3^--N retained by biochar. These findings call for an investigation of various crops under different biochar management regimes.

Understanding how biochar improves yields requires an integration of soil biology and chemistry. Biochar can have direct and indirect effects on soil microorganisms, including i) providing microbes with a direct C substrate; ii) adsorbing nutrients for microbial consumption; and iii) providing habitats for different microbial communities (Gomez et al. 2014). Biochar addition increases pH in acidic soils, which typically enhances microbial biomass and activity (Dempster et al. 2012; Gomez et al. 2014; Pokharel and Chang 2019; Watzinger et al. 2014). Additionally, biochar decreases bulk density, potentially increasing soil thermal conductivity and water retention, which may increase yield under unfavorable environmental conditions (Zhang et al. 2013).

While there have been many findings of increased yield with biochar addition, there are also many studies that found no significant changes to crop production (Alburquerque et al. 2013; Chan et al. 2007; Jones et al. 2012; Karer et al. 2013; Liu et al. 2014). There are still indirect advantages of biochar addition, such as reduced nitrogen (N) leaching when amended with N fertilizers (Güereña et al. 2013). Despite these benefits, many farmers are hesitant to apply biochar, given its high cost (Shahzad et al. 2018). Therefore, due to the recalcitrant nature of biochar, lack of nutrients initially available, and its high production cost, research should focus on the addition of biochar combined with other amendments (inorganic and organic fertilizers) for profitable crop growth (Ahmed and Schoenau 2015; Romero et al. 2021b), as its benefits may outweigh the drawbacks in terms of long-standing sustainable agricultural practices.

New research has been dedicated to finding different, more economical ways to apply biochar. One method is where biochar is consumed by animals and released into the corresponding manure, that is then applied as an organic fertilizer (Mohammed and Tak 2018). Terry et al. (2020) did not see any adverse effects on cattle health when supplementing cattle diets with biochar. Results from Romero et al. (2021a) showed that biochar did not change physically or chemically significantly as it passes through the rumen of cattle, meaning the resulting biochar in the manure retains its beneficial properties when amended to soil. Furthermore, in a biochar-feed experiment, Joseph et al. (2015a) reported that biochar adsorbed a range of nutrients in the rumen, and these nutrients are likely retained when applied to the soil.

To date, biochar research has mainly investigated highly weathered, nutrient-poor tropical soils, and research on biochar use in temperate agriculture is lacking (Ahmed and Schoenau 2015; Karer et al. 2013). But the use of biochar in prairie soils, in particular, is new, with mixed results in improving nutrient cycling, leaving much yet to be discovered (Romero et al. 2021b) and therefore making this research in Western Canada novel and important in the future of plant soil relations.

The objectives of this experiment were to analyze i) changes in crop grain protein content and yields as a function of additions of biochar, biochar-manure (BM) or regular manure (RM) at different biochar rates and ii) how soil and microbial function are influenced by biochar additions. First, I hypothesized that that manure would result in the highest canola and wheat yields due to the addition of labile OM for microbial activity, followed by BM due to additional nutrients from the rumen of cattle that are retained in the manure (Terry et al. 2020). Second, I theorized that that the highest grain protein contents would come from RM additions, followed by BM, due to the low N in BM and its ability to adsorb water and nutrients (Shahzad et al. 2018). Finally, I hypothesized that adding biochar and manure would increase soil microbial biomass due to creating a more favorable environment and new sources of C. It was predicted there would be an increase of soil microbial utilization of amino acids and soil fertility at high application rates in biochar due to improved use efficiency and retention of nutrients (Alburquerque et al. 2013).

3.3 Methods

3.3.1 Experimental design and treatments

The impact of manure treatments on crop yield was investigated using a randomized complete block design with four replicates per treatment. Soil was classified under the Canadian Soil Classification System, and as a loamy sand Gray Luvisol with a bulk density ranging from 1.27-1.41 g $cm^{-3,}$ (Dyck et al. 2012; Li et al. 2018). The study site at the Breton Research Station

(53°07′N, 114°28′W) has an average (1967–2018) growing season from 1 Apr–31 Aug, mean precipitation of 370 mm, and mean temperature of 3.1 °C (Dyck et al. 2020). In 2009; the plots produced oats, grass, and wheat with no fertilization. From 2010-2011, oats and barley were harvested, followed by a fallow period. From 2013-2015, barley-canola rotations were fertilized with 80 kg N ha^{-1} urea in 2015. Wheat-barley-barley were grown from 2016-2018 with an application of 50 kg N ha^{-1} urea in 2017 and 2018.

Treatments were incorporated in a completely randomized block design 5 cm into the field plots on September 13, 2019 ("Fall 2019") with (1) stockpiled manure from cattle on traditional western feedlot diet (RM) at a rate of 5.4 Mg ha^{-1} (target of 100 kg total N ha^{-1}), (2) stockpiled manure from cattle on traditional western feedlot diet with 2% biochar (BM) at a rate of 4.9 Mg ha^{-1} (target of 100 kg total N ha^{-1}), (3) pure biochar at a rate of 10 Mg ha^{-1} (BC10), (4) pure biochar at a rate of 5 Mg ha^{-1} (BC5), (5) a combination of (1) and (4), (6) a combination of (2) and (3), (7) a combination of (2) and (4), and (8) a control (soil without any organic or inorganic amendments – CT). The biochar utilized came from southern yellow pine (*Pinus echinate*) pyrolysis using a patented Engineered Biocarbon technique (Table 3.1).

The various manure treatments came from a feedlot study conducted at Lethbridge Research and Development Centre of Agriculture and Agri-Food Canada (AAFC) near Lethbridge, AB. Eighty yearling steers were used in a 235-day feeding trial (Terry et al. 2020). One of the amendments involved a traditional western cattle diet (60% barley silage, 85% barley grain, and 5% mineral supplement) (Terry et al. 2020). The other amendment included the same diet supplemented with 2% biochar (dry-matter basis). The same pinewood biochar used in the AAFC trials (BM manure) was used in the biochar plots (BC5, BC10, RM+BC5, BM+BC10, and RM+BC10). Biochar was provided by National Carbon, Inc. (Greenwood Village, CO) for the feedlot and field trials for Its patented post-pyrolysis treatment step in a front-end biomass pyrolysis (<650 ◦C) (Romero et al. 2021a). Inorganic N of cattle feedlot manure is around 40% of TN (Eghball et al. 2002), so RM and BM had approximately 40 kg available N ha^{-1} with no available N in the BC (under detection limit) and CT (<0.1 kg available N ha^{-1}) applications in the first year, and 16 kg available N ha^{-1} in the following year.

Soil temperature and moisture content data were collected using RT1 and EC5 sensors, respectively, with EM50 data loggers placed in each treatment plot one week after amendment (METER, Pullman, WA, USA). Fields were planted with CDC Go wheat on 3 June 2020 ("Summer 2020") and Round-up ready Canola on May 23, 2021 ("Summer 2021"), using a field plot seeder. At maturity on 4 October 2020 ("Fall 2020") and 10 Sept 2021 ("Fall 2021"), wheat and canola respectively, were harvested from two 1x1 m areas in each treatment plot for a composite sample using a hand-sickle. The remaining wheat and canola in each treatment plot were harvested with a combine, but the straw was retained on the plot throughout the trial. Soil samples (0−15 cm) were collected pre-seeding (Summer 2020) and post-harvest (Fall 2020 & 2021) and composited to be stored at 4 °C until they were processed in the lab.

3.3.2 Crop analyses

Wheat and canola samples were dried in burlap sacks at room temperature (22 °C) and threshed for crop biomass. Protein content was analyzed using near-infrared spectroscopy (NIR) (Williams et al. 1985). For the estimation of aboveground dry matter partitioning, harvest index (HI) was calculated according to Thilakarathna et al. (2021) in Eq. [1]:

$$Harvest\ Index = \frac{Grain\ yield\ DM}{Aboveground\ DM} \tag{1}$$

where $Harvest\ Index$ = wheat harvest index (kg grain DM kg^{-1} grain and straw DM), $Grain\ yield\ DM$ = grain yield from soil treatment (kg ha^{-1}), and $Aboveground\ DM$ = grain and straw yield from soil treatment (kg ha^{-1})

3.3.3 Soil analyses

For each soil collection period (Fall 2019, Summer 2020, Fall 2020, and Fall 2021), the soil was sub-sampled from two cores and mixed for homogeneity in each of these 32 plots, then transported back to the laboratory in a cooler the same day. In the lab, soil sub-samples were passed through a 4-mm sieve to remove debris and mixed for homogeneity, then stored in a refrigerator (4 °C). Soil sub-samples were dried at 105 °C for 48 hours to determine moisture content. Another set of sub-samples was air-dried for 48 hours, ground (< 2 mm) with a Ball Mill MM200 (Brinkmann Retsch, Haan, Germany), and stored in labelled 20-mL scintillation vials for elemental analysis. Total C and N were quantified by dry combustion with a Thermo Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (Sparks et al. 2020).

To measure thermal stability of SOM, differential scanning calorimetry (DSC; STA 6000, Perkin Elmer, Waltham, MA USA) was used on approximately 20 mg of dried soil (Fernández et al. 2011). The heat of combustion was calculated by integrating the DSC curve over the exothermic region. Thermal stability of the soil was calculated as a ratio of the heat of combustion for the recalcitrant region (410 - 725 °C) to the labile region (150 - 410 °C) of organic matter.

FE20 and FE30 meters (Mettler Toledo Columbus, OH, USA) measured soil pH and EC in a 1:2 (w:v) soil to water extract ratio after the sample was shaken for 1 hour, vacuum filtered and allowed to settle for 30 min. To measure anions, cations, and microbial biomass, 500 g composite soil samples were incubated at 20 °C for 72 hours (Solaiman 2007). Water content exceeded 40-50% field capacity (data not shown), so no additional water was added during incubation. Ion-exchange membranes were added to each soil sample, removed at the end of incubation, and then subject to 15 mL 0.5 M HCl extraction. The extracted solution was analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Thermo iCAP6300 Duo, Thermo Fisher Scientific, Waltham, MA, USA) to measure P, K, and S values (Qian et al. 1992). Ammonium concentrations were measured using the salicylate-hypochlorite method (Bower and Holm-Hansen 1980) and NO_3-N and NO_2-N were measured using the hydrazine reduction method (Kamphake et al. 1967) on a colorimetric autoanalyzer (Gallery Plus, Thermo Fisher Scientific, Waltham, MA, USA).

Approximately 20 g (dry wt. eq) of soil was used post-incubation for microbial biomass analysis (Solaiman 2007). One set of 20 g samples (dry wt. eq) were fumigated with 30 ml chloroform in 50 ml glass beakers for at least 24 hours. Another set of 20 g samples (dry wt. eq) was placed directly in 50 ml glass beakers to represent unfumigated samples. The two sets of samples were then extracted following Voroney et al. (2008) methodology with 0.5 M K_2SO_4 solution in a 1:2 (w:v) soil:extract ratio, shaken at 250 rpm for 1 hour, filtered using Whatman No. 42 filter paper and analyzed using a Shimadzu Total Organic Carbon Analyzer (Shimadzu Corporation Kyoto, Japan). The difference between fumigated and unfumigated C and N contents of each sample represented microbial biomass C and N, respectively (Solaiman 2007).

Community Level Physiological Profiles (CLPP) were measured with a microplate-based multi-substrate induced respiration experiment using a plate-reading spectrophotometer (Biotek Instruments, Winooski, VT, USA) at a 570nm wavelength (Campbell et al. 2003). Sixteen substrates, including carbohydrates (L-(+)-arabinose, D-(-)-fructose, D-(+)-galactose, D- (+)-glucose, D-(+)-trehalose), carboxylic acids (N-acetyl glucosamine, L-alanine, Y-amino butyric acid, L-arginine, L-cysteine-HCL, L-lysine-HCL), amino acids (Citric acid, α- ketoglutaric acid, L-malic acid, Oxalic acid), and deionized H_2O, were analyzed. The CO_2 production rates (μg CO_2-C g^{-1} h^{-1}) were measured on a Microplate Reader (Synergy/HT, BioTek) through pH changes expressed by colour changes in the indicator gel.

3.3.4 Statistical analyses

Assumptions of normal distribution and homogeneity of variance of the residuals were confirmed with Shapiro and Bartlett/Levene tests (Logan 2011), and if the models did not meet assumptions, log or sqrt transformations were applied to the response variables. A one-way analysis of variance (ANOVA) was used to assess soil and manure properties. A blocked ANOVA, where treatment was a fixed factor and block was a random factor, was used if the block was a significant factor. A linear one-way ANOVA was used instead if the block was not significant. Differences were detected at $P < 0.05$, and when significant differences were present, the results were further analyzed using a Tukey-Kramer test to determine which treatments were significantly different.

Relationships between the soil properties (temperature, moisture, and biogeochemical values) and wheat and canola yields were examined using Spearman's rank correlations. Soil temperature was correlated using total days greater than 15 °C and soil moisture was correlated using total days greater than 30% volumetric water content for each respective growing season (Jun 3 – Oct 4 2020 and May 23 – Sept 10 2021). These thresholds were selected to represent an amended growing degree days and chosen due to their influence on crop production in terms of temperature and moisture stress (Atkinson 2018; Reddy et al. 1985; White et al. 2019). Statistical calculations were performed using R v. 4.2.1 (R Core Team 2021).

Microbial community profiles were also examined using ordinal statistics (PC-ORD software v.6 (MjM Software, Gleneden Beach, OR, USA). Nonmetric multidimensional scaling (NMS) displayed group dissimilarity in two dimensions. Multi-response permutation procedure (MRPP, $P = 0.05$) with a Bray-Curtis distance measure determined significant differences among treatments (Logan 2011).

3.4 Results

3.4.1 Environmental conditions

Air temperatures ranged from 13.9 °C to -2.8 °C to from mid-September to mid-October 2019 (Fig. 3.1). The soil followed a similar temperature trend, freezing in early November 2020 (Fig. 3.2a). In the Fall 2019 months (Sept-Nov), precipitation averaged 43 mm (Fig. 3.1). Soil moisture contents ranged from 0.11 – 0.31 m^3 m^{-3} in Fall 2019 (Fig. 3.2b). The average winter precipitation of 25.5 mm was lower in December 2019 and January 2020 compared to 44.5 mm in February and March 2020 (Fig. 3.1b).

During the 2020 growing season (Apr-Sept; Fig. 3.1), daily temperatures (11.0 °C) were 6.4% lower than the average (11.7 °C), but precipitation was 35% higher than the long-term average (552.6 mm vs. 410.4 mm). The total number of days greater than 15°C ranged from 73-82, with the greatest from the BC10 and RM+BC10 and the lowest from the BC5 ($P > 0.05$; Table 3.8). The total number of days greater than 30% volumetric water content ranged from 36-49 amongst treatments, with the highest from BC10 and the lowest from the CT ($P > 0.05$; Table 3.8). Mid-July to late August had some of the highest air (21 °C; Fig. 3.1) and soil temperatures

(23.5 °C; Fig. 3.2a). There were frequent wetting and drying cycles throughout spring and Summer, represented by soil moisture peaks in late Apr, early and late May, and early June and large differences in soil moisture amongst treatments (Fig. 3.2b & Table 3.8).

On the contrary, the 2021 growing season (Apr-Sept; Fig. 3.1) had 5.9% higher than typical temperatures (12.4 °C) and 22.5% lower than usual precipitation (318 mm) rates. 2021 had larger ranges of air temperature (up to 28.8 °C; Fig. 3.1), and soil temperatures (up to 29.4 °C; Fig. 3.2a) compared to 2020. The total number of days greater than 15°C ranged from 84-92, with the highest from CT and BC10 and the lowest from RM ($P > 0.05$; Table 3.8). The total number of days greater than 30% volumetric water content ranged from 6-20, with the highest from BC10 and lowest from RM+BC10 ($P > 0.05$; Table 3.8).

Wheat grain and harvest index was positively correlated to soil temperature and negatively correlated to moisture ($P > 0.05$), while protein was strongly correlated to temperature ($P < 0.01$; Table 3.7). Canola biomass was negatively correlated ($P > 0.05$) to soil temperature and moisture, but grain, protein, and harvest index was positively correlated ($P > 0.05$) to soil moisture.

3.4.2 Initial soil and manure properties

Soil pH differed significantly among all treatments ($P < 0.001$), with the control having the lowest (6.33), followed by RM (6.91), BM (7.05), and BC (7.18) (Table 3.2). Manures (RM and BM) had similar ($P > 0.05$) TC, TN, and C/N and had, on average, 28.8% and 35.1% more TC and TN than soil (Table 3.2). BM contained significantly more (11.2%) TC than RM; however, the amount of TN from biochar was under detection limits (Table 3.2). Organic matter ($P < 0.05$) was lowest in soil (5.10%), 36.7% for BC, 65.2% for BM, and highest for RM (78.6%;

Table 3.2). However, OM stability did not differ ($P > 0.05$) between manures (RM had a ratio of 0.18 of recalcitrant to labile OM and BM a ratio of 0.21 of recalcitrant to labile OM) but was highest in BC ($P < 0.05$; 1.26; Table 3.2).

3.4.3 Crop biomass, grain yield, protein content, and harvest index

In the 2020 wheat harvest, there were no significant differences in wheat biomass (Fig. 3.3a), ranging between 2513 and 3215 kg ha^{-1}, or harvest index (Fig. 3.3d). In the 2021 canola harvest, there were no significant differences ($P > 0.05$) in biomass (Fig. 3.4a), grain yield (Fig. 3.4b), protein (Fig. 3.4c), or harvest index (Fig. 3.4d).

Wheat grain yield was greatest in RM+BC10 ($P < 0.05$; 1485 kg ha^{-1}) and BM+BC10 (1415 kg ha^{-1}; Fig. 3.3b), and lowest in BM (579 kg ha^{-1}). RM+BC10 and BM+BC10 had the highest grain yields, yet the CT had the highest protein (12%; $P < 0.05$) and BM+BC5 was lowest (9.5%). Focusing on aboveground dry matter partitioning estimated as wheat harvest index (HI), BC5 was highest ($P > 0.05$; 0.48) and BM was lowest (0.25; Fig. 3.3d).
RM and RM+BC10 had the highest ($P > 0.05$) canola biomass (3215 and 3062 kg ha^{-1}, respectively) and CT was the lowest (1975 kg ha^{-1}). The CT also had the lowest ($P > 0.05$) canola grain yield (508 kg ha^{-1}; Fig. 3.4b) and protein content (21 %; $P < 0.05$) protein. The highest ($P > 0.05$) grain yield came from RM (687 kg ha^{-1}) and protein from RM+BC10 (21%).

3.4.4 Soil chemical and biological properties

Treatments were only significantly different for P and S during Fall 2019 (Table 3.3) and K during summer 2020 (Table 3.4) Treatments RM, BM, and BM+BC10 had the highest ($P < 0.05$) exchangeable S (average of 24.6 µg 10 cm^2 d^{-1}) and BC5 had the lowest (15.6 µg 10 cm^2 d$^-$

^{1}). BM+BC10 had the highest (1.47 μg 10 cm^2 d^{-1}) and BM had the lowest P (0.18 μg 10 cm^2 d^{-1}). Nitrogen ranged from 31.4 – 63.7 μg 10 cm^2 d^{-1} and K from 22.3 – 42.4 μg 10 cm^2 d^{-1}. In summer 2020 (Table 3.4), BC5 had the highest ($P < 0.05$; 26.4 μg 10 cm^2 d^{-1}) and CT and RM had the lowest K (13.1 μg 10 cm^2 d^{-1}). Nitrogen ranged from 5.50– 11.1 μg 10 cm^2 d^{-1}, P ranged from 0.35 – 0.62 μg 10 cm^2 d^{-1}, and S ranged from 1.92– 2.67 μg 10 cm^2 d^{-1}. In fall 2020 (Table 3.5), N (3.81 – 7.17 μg 10 cm^2 d^{-1}) and K decreased (10.9-17.7 μg 10 cm^2 d^{-1}), P (0.30 – 0.73 μg 10 cm^2 d^{-1}) and S remained similar (2.4 – 2.88 μg 10 cm^2 d^{-1}). In fall 2021 (Table 3.6), N ranged from 13.8 – 31.9μg 10 cm^2 d^{-1}, P ranged from 0.41 – .98 μg 10 cm^2 d^{-1}, K ranged from 22.3 – 42.4 μg 10 cm^2 d^{-1}, and S ranged from 7.17–9.65 μg 10 cm^2 d^{-1}.

Microbial biomass C (MBC) and N (MBN) differed significantly among treatments on all sampling dates (Tables 3.3-3.5), except for MBN in Fall 2020 ($P > 0.05$; Table 3.5) and Fall 2021 ($P > 0.05$; Table 3.6). The MBC was highest in BC5 (1201 mg kg^{-1}) and BM+BC10 (981 mg kg^{-1}) and lowest in BC10 (632 mg kg^{-1}), while MBN was highest for BM (170 mg kg^{-1}) and RM+BC10 (219 mg kg^{-1}) treatments and lowest in CT (91.3 mg kg^{-1}) at amendment (Table 3.3). Both MBC and MBN increased during Summer 2020 (Table 3.4) and Fall 2021 (Table 3.6), and MBC and MBN increased in Fall 2020 (Table 3.5).

The EC was significantly higher ($P < 0.05$) in RM (453 µS cm^{-1}), BM (495 µS cm^{-1}), and BM+BC10 (739 µS cm^{-1}) during Fall 2019, with a large decrease in all treatments during Fall 2020 (Table 3.5), followed by an increase during Fall 2021 (Table 3.6). The EC was not significantly different among treatments ($P > 0.05$) during Summer 2020 (Table 3.4) or Fall 2021 (Table 3.6). The pH did not differ significantly among treatments ($P > 0.05$) during any sampling times except Fall 2021 ($P < 0.001$), when values in the manure treatments were significantly higher than BC10 (6.98 and 6.74, respectively). pH increased, on average, from Fall 2019 (Table 3.3) to Fall 2021 (Table 3.6).

Total C and N were stable throughout the experiment, with only TC significantly different ($P < 0.05$) among treatments from Fall 2019 (Table 3.3) to 2021 (Table 3.4), except for Fall 2020 (Table 3.5; $P > 0.05$). At the start in Fall 2019 (Table 3.3), BM+BC10 had the highest ($P < 0.05$) TC (53.0 g kg^{-1}), then RM+BC10 (30.2 g kg^{-1}) at the end of Fall 2021 (Table 3.6). TN ranged between 1.7 – 2.04 g kg^{-1} throughout the growing season (Tables 3.4-3.6) but did not differ ($P > 0.05$) among treatments during the entire experiment.

The wheat biomass, grain, and harvest index were not ($P > 0.05$) correlated to any parameter, but the protein was negatively correlated to microbial biomass C and N ($P < 0.001$, Table 3.7). Canola biomass was correlated ($P < 0.05$) to microbial biomass N, protein and harvest index were negatively correlated to N and P respectively (Table 3.7).

3.4.5 Microbial functioning

Differences between treatments and soil microbial functioning were analyzed using a Bray-Curtis distance measure (Fig. 3.5) and an ANOVA (Fig. 3.6). Soil microbial functions did not differ significantly ($P > 0.05$) among treatments for Fall 2019 or Summer 2020 (MRPP data not shown). However, microbial functions differed significantly ($P < 0.05$) among CT versus RM+BC10 ($P = 0.015$), BC5 ($P = 0.020$), BM+BC5 ($P = 0.031$), and BC10 ($P = 0.032$) in Fall 2020 vs. RM+BC10 ($P = 0.027$), BC5 ($P = 0.036$), BM+BC5 ($P = 0.045$) in Fall 2021 towards amino acid utilization. PC1 and PC2 axes explained 92.3%, 89.3%, 76.0%, and 78.0% of the variation within the eight treatments for Fall 2019, Summer 2020, and Fall 2020 sampling dates, respectively.

When compared as cumulative respiration, BM+BC5, RM+BC10, and BM+BC10 had the greatest respiration rates in amino acids (*P* < 0.05; 132, 124, and 121 μg $CO2\ g^{-1}\ ha^{-1}$ respectively) and the CT was the lowest (Fig. 3.6c) at 89 μg $CO2\ g^{-1}\ ha^{-1}$. The manures (RM and BM) and biochars (BC5 and BC10) were not significantly different (*P* < 0.05) from one another. All treatments were similar (*P* > 0.05) when looking at carbohydrates (Fig. 3.6a; ranging from 103 to 152 μg $CO2\ g^{-1}\ ha^{-1}$) and carboxylic acids (Fig. 3.6b; ranging from 121 to 165 μg $CO2\ g^{-1}\ ha^{-1}$).

3.5 Discussion

3.5.1 Environmental impacts and crop growth

This study supports the hypothesis of increased recalcitrance of BM compared to RM when applied to soil within the first year; however, this recalcitrance contradicts the initial hypothesis that RM and BM would have a higher yield than biochar alone, given the labile properties of manure. Precipitation during the 2020 growing season (Apr 1-Sept 30) was 35% higher than the long-term average (552.6 mm vs. 410.4 mm, respectively). Breton's clay-dominated soil likely benefited from reduced soil compaction, waterlogging, and improved root growth (not measured) (Chan et al. 2007) in biochar amended treatments, resulting in higher wheat grain yield in BC, RM+BC and BM+BC treatments and protein in the biochar treatments (*P* < 0.05; Fig. 3.3b & c). Differences were present in wheat grain yield (*P* < 0.01; 1153 and 1203 kg ha^{-1} for BC10 and BC5, respectively; Fig 4.3b) and protein content (*P* < 0.01; 11.6 and 10.8 % for BC10 and BC5, respectively; Fig. 3.3c), which were significantly higher when BM was added with BC than BM (579 kg ha^{-1} grain and 9.9 % protein) or RM (1026 kg ha^{-1} grain and

10.3 % protein) alone. Likewise, the RM plots trended higher canola biomass, grain, and harvest index than BM plots ($P > 0.05$; Fig. 3.4) due to a warmer and drier season than the average year.

Temperatures during the growing season (11.0 °C) were 6.4% lower than the long-term average (11.7 °C) in 2020 (Fig 3.1), potentially limiting the wheat yield (Meng et al. 2017), as wheat grain (0.293) and harvest index (0.287) were positively correlated ($P > 0.05$), and protein strongly related (0.481; $P < 0.01$; Table 3.7), to soil temperature. This explains the higher ($P < 0.001$) protein content for CT and BC10 (Fig. 3.3). The northwest regional yield for spring wheat was only 73.2% of the 5-year index and 72.5% of the 72.5 of the 10 year index compared to the 108% of the entire province (Alberta Agriculture 2020), drawing attention to the importance of regional climates. In support of these findings, a study by Gauer et al. (1992) found that increased moisture also decreased protein by about 1% for N applied at 80-120 kg ha^{-1} and all variables were negatively correlated with soil moisture ($P > 0.05$; Table 3.7). This decrease was also seen in the 2020 average wheat protein of 13.2%, compared to 14.2% in 2019 in western Canada (Canadian Grain Commission 2020).

Environmental factors, such as temperature and moisture, account for 46-90% of the variability in microbial biomass in the Breton plots (adjacent to the Breton Research Station), so different results are expected during different temperature and precipitation trends (McGill et al. 1986). For example, Meng et al. (2017) found that average yields decreased by 0.6% for canola and spring wheat when there was a 20% increase in the number of days with high temperatures. Abiotic water and salt stress can also impact canola oil quantity and quality (El Sayed et al. 2021; Hammac et al. 2017). Despite the high temperatures, canola had 3.2% more protein in 2020 than 2019 across western Canada (Canadian Grain Commission 2021), suggesting further research on potential precipitation impacts in future crops.

In contrast to 2020, the 2021 canola year had 5.9% higher than average temperatures (12.4 °C) and 22.5% lower than average precipitation (318 mm; Fig 3.1). This precipitation resulted in negatively correlated biomass (-0.237) and grain (-0.112) to temperature and positive correlations for grain (0.178), protein (0.229), and harvest index (0.066) to moisture ($P > 0.05$; Table 3.7). The northwest regional yield for canola was not present in the Alberta Crop 2021 report, however, there was a low to very low soil moisture ranking for this season (Alberta Agriculture 2021). Typically, drought reduces stomatal conductance, reducing photosynthetic rate and plant growth (Mansoor et al. 2020). The addition of biochar has been shown to increase photosynthetic pigment concentrations, chlorophyll content, and relative water content, which could aid in regulating increased air and soil temperatures (Mansoor et al. 2020; Zhao et al. 2020), and manure addition can aid in water holding capacity, and water use efficiency (El Sayed et al. 2021). The improvement in water usage is present in the biomass data, where the control treatment had the lowest (1975 kg ha^{-1}) biomass compared to amended plots. A lack of differences ($P > 0.05$) among treatments in biomass, grain biomass, and nutrients (Fig. 3.4); however, suggests that biochar and manure effects were less important in this study during a drier-hotter year than during a wetter-colder season.

In conclusion, wheat grain, protein, and harvest index were all positively correlated to soil temperature and negatively with moisture ($P > 0.05$; Table 3.7). The opposite was observed during the following year, where RM and BM+BC5 had the highest canola biomass and grain ($P > 0.05$; Table 3.4a-b). Canola biomass and grain were negatively correlated to soil temperature and moisture ($P > 0.05$; Table 3.7), suggesting that fewer recalcitrance sources of OM (i.e., lower biochar amounts) may improve yield slightly during warmer years.

3.5.2 Soil fertility

Exchangeable N increased by nearly 10x from Fall 2020 to Fall 2021, but total N remained similar between the years (Tables 3.5 & 3.6). This change in exchangeable N came from the retained straw from the wheat trial. In a study by Malhi et al. (2006), canola planted after wheat straw retained plots had a 29% greater N uptake than when planted in wheat straw removed plots. In addition to this greater N uptake, canola had 33 and 19% higher seed and straw yield, respectively, when wheat straw was retained (Malhi et al. 2006), supporting the expectation of a higher canola grain compared to wheat grain yield. Ahmed and Schoenau (2015) noted that few nutrients are left for wheat after a canola plot, so crop order is crucial in this application.

Phosphorus and sulfur deficiencies in western Canadian soils are rare, and it is unlikely these differences influenced yields (Carew et al. 2009; Dyck et al. 2020). No significant ($P > 0.05$) correlations were found between P and S and wheat or canola yields (Table 3.7). It is important to recognize that optimal N application rates depend on OM application rates, yield potential, and rotations, and P and K concentrations are based on soil type, and S concentrations depend on field history (Jones and Olson-Rutz 2016), so yields will vary for each situation.

The influence of biochar on nutrient availability has had mixed results. Karer et al. (2013) found an increased P and K in the first year, while Ahmed and Schoenau (2015) found a slight decrease in N and P. Like Karer et al. (2013), no significant differences in P and K concentrations among treatments were found during wheat or canola harvests. Canola is a better nutrient scavenger than wheat, so deficiencies in P and K are rare (Jones and Olson-Rutz 2016). Future studies should study the interactive impact of nutrient management options and contrasting, variable weather, on N (as further discussed in 3.5.3) as a significant correlation ($P > 0.05$) between N and canola protein (Table 3.7) was uncovered.

Biochar increases pH when the initial pH of the soil is lower than the amended biochar (Karer et al. 2013). In this study, biochar increased soil pH from 6.25 to 6.78. However, as the optimal pH for wheat is 6-7 (Galinato et al. 2011), it is understandable there were no differences amongst biochar treatments. The optimal pH of canola is wider than wheat, around 5.5-8.5 (Jones and Olson-Rutz 2016). This broader range helps explain its lack of biomass and grain differences among treatments. However, the small (1.2%) increase in pH compared to prior harvest (Table 3.5 & 3.6), lack of statistical significance strength in correlations (Table 3.7) between pH and both canola and wheat yields, and the minimal pH differences among treatments were unlikely to play an important role in the short term (Tables 3.6 & 3.7).

3.5.3 Microbial biomass and functions

In this study, there were differences ($P < 0.05$) in microbial biomass among treatments and in microbial functioning between CT compared to RM+BC10, BC5, BM+BC5, and BC10 (MRPP data not shown). Two years after the amendment application during the canola growing season (2021), differences in microbial biomass among treatments were no longer present ($P > 0.05$), yet significant differences in microbial functioning between CT compared to RM+BC10, BC5, BM+BC10 ($P < 0.05$; MRPP data not shown). When compared as a cumulative respiration, the RM+BC10, BC5, BM+BC10 all showed higher activity towards amino acids (Fig. 3.6c). Therefore, the hypothesis of a shift toward amino acid utilization with biochar additions was supported in both study years (Fig. 3.5b & c) and as a whole (Fig. 3.6c).

Similar results were reported by Abujabhah et al. (2016) and Sun et al. (2019) who noted that biochar generally increased amino acid utilization and decreased carboxylic acid use. Additionally, Anders et al. (2013) found no changes in microbial biomass with biochar additions in temperate soils, but did observe a change in microbial community structure due to changes in nutrient availability. Especially given the negative correlation between microbial biomass and wheat protein ($P < 0.01$, Table 3.5), the increase in microbial biomass limited protein production, thus calling for further investigation of microbial communities. Furthermore, the hypothesis that microbial biomass would be higher in BM compared to RM was supported across the entire growing seasons (Table 3.3-36), and related to changes in habitat (pH, bulk density, OM content) and C quality, which is explored in the other Chapters.

Increasing total amino acids in soil is positively related to N use efficiency (NUE) (Sun et al. 2019) in N limited cold environments (Marschner 1995). Mechanisms include partitioning of different forms of N between roots and shoots (Marschner 1995) and can result in higher grain protein (Kibite and Evans 1984), describing the higher wheat protein content found with BC treatments (11.6 and 10.8 % for BC10 and BC5, respectively) compared to BM (9.9%) or RM (10.3%). There were no differences among treatments for canola protein content, but under drought conditions, there have been studies indicating a decreased agronomic NUE in canola (Ma and Herath 2016). Similar to Hammac et al. (2017), further studies should investigate the possibility of water and temperature variabilities overriding and altering soil nutrients and microbial activity in crop production.

Positive correlations between biochar applications up to 20 Mg ha^{-1} and NUE have been noted (Sun et al. 2019). This matches the findings of higher wheat grain yields and protein content in BC10 than BC5, with RM and BM (Fig. 3.3). Additionally, Jones et al. (2012) found that biochar rates of 50 Mg ha^{-1} had excessive chlorosis, low foliar N, and reduced root growth, so future work investigating peak amounts of biochar application for optimal growth in various crops is needed.

Nitrogen availability and NUE are critical limiting factors in crop production (Carew et al. 2009; Hammac et al. 2017; Karer et al. 2013), with excess N causing declines in wheat yield and variable results in canola yield (Jones and Olson-Rutz 2016). Protein content increases in with N fertilizer rates, up to 120 kg N ha^{-1} (Gauer et al. 1992) and canola protein content peaks around 40 kg N ha^{-1} (Malhi et al. 2006). There was a negative ($P < 0.01$) relationship found between canola protein and inorganic N, suggesting that there may have been excess N in some treatments. Shahzad et al. (2018) observed a higher wheat protein content with BC and inorganic N fertilizer due to the immediate availability of N from the inorganic N source.

However, N input only explains protein increases when N is adsorbed by the plant in excess (Terman et al. 1969). As such, total N in soil does not represent actual availability (Ahmed and Schoenau 2015), and thus did not play an important role in protein content in this study (given the similar TN among treatments) but rather, as previously discussed, NUE from BC amendments. Findings of improved N recovery from Güereña et al. (2013) suggest the retention of organic N in biochar and minerals. This retention of N combined with minimal impact on inorganic N immobilization and long-term C stability with biochar shows potential for continued profitability (Dempster et al. 2012; Hood-Nowotny et al. 2018).

3.6 Conclusion

In conclusion, the use of biochar-manure could be a viable option for crop growth, but may only be beneficial if used in combination with BC. The results suggest warmer and drier years could interact and lead to better performance when using BM+BC5, while under wetter-colder conditions, BM+BC10 can be advantageous. However, irrespective of the biochar rates, implementing biochar-manure application is more important in wet and cold years.

The influence of unpredictable weather patterns due to escalating climate change will increasingly drive the effect of agricultural amendments on crop performance. Given the potential long-term C stability benefits of biochar application, field studies should be conducted to examine its effects over decades or even centuries. Future research should further investigate the impact of BM application on NUE and soil physical properties across a broad range of soils, crop species, and biochar types. Additional research should look at canola oil quantity and quality given its role as a key oil and protein meal globally (Hammac et al. 2017).

Table 3.1. Background information and physicochemical properties of the biochar used in the biochar and biochar-manure treatment (Mohammed and Tak 2018; Romero et al. 2021a).

Background Parameter	
Pyrolysis technology	Engineered Biocarbon™ (<650 ∘ C)
Feedstock	Southern yellow pine (*Pinus echinata*)
Surface area (m^2 g^{-1})	200–250
Bulk density (kg m^{-3})	160–256
Porosity (vol %)	60–70
pH	7.18
EC (dS m^{-1})	0.43
C (% dry mass)	73.3
H (% dry mass)	3.6
N (% dry mass)	0.2
O (% dry mass)	21.2
H/C (ratio)	0.29
Total P (g kg)	0.22
NO_3–N (mg kg^{-1})	1.38
NH_3–N (mg kg^{-1})	4.03
PO_4–P (mg kg^{-1})	42.14

Table 3.2. Comparison of parameters in manure and biochar treatments used as soil amendments (means ±SE; $n = 4$).

Properties	pH	TC (g kg^{-1})	TN (g kg^{-1})	C/N	OM (%)	OM Stability (ratio)
Soil	6.33±0.02^{d}	9.27±0.61^{c}	0.71±0.05^{b}	13.0±0.34^{b}	5.10±0.49^{d}	0.43±0.06^{b}
Manure (RM)	6.91±0.02^{c}	293±5.87^{b}	18.6±1.27^{a}	15.9±0.73^{a}	78.6±0.45^{a}	0.18±0.01^{c}
Biochar-Manure (BM)	7.05±0.01^{b}	326±10.6^{b}	20.2±0.64^{a}	16.2±0.96^{a}	65.2±0.31^{b}	0.21±0.05^{c}
Biochar (BC)	7.18±0.02^{a}	742±9.58^{a}	UDL‡	UDL‡	36.7±0.34^{c}	1.26±0.01^{a}
***P*-Value**	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

Note: Amendments: manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar. Abbreviations: BC, black C; OM, organic matter; TC, total carbon; TN, total nitrogen. ‡Under detection limit. Means followed by a common letter within a column are not significantly different ($P < 0.05$). Significance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3.3. Biological and physical properties of treatments from September 13, 2019 collection (means ±SE; n = 4).

Treatment	N	P	K	S	MBC	MBN	EC	pH†	TC	TN
	(µg 10 cm² d⁻¹)						(µS cm⁻¹)		(g kg⁻¹)	
CT	44.1±8.36	0.43±0.09^{abc}	22.4±1.73	19.9±0.94^{ab}	230±7^{c}	37±1^{d}	377±116^{ab}	6.25±0.02	17.4±0.9^{c}	1.70±0.1
RM	56.4±7.49	0.77±0.15^{ab}	27.5±5.02	24.7±2.01^{a}	295±22^{bc}	52±4^{bcd}	453±60^{a}	6.21±0.09	18.4±1.0^{c}	1.78±0.1
BM	57.1±11.3	0.75±0.15^{ab}	28.1±2.58	25.1±1.82^{a}	364±38^{c}	68±8^{ab}	495±65^{a}	6.22±0.05	19.4±1.5^{ab}	1.85±0.1
RM+BC10	41.7±0.85	0.72±0.17^{ab}	42.4±14.2	23.3±2.25^{ab}	502±102^{a}	95±27^{a}	373±15^{ab}	6.27±0.06	37.3±4.0^{ab}	1.90±0.2
BC5	31.4±5.29	0.18±0.02^{c}	22.3±4.66	15.6±1.31^{b}	234±17^{c}	38±3^{d}	152±22^{b}	6.24±0.01	25.8±0.3^{bc}	1.70±0.2
BM+BC5	47.9±0.98	0.92±0.09^{ab}	23.4±3.42	24.0±1.99^{a}	334±22^{abc}	59±4^{abc}	340±48^{ab}	6.29±0.06	24.5±2.2^{bc}	1.84±0.1
BC10	36.9±4.04	0.26±0.03^{bc}	21.6±2.44	17.9±1.49^{ab}	235±19^{c}	40±3^{cd}	295±74^{ab}	6.17±0.06	37.1±3.8^{ab}	1.80±0.1
BM+BC10	63.7±6.83	1.47±0.72^{a}	39.0±7.57	24.5±1.96^{a}	347±31^{ab}	59±7^{abcd}	739±234^{a}	6.36±0.03	53.0±14.2^{a}	2.02±0.2
***P*-Value**	0.579	<0.001***	0.207	0.004*	<0.001***	<0.001***	0.004**	0.358	<.0001***	0.199

Note: Abbreviations: EC, electroconductivity; K, potassium; N, nitrogen; P, Phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; TC, total carbon; TN, total nitrogen; OM, organic matter.
Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Means followed by a common letter within a column are not significantly different (P < 0.05). Significance: *, P < 0.05; **, P < 0.01; ***, P < 0.001. †Soil pH was measured in an 1:5 soil:water (v:v) ratio.

Table 3.4. Biological and physical properties of treatments from June 3, 2020 collection (means ±SE; n = 4).

Treatment	N	P	K	S	MBC	MBN	EC	pH†	TC	TN
	(µg 10 cm^2 d^{-1})						(µS cm^{-1})		(g kg^{-1})	
CT	5.50±0.56	0.43±0.10	13.1± 3.29	1.12±0.21	698±74^{ab}	121±18^{ab}	424±54	6.28±0.01	19.0±0.8^{d}	1.84±0.1
RM	7.62±1.73	0.42±0.09	13.1±1.81	2.12±0.38	996±96^{ab}	180±18^{abc}	402±48	6.33±0.02	19.3±0.6^{cd}	1.89±0.1
BM	6.36±1.17	0.41±0.05	14.5±1.81	2.43± 0.48	1197±111^{a}	236±24^{a}	255±43	6.35±0.05	23.1±1.8^{bcd}	2.04±0.2
RM+BC10	9.73±2.43	0.56±0.09	16.8± 2.17	2.07±0.54	1066±113^{ab}	229±2^{ab}	390±119	6.30±0.04	35.7±1.4^{a}	2.02±0.1
BC5	7.78±1.49	0.35±0.11	26.4± 4.77	1.92±0.37	820±161^{ab}	132±27^{abc}	316±55	6.37±0.02	28.4±3.8^{abcd}	1.77±0.2
BM+BC5	11.1±3.17	0.62±0.15	17.6±2.29	2.67±0.48	1201±187^{a}	228±36^{a}	465±76	6.34±0.03	26.7±2.6^{abcd}	1.91±0.1
BC10	6.76±1.75	0.33±0.02	20.8±1.41	2.58± 0.19	555±24^{b}	95±10^{d}	233±40	6.37±0.01	32.2±2.2^{ab}	1.82±0.1
BM+BC10	9.91±1.53	0.62±0.10	23.7± 5.61	2.11±0.41	1017±113^{ab}	193±25^{abc}	374±41	6.34±0.01	29.3±1.2^{abc}	1.91±0.1
***P* - Value**	0.408	0.255	0.036*‡	0.252	0.006**	<.0001***	0.218	0.200	<.0001***	0.625

Note: Abbreviations: EC, electroconductivity; K, potassium; N, nitrogen; P, Phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; TC, total carbon; TN, total nitrogen; OM, organic matter.

Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

†Soil pH was measured in an 1:5 soil:water (v:v) ratio. Means followed by a common letter within a column are not significantly different (P < 0.05).

‡Tukey's Honest Significant Difference (HSD) test did not show significant differences between treatments even though $P < 0.05$.

Significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 3.5. Biological and physical properties of treatments from October 4, 2020 collection (means ±SE; n = 4).

Treatment	N	P	K	S	MBC	MBN	EC	pH†	TC	TN
	(μg 10 cm² d⁻¹)				(g kg⁻¹)		(μS cm⁻¹)		(g kg⁻¹)	
CT	3.81±0.81	0.45±0.14	10.9±1.54	2.40±0.49	545±50	92±10^{b}	91±26^{b}	6.85±0.03	17.1±1.2	1.63±0.1
RM	4.42±0.28	0.58±0.20	15.7±3.30	2.64±0.32	818±63	129±10^{ab}	75±20^{b}	6.87±0.01	18.9±0.5	1.81±0.1
BM	4.83±0.53	0.52±0.11	14.3±1.60	2.40±0.33	936±42	148±13^{ab}	67±4^{b}	6.80±0.03	19.6±1.0	1.85±0.1
RM+BC10	7.17±2.77	0.73±0.19	17.7±2.89	2.84±0.24	870±107	127±21^{ab}	100±14^{ab}	6.77±0.03	28.0±1.4	1.83±0.2
BC5	3.84±0.40	0.33±0.07	13.8±4.58	2.63±0.30	901±148	106±18^{ab}	129±39^{ab}	6.75±0.07	31.1±4.5	2.02±0.2
BM+BC5	5.48±1.11	0.45±0.14	13.3±1.49	2.88±0.35	1067±67	160±16^{a}	73±14^{b}	6.79±0.04	23.8±2.4	1.87±0.2
BC10	3.53±0.41	0.30±0.04	13.0±1.29	2.51±0.26	807±99	115±16^{ab}	250±25^{a}	6.76±0.10	30.3±3.7	1.80±0.2
BM+BC10	4.13±0.26	0.31±0.07	13.9±4.14	2.59±0.08	917±166	126±20^{ab}	177±52^{ab}	6.65±0.07	28.7±3.1	2.01±0.1
***P* – Value**	0.255	0.365	0.848	0.952	0.076	0.033*	0.013*	0.157	0.183	0.410

Note: Abbreviations: EC, electroconductivity; K, potassium; N, nitrogen; P, Phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; TC, total carbon; TN, total nitrogen; OM, organic matter.

Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

†Soil pH was measured in an 1:5 soil:water (v:v) ratio. Means followed by a common letter within a column are not significantly different (P < 0.05). Significance: *, $P < 0.05$.

Table 3.6. Biological and physical properties of treatments from September 10, 2021 collection (means ±SE; *n* = 4).

Treatment	N	P	K	S	MBC	MBN	EC	pH[†]	TC	TN
	(μg 10 cm^2 d^{-1})						(μS cm^{-1})		(g kg^{-1})	
CT	22.7±3.25^{a}	0.41±0.05	23.0±3.51	7.17±1.09	2911±197	252±31	390±35	6.90±0.03^{ab}	16.8±0.7^{b}	1.62±0.1
RM	18.9±2.80^{a}	0.98±0.37	19.2±1.06	7.76±0.82	3317±155	369±11	294±52	6.98± 0.04^{a}	18.1±0.3^{b}	1.77±0.1
BM	17.5±4.39^{a}	0.53±0.05	19.2±2.65	8.88±1.01	3373±133	335±32	526±85	6.99±0.01^{a}	18.9±1.5^{ab}	1.78±0.1
RM+BC10	13.8±2.92^{a}	0.43± 0.03	22.6±1.29	7.81±0.86	3622±272	335±21	354±106	6.78±0.03^{bc}	30.2±4.7^{a}	1.89±0.1
BC5	37.8±11.7^{a}	0.53±0.17	18.3±0.82	9.65±2.94	3151±220	289±40	290±82	6.77± 0.06^{bc}	23.9±1.8^{ab}	1.72±0.1
BM+BC5	25.3±9.20^{a}	0.85±0.22	21.5±1.30	9.00±1.49	3588±181	367±27	279±73	6.88±0.03^{abc}	24.2±2.3^{ab}	1.78±0.1
BC10	31.9±4.91^{a}	0.44±0.06	23.6±2.50	8.01±0.13	3405±286	282±41	202±35	6.74± 0.02^{c}	24.2±1.8^{ab}	1.89±0.1
BM+BC10	20.8±1.81^{a}	0.84±0.21	22.2±5.29	9.34±1.83	3449±87	338±19	336±64	6.86±0.02^{abc}	24.6±2.0^{ab}	1.85±0.1
P-Value	0.221	0.129	0.685	0.933	0.306	0.119	0.602	<0.001***	0.002**	0.428

Note: Abbreviations: EC, electroconductivity; K, potassium; N, nitrogen; P, Phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; TC, total carbon; TN, total nitrogen; OM, organic matter.
Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.
[†]Soil pH was measured in an 1:5 soil:water (v:v) ratio. Means followed by a common letter within a column are not significantly different (P < 0.05). Significance: **, $P < 0.01$; ***, $P < 0.001$.

Table 3.7. Spearman's rank correlation coefficients of relationships between wheat and canola biomass, grain, protein, and harvest index (HI) and soil properties (means ±SE; n = 4).

	Variable	N	P	K	S	MBC	MBN	EC	pH†	TC	TN	Temp	Moisture
Wheat 2020	Biomass	-0.081	0.259	0.056	0.076	-0.042	-0.116	-0.259	0.041	-0.136	0.003	-0.099	-0.303
	Grain	-0.018	0.096	0.044	0.071	-0.129	-0.114	0.112	-0.020	0.129	-0.018	0.293	-0.151
	Protein	-0.232	-0.141	-0.225	-0.189	-0.531**	-0.507**	0.381*	0.126	-0.121	-0.248	0.481**	-0.161
	HI	-0.121	0.040	-0.077	-0.108	-0.348	-0.206	0.086	0.029	0.014	0.0183	0.287	-0.239
Canola 2021	Biomass	0.032	0.070	0.19	0.198	0.091	0.367*	-0.054	0.044	-0.013	0.0314	-0.237	-0.042
	Grain	0.185	-0.249	0.26	-0.017	0.065	0.251	-0.095	-0.153	-0.066	-0.080	-0.112	0.178
	Protein	-0.442*	-0.028	0.232	-0.163	0.261	0.064	0.019	0.037	-0.019	0.224	0.134	0.229
	HI	0.285	-0.241	0.134	-0.172	0.050	-0.015	-0.063	-0.333	0.068	0.042	0.177	0.066

Note: Abbreviations: EC, electroconductivity; K, potassium; N, (organic) nitrogen; P, Phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; TC, total carbon; TN, total nitrogen; OM, organic matter.
†Soil pH was measured in an 1:5 soil:water (v:v) ratio. Significance: *, $P < 0.05$; **, $P < 0.01$.

Table 3.8. Soil temperature of total days greater than 15°C and soil moisture of total days greater than 30% volumetric water content for each respective growing season (Jun 3 – Oct 4, 2020, and May 23 – Sept 10, 2021) (means ±SE; n = 4).

Growing Season	**Jun 3 – Oct 4 2020 (Wheat)**		**May 23 – Sept 10 2021 (Canola)**	
	Temperature	Moisture	Temperature	Moisture
Treatment	days			
CT	81.0±1.3	36.0±0.8	92.0±1.6	13.5±0.2
RM	75.0±2.5	41.7±3.0	84.0±5.7	10.3±4.5
BM	75.7±2.8	48.7±2.9	90.3±0.6	16.0±8.6
RM+BC10	81.0±1.4	44.0±4.7	91.8±1.2	6.0±1.9
BC5	73.8±5.9	42.3±4.6	86.0±2.9	7.0±4.5
BM+BC5	78.0±0.9	40.3±5.6	91.0±1.1	10.0±5.2
BC10	82.7±0.9	49.0±2.0	92.0±1.6	20.7±5.9
BM+BC10	77.0±2.9	41.8±4.5	89.5±2.9	13.3±6.3
***P*-Value**	0.348	0.301	0.182	0.212

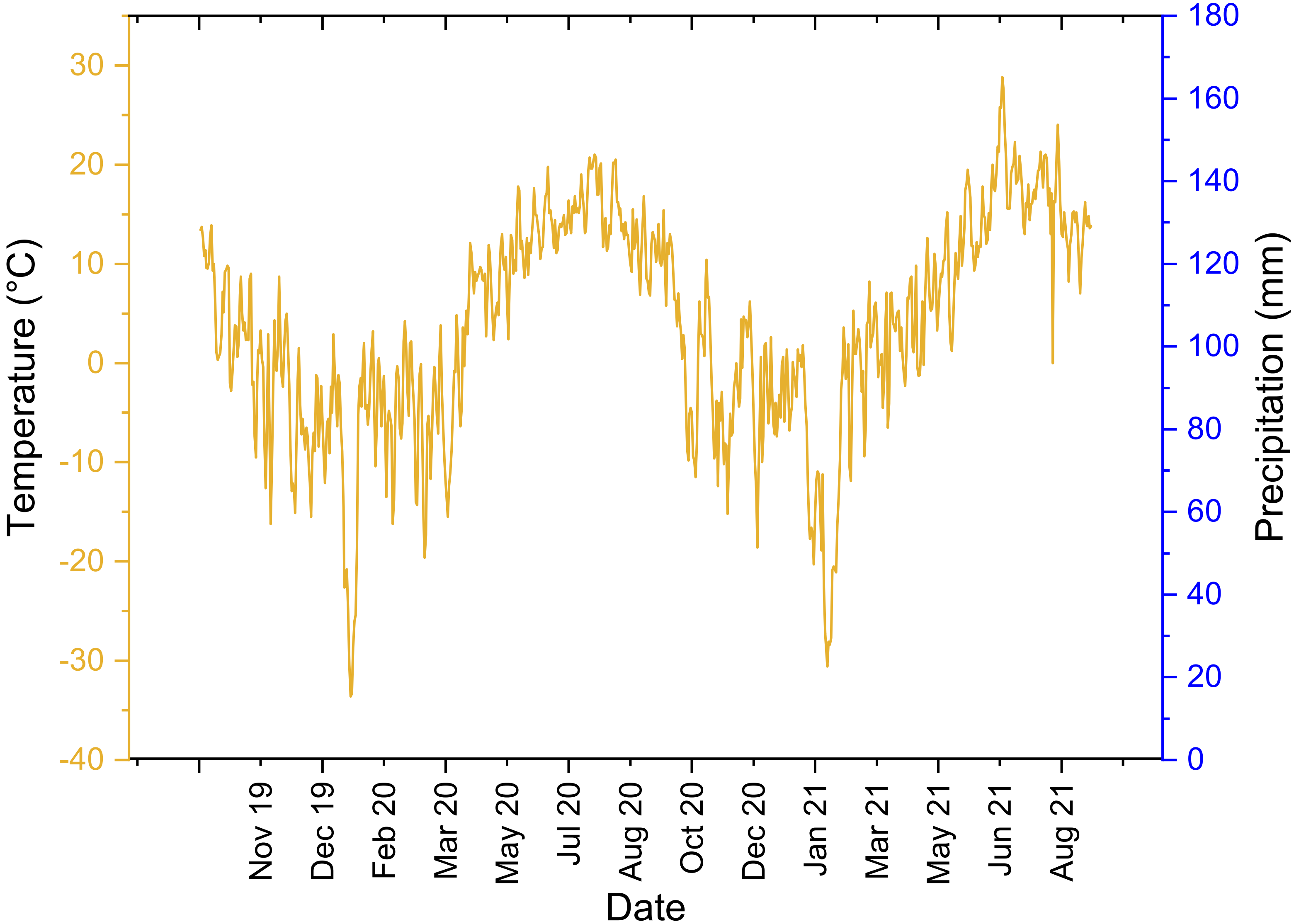

Figure 3.1. The average daily temperature and monthly precipitation during Fall 2019-2021.

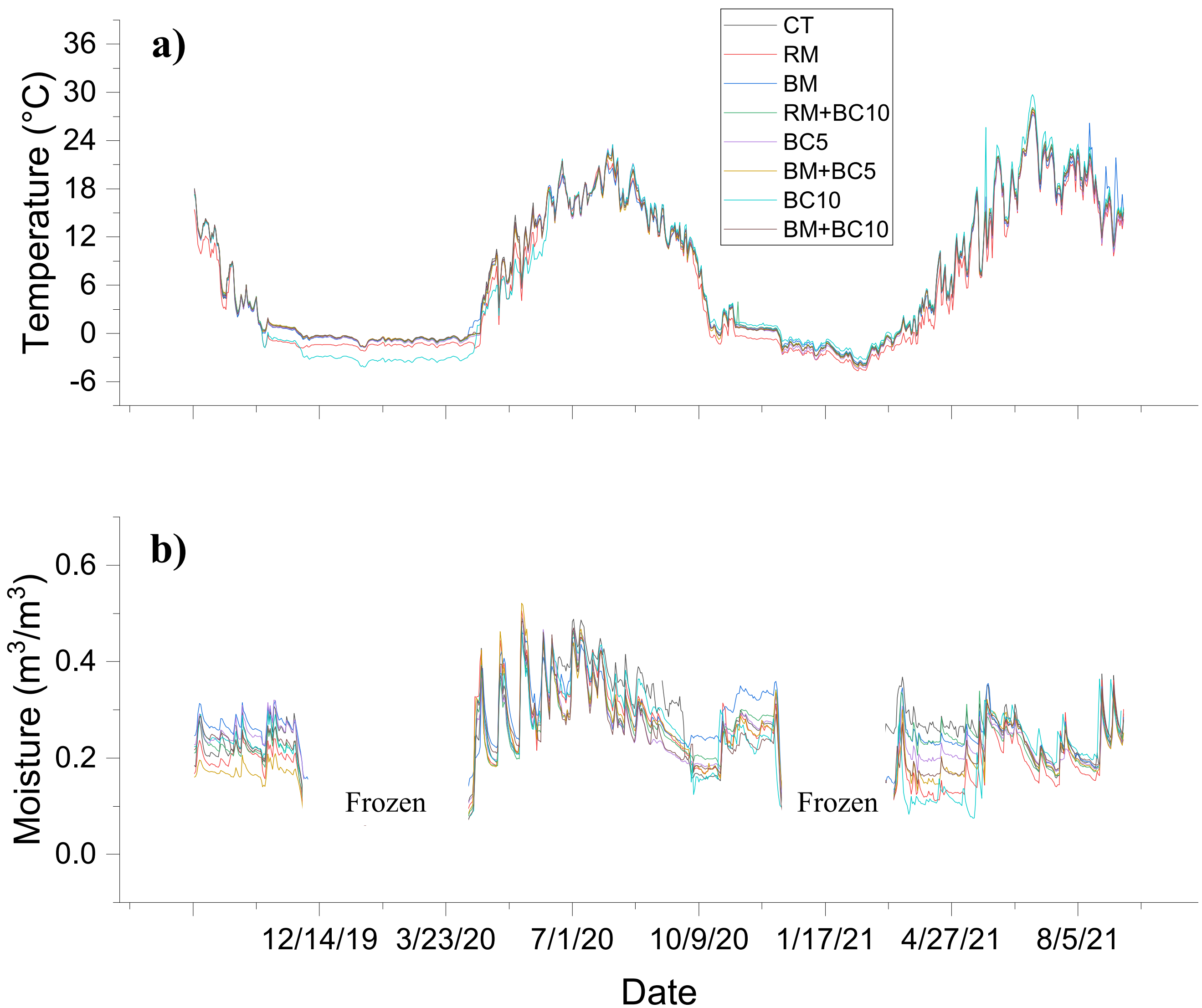

Figure 3.2. Daily soil temperature (a) and mean soil moisture (b) (10 cm) by treatment from Fall 2019 to 2021. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

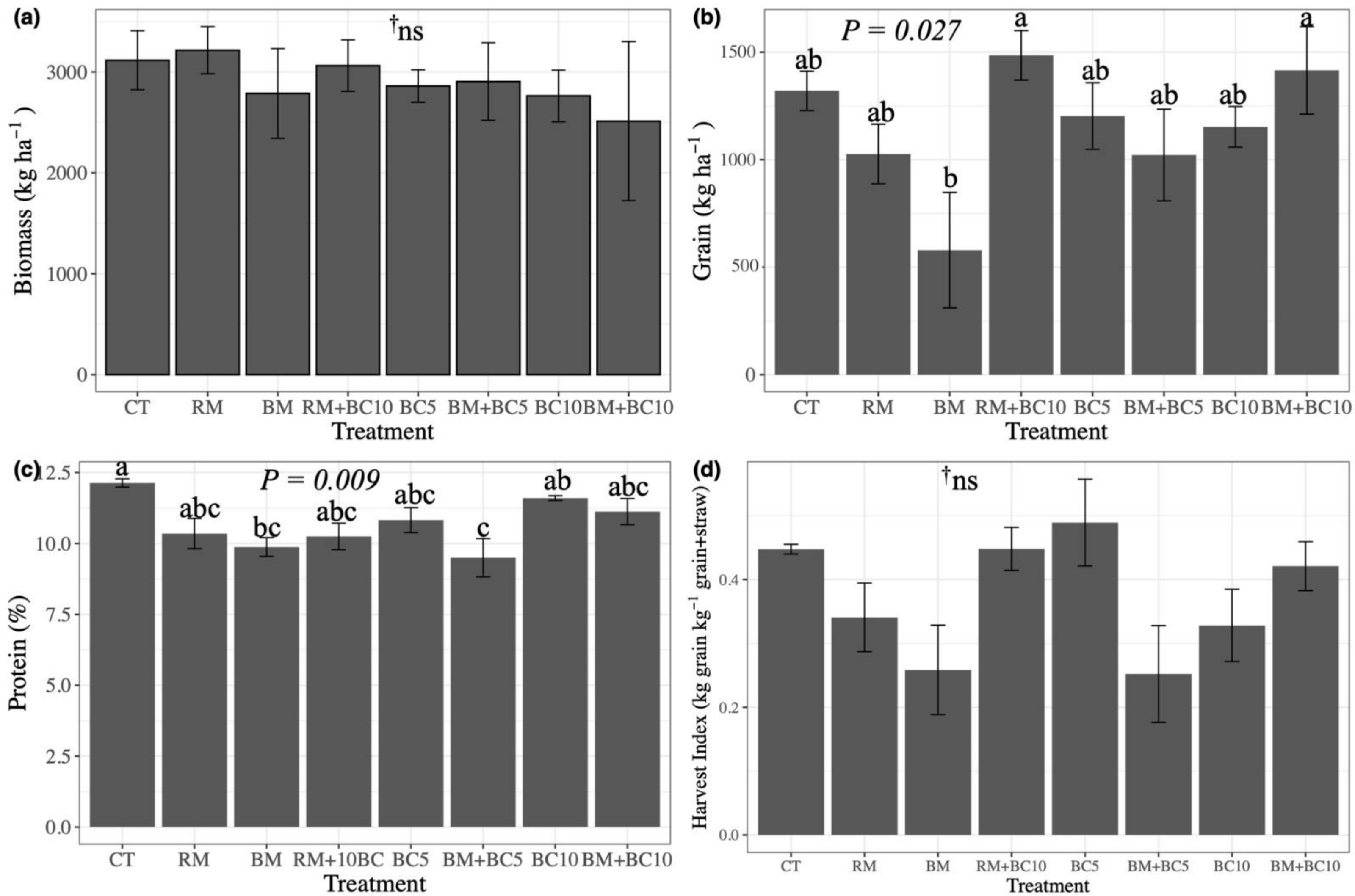

Figure 3.3. Mean (a) wheat biomass yield, (b) grain yield, (c) protein content and (d) harvest index (n=4) of CDC GO wheat as affected by various amendments with standard error in 2020. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Different letters denote significant differences among treatments, $P < 0.05$ and *ns indicate not significant, $P > 0.05$.

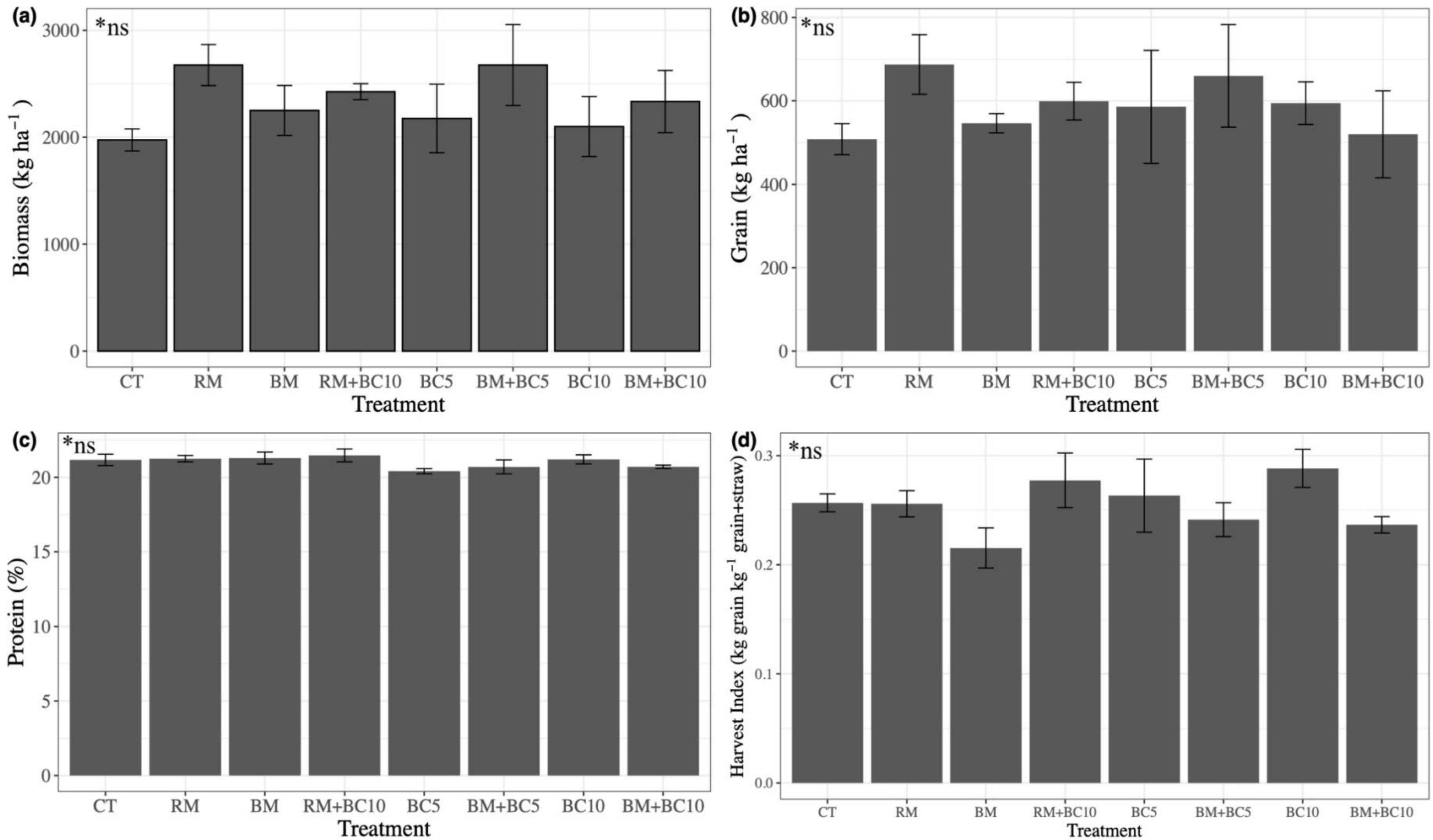

Figure 3.4. Mean (a) canola biomass yield, (b) grain yield, (c) protein content and (d) harvest index (*n*=4) of Roundup Ready Canola as affected by various amendments with standard error in 2021. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. *ns indicate not significant, $P > 0.05$.

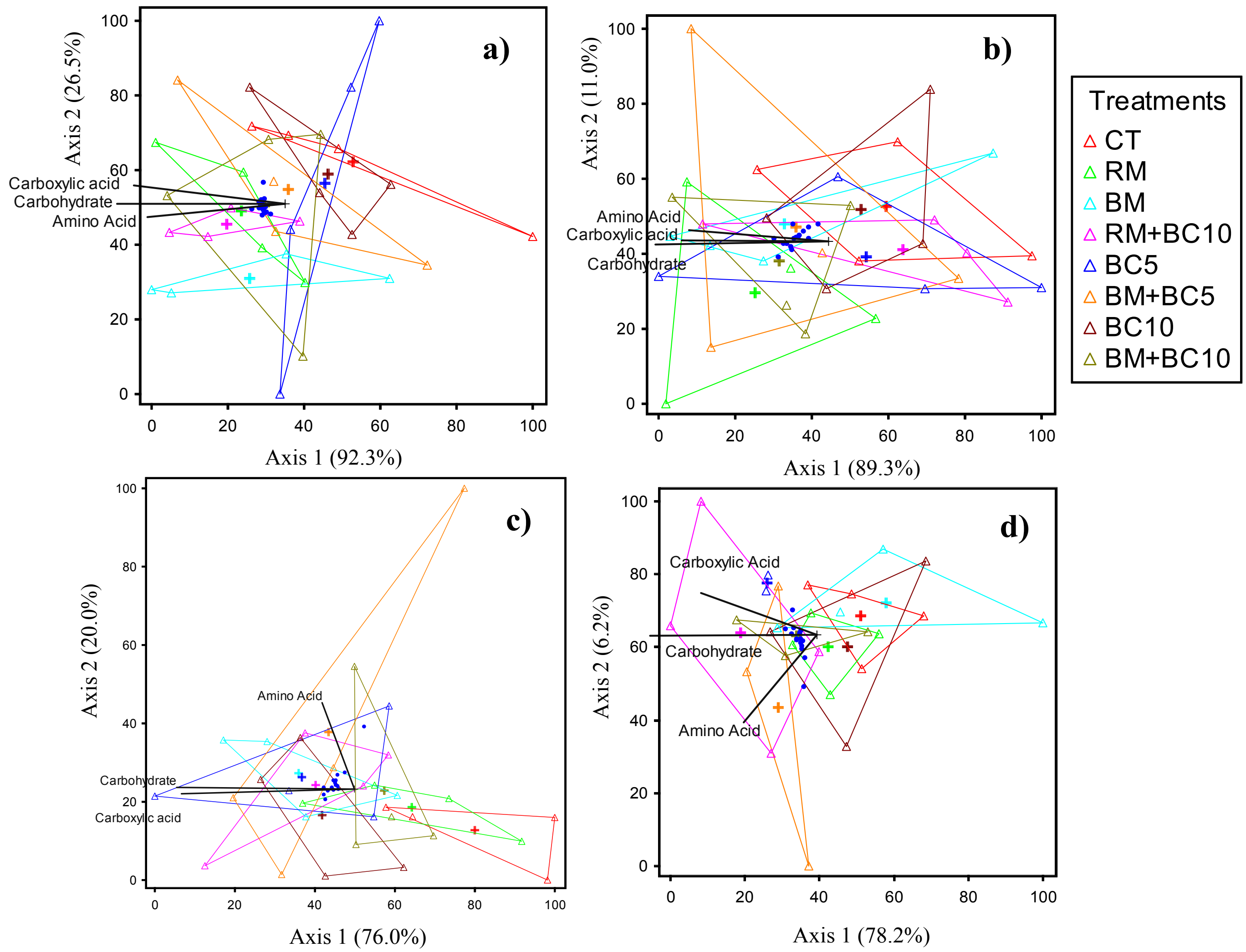

Figure 3.5. Non-metric multidimensional scaling ordination bi-plot of soil microbial function (as measured by CO_2 production) using a Bray-Curtis distance measure on a) Fall 2019, b) Summer 2020, c) Fall 2020, and d) Fall 2021. Vector associations are weighted by length and include responses to carbohydrates, carboxylic acids, and amino acids. Treatments: CT, control, RM, manure from cattle fed traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

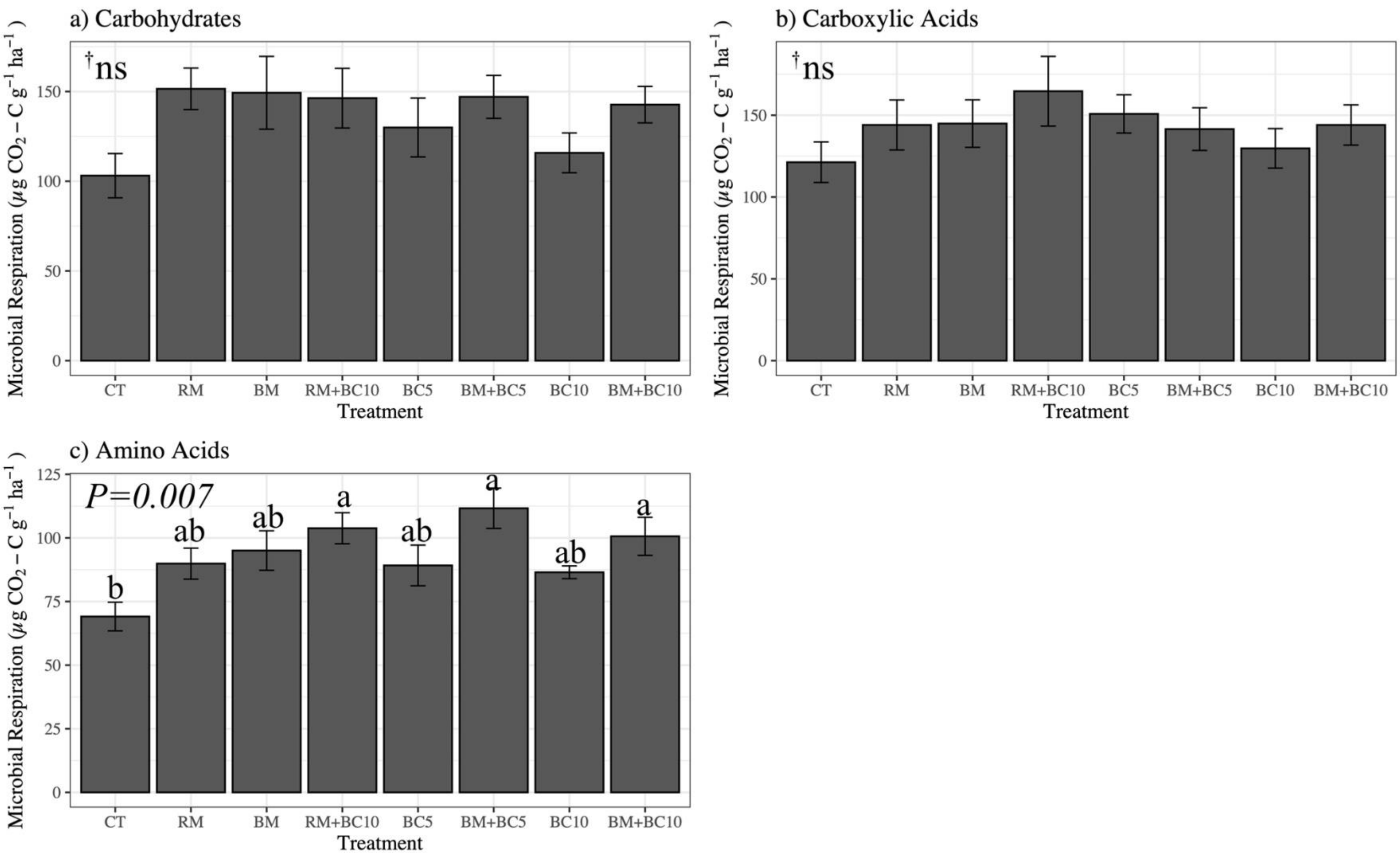

Figure 3.6. Mean cumulative soil microbial function (as measured by CO_2 production) responses to a) carbohydrates, b) carboxylic acids, and c) amino acids. Treatments: CT, control, RM, manure from cattle fed traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Letters denote significant differences between treatments, P <0.05. †ns indicate not significant, P > 0.05.

Chapter 4: Biochar and manure from cattle fed biochar as an agricultural soil fertility amendment reduce greenhouse gas emissions in a Gray Luvisol field trial

4.1 Abstract

Greenhouse gas (GHG) emissions from agricultural practices contribute 14% of anthropogenic emissions, and novel practices are being considered, including feeding cattle a modified diet, to reduce these emissions. This Chapter looks at the effect of adding manure from cattle fed a regular diet (RM) and a diet supplemented with 2% biochar (BM) or amended in the field with 5 (BC5) or 10 Mg ha^{-1} (BC10) of biochar on GHG emissions in a Gray Luvisol agricultural field experiment. Emissions of CO_2, CH_4 and N_2O were monitored over the growing season and soil samples were collected at the end to analyze exchangeable NPKS, microbial biomass, total C and N, electrical conductivity (EC), and pH. Wheat (*Triticum aestivum*) was planted to calculate the yield emission intensity/yield-based emission factor (EF_{yield}) and the cumulative GHG emissions/area-based emission factor (EF_{area}). A key finding was the inhibition of CH_4 oxidation from BM+BC5 and BM+BC10. The biochar in BM may have acted as a biocide to methanotrophs, causing a reduction in the release of CH4 over time. Yet, there were no significant differences in N2O emissions, amongst treatments. Therefore, BM+BC5 and BM+BC10 applications may impact total GHG emissions and improve grain productivity and protein content compared to BM alone.

4.2 Introduction

The last three decades have been warmer than any preceding decade, highlighting the global human impact on climate change (IPCC 2014). The heating of the earth's surface is called the greenhouse gas (GHG) effect, where GHGs, primarily carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4), absorb infrared radiation emitted from the surface and reradiate it back to the earth (Manabe 2019). These GHGs trap heat at different rates, with CH_4 having 28x and N_2O having 265x the potential of CO_2 (IPCC 2014). In the 1970s, atmospheric scientists realized N_2O's detrimental impact on the ozone layer (Bremner 1997). Increasing livestock and thawing permafrost have increased CH_4 emissions 150% since the 1750s (Kammann et al. 2017); however, sustainable strategies such as organic fertilizer amendments and crop rotations could reduce emissions up to 4500–6000 Mt CO_2-eq yr^{-1} in agricultural operations by 2030 (Vaccari et al. 2011).

Biochar, the result of thermal alteration (pyrolysis) of organic material (OM) with little or no oxygen, is an agricultural soil additive that might help to combat climate change and mitigate greenhouse gases (Gomez et al. 2014; Lehmann and Joseph 2015; Pereira et al. 2014). The first documented use of biochar was ~2,500 to 500 years B.P., in the *Terra Preta del Indio* soils of Brazil, with high OM and fertility despite the surrounding highly-weathered Oxisols (Lehmann et al. 2003). Despite the increased microbial activity of the *Terra Preta del Indio* soils, low C respiration rates were found when compared to adjacent native tropical soils due to increases in microbial efficiency (CO_2 release per unit of soil carbon) from biochar (Atkinson et al. 2010; El-Naggar et al. 2015).

Biochar recalcitrance can offset CO_2 emissions and sequester C in the soil (Abagandura et al. 2019; Spokas and Reicosky 2009). Through its recalcitrance, the addition of biochar can result in negative priming, the decrease in mineralization of native organic C following the addition of organic matter (Dodor et al. 2018). In contrast, manure C mineralization is much more complete and rapid than biochar C, as seen in experiments by Weber et al. (2021a) and Troy et al. (2013). Increased CO_2 emissions can also occur with mineralization of labile C, changes in microbial populations, or SOC priming (Troy et al. 2013). Biochar's ability to reduce N_2O emissions through decreased denitrification is related to environmental conditions and soil properties (Dempster et al. 2012; Kammann et al. 2012; Romero et al. 2021b). However, it can also increase or not affect N_2O emissions depending on pH, temperature, NO_3^- concentration, oxygen concentration, organic C availability, and water content (Ameloot et al. 2013; Bergaust et al. 2010; Bremner 1997; Troy et al. 2013).

The effect of biochar on CH_4 has been poorly investigated compared to CO_2 and N_2O and soil can become a sink or source depending on the ratio of methanogens to methanotrophs (Feng et al. 2012; Jeffery et al. 2016). Biochar applications to acidic soils can increase porosity, raise pH, and decrease Al^{3+} solubility, reducing the populations of methanotrophic bacteria (Jeffery et al. 2016; Yu et al. 2013). In summary, abiotic changes to the soil, changes to microbial communities, and direct absorption of various chemicals and gases in biochar pores (Table 1.1) can lead to potential GHG mitigation (Abujabhah et al. 2016; Bruun et al. 2011; Calvet 1989; Jeffery et al. 2016).

Unfortunately, amending biochar directly to soil poses potential air and water pollution risks; due to its light, fine, particulate nature, it is very costly as a sole amendment and lacks immediate nutrients needed for crop production (Bruun et al. 2011; Pereira et al. 2014; Shahzad et al. 2018). To alleviate these concerns, biochar has been supplementary to the livestock industry through the addition to bedding, manure, and diet, which have become promising means of distribution (Joseph et al. 2015b; Schmidt et al. 2019; Weber et al. 2021b). In a study investing cattle-fed biochar, Romero et al. (2021a) discovered the presence of unchanged biochar in manure after being passed through the rumen, showing potential for biochar-manure applications. (Reeve et al. 2012). Little research; however, looks at the effect of manure from cattle fed with biochar on GHG emissions when such manure is applied for crops in temperate climates (Lentz et al. 2014). By studying the impact of biochar and biochar loaded manure on field GHG emissions and soil properties in an annual cropland, we can develop a clearer understanding of its role in sustainable agricultural practices.

The objectives of this experiment were to investigate how i) biochar (BC), biochar-manure (BM), or regular manure (RM) soil amendment at different application rates alter greenhouse gas emissions and ii) how these are influenced by environmental factors. I hypothesized that that GHG emissions would be greatest in soil amended with RM and RM + BC, then BM and BM + BC (due to the retained biochar in BM) and lowest in BC. I further theorized greater biochar application rates will lower denitrification and methanogenesis rates, reducing CO_2, CH_4, and N_2O (Abujabhah et al. 2016; Bruun et al. 2011; Jeffery et al. 2016; Kammann et al. 2012; Singh et al. 2010).

4.3 Methods

4.3.1 Experimental design and treatments

This study investigated the effect of manure treatment on greenhouse gas emissions using a randomized complete block design with four replicates. The site at the Breton Research Station (53°07′N, 114°28′W) was amended on 13 September 2019 on a Gray Luvisol with a loamy sand texture. The study plot’s known history dates to 2009; the plots produced oats, grass, and wheat with no fertilization. From 2010-2011, oats and barley were harvested, followed by a fallow period. From 2013-2015, barley-canola rotations were fertilized with 80 kg N ha^{-1} urea in 2015. Wheat-barley-barley were grown from 2016-2018 with an application of 50 kg N ha^{-1} urea in 2017 and 2018.

These treatments included: (1) stockpiled manure (RM) from cattle on a typical western Canadian feedlot diet at a rate of 5.4 Mg ha^{-1} (target of 100 kg total N ha^{-1}); (2) stockpiled manure from the same feedlot diet, but supplemented with 2% biochar (BM) at a rate of 4.9 Mg ha^{-1} (target of 100 kg total N ha^{-1}); (3) biochar a rate of 10 Mg ha^{-1} (BC10); (4) biochar at a rate of 5 Mg ha^{-1} (BC5); (5) a combination of (1) and (4); (6) a combination of (2) and (3); (7) a combination of (2) and (4); and (8) a control (CT-soil without manure). Atmospheric data was collected from a nearby weather station, and soil temperature and moisture content data were collected using RT1 and EC5 sensors, respectively, with EM50 data loggers (METER, Pullman, WA, USA).

A feedlot study conducted at Lethbridge Research and Development Centre of Agriculture and Agri-Food Canada (AAFC) near Lethbridge, AB, provided the various manures. Eighty yearling steers were used in a 235-day feeding trial (Terry et al. 2020) One of the manures came from a regular western cattle diet consisting of 60% barley silage, 85% barley grain, and 5% mineral supplement (Terry et al. 2020), and the other manure came from the same diet supplemented with 2% biochar (dry-matter basis). Inorganic N of cattle feedlot manure is around 40% of TN (Eghball et al. 2002), so RM and BM had approximately 40 kg available N ha^{-1} with no available N in the BC (under detection limit) and CT (<0.1 kg available N ha^{-1}) applications.

Southern yellow pine (*Pinus echinate*) biochar was used in the AAFC trials (BM manure) and BC plots (BC5, BC10, RM + BC5, BM + BC10 and RM + BC10). National Carbon, Inc. (Greenwood Village, CO) recommended and provided the biochar for the feedlot and field trials from its patented post-pyrolysis treatment step in a front-end biomass pyrolysis (<650 ◦C) (Romero et al. 2021a). Characteristics of the biochar are provided in Table 3.1.

4.3.2 Initial soil and treatment analyses

Soil and treatments were air-dried for 48 hours, ground (<2 mm) with a Ball Mill MM200 (Brinkmann Retsch, Haan, Germany), and stored in 20 mL scintillation vials for analyses. A Thermo Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) measured total C and N values using dry combustion (Sparks et al. 2020). Differential Scanning Calorimetry (DSC; STA 6000, Perkin Elmer, Waltham, MA USA) measured the heat of combustion by integrating the DSC curve over the exothermic region in approximately 20 mg of dried soil (Fernández et al. 2011). The ratio of the heat of combustion of the recalcitrant region (410 °C-725 °C) to labile region (150 °C-410 °C) of organic matter was calculated as OM stability.

4.3.3 Chemical and biological analyses of soil and crops

Samples were oven-dried at 105 °C for 48 hours to determine moisture content, then ground (<2 mm) with a Ball Mill MM200 (Brinkmann Retsch, Haan, Germany). Samples were stored in 20-mL scintillation vials for total C and N dry combustion analysis with a Thermo Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (Sparks et al. 2020). Following a protocol from Fernández et al. (2011), differential scanning calorimetry (DSC; STA 6000, Perkin Elmer, Waltham, MA USA) was used to calculate thermal stability. FE20 and FE30 meters (Mettler Toledo Columbus, OH, USA) measured soil pH and EC in a 1:2 (w:v) soil to water extract ratio after the sample was shaken for 1 hour, vacuum filtered and allowed to settle for 30 min.

To measure exchangeable NPKS and microbial biomass, sub-samples were incubated at 20 °C for 72 hours (Solaiman 2007). Ion-exchange membranes were added and extracted with 15 mL of 0.5 M HCl using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Thermo iCAP6300 Duo, Thermo Fisher Scientific, Waltham, MA, USA) to measure P, K, and S values (Qian et al. 1992) at the end of the incubation. Ammonium concentrations were measured using the Salicylate-Hypochlorite method (Bower and Holm-Hansen 1980) and NO_3^{-}—N and NO_2^{-}—N were measured using the Hydrazine reduction method (Kamphake et al. 1967) on a colorimetric autoanalyzer (Gallery Plus, Thermo Fisher Scientific, Waltham, MA, USA). For microbial biomass C and N, 20 g samples (dry wt eq) were fumigated with 30 ml chloroform to a 50 ml glass beaker for at least 24 hours and were compared to an unfumigated set (Solaiman 2007). The samples were mixed in a 0.5 M K_2SO_4 solution (1:2 (w:v) soil:extract ratio), shaken at 250 rpm for 1 hour, filtered using Whatman No. 42 filter paper (Voroney et al. 2008) and analyzed using a Shimadzu Total Organic Carbon Analyzer (Shimadzu Corporation Kyoto, Japan).

Fields were planted with CDC Go wheat on 3 June 2020 using a field plot seeder and harvested on 27 September 2020, in two 1x1 m plots for a composite sample with a hand-sickle from each treatment plot. Wheat samples were dried in burlap sacks at room temperature (22 °C) and threshed for grain biomass, while protein yield was analyzed using near-infrared spectroscopy (NIR) (Williams et al. 1985). Harvest index (HI) was calculated according to Thilakarathna et al. (2021) as follows in Eq. [1]:

$$Harvest\ Index = \frac{Grain\ yield\ DM}{Aboveground\ DM} \tag{1}$$

Where $Harvest\ Index$ = wheat harvest index (kg grain DM kg^{-1} grain and straw DM), $Grain\ yield\ DM$ = grain yield from soil treatment (kg ha^{-1}), and $Aboveground\ DM$ = grain and straw yield from soil treatment (kg ha^{-1}).

4.3.4 Greenhouse gas collection and calculations

Gas collection utilized a non-flow-through, non-steady-state chamber method (static, closed) due to its simple and versatile small size (Chapuis-Lardy et al. 2007; Charteris et al. 2020; de Klein et al. 2020). Chambers were custom-built at 10,000 cm^3 (64.1 cm length x 15.6 cm width x 10.0 cm height) to be large enough to lower spatial heterogeneity, but small enough to capture >3 μg N m^{-2} h^{-1} within 30-minute closure times (Charteris et al. 2020). A capillary vent was connected to the lid to allow gas flow between the atmosphere and inside the chamber.

Sampling occurred at the same time, between 1000 and 1200 h, daily to capture the mean flux in temperate climates (Charteris et al. 2020). Chamber installation and measurement followed Roman-Perez et al. (2021)'s protocol, with one chamber in each plot for a total of 32 chambers (Charteris et al. 2020). A chisel and rubber mallet were used to push the chamber 5 cm into the soil (Charteris et al. 2020; Kim et al. 2021). Chambers remained in place for the duration of the experiment from September 2019-2020 and were only removed for one day during seeding and harvest (Kim et al. 2021; Lin et al. 2017).

Gas samples were collected weekly and biweekly from spring thaw to winter freeze to capture potential differences between treatments throughout the field season (de Klein et al.

2020). Emissions were assumed to be negligible during the winter months with minimal biological activity (Lin et al. 2017). During periods of high activity, including soil disturbance, rainfall, spring thaw, or amendment addition, chamber sampling frequency increased to twice weekly to improve GHG emissions accuracy (Charteris et al. 2020). When plants began to grow, they were kept in the chamber to understand crop growth on GHG emissions (Hernandez-Ramirez et al. 2009). Atmospheric conditions were recorded by a permanent on-site weather station adjacent to the research plots (Environment and Climate Change Canada 2020).

For each sampling period, three 20 mL gas samples were collected with a 50 mL syringe through a rubber septum on the chamber lid at 16, 32, and 48 min (Charteris et al. 2020). Additionally, three ambient gas samples were collected at the start of the sampling period from approximately 10 cm above the ground to represent time zero (T0) (Charteris et al. 2020; Roman-Perez et al. 2021). All samples collected during each sampling period were transferred to 12-mL pre-evacuated soda glass vials and stored in a refrigerator (5 °C) until analysis (Exetainer, Labco, High Wycombe, UK) (Harvey et al. 2020).

A Varian CP-3800 gas chromatograph (Varian, Palo Alto, CA, USA), equipped with a thermal conductivity detector (TCD), an electron capture detector (ECD), and a flame ionization detector (FID), was used to measure CO_2, N2O and CH_4 concentrations, respectively. Quality control standards (Air-gas Specialty Gases, Chicago, IL) were 399.1 and 1001 ppm for CO_2, 1.52 and 2.01 ppm for CH_4, and 0.468 and 1.14 ppm for N_2O (Harvey et al. 2020). Standards were run for every 30 samples, and minimal detectable flux was approximately 97.86, 0.94, and 0.27 g ha^{-1} d^{-1} for CO_2, CH_4, and N_2O, respectively.

The CO_2, N_2O, and CH_4 fluxes, in units of g CO_2-C kg^{-1} h^{-1}, N_2O-N kg^{-1} h^{-1}, and CH_4-C kg^{-1} h^{-1}, respectively, were calculated using a modified ideal gas law in Eq. [2] (Kim et al. 2021; Lin et al. 2017; Venterea et al. 2020).

$$F = \frac{S \, x \, P \, x \, V}{A \, x \, R \, x \, T} \tag{2}$$

Where F = flux rate (g CO_2-C, N_2O-N, or CH_4-C ha^{-2} d^{-1}), S = slope of the linear or quadratic regression at time zero (Venterea et al. 2020), P = ambient pressure (Pa), V = volume of chambers (L), A = surface area with the chamber (m^2), R = gas constant, and T = temperature (K).

Linear interpolation was used between sampling dates to create a complete time series (Dorich et al. 2020). Area-based emission factor for N/ cumulative GHG emissions, was calculated according to Thilakarathna et al. (2021) in Eq. [3]:

$$Cumulative\ GHG\ emissions/\ EF_{area} = \frac{(E_{treatment} - E_{control})}{N_{input}} x\ 100 \tag{3}$$

Where EF_{area} = area-based emission factor (% kg N_2O-N kg^{-1}), $E_{treatment}$ = emissions from the amended soil (kg N_2O-N ha^{-2} d^{-1}), $E_{control}$ = emissions from soil without manure (kg N_2O-N ha^{-2} d^{-1}), and N_{input} = N of treatment applied (kg N ha^{-1}). Biochar had no detectable N, so it was not included in the analysis. The yield-based emission factor for N/ yield emission intensity, was calculated according to Thilakarathna et al. (2021) in Eq. [4]:

$$Yield\ emission\ intensity/EF_{yield} = \frac{Treatment\ emission}{Grain\ Yield} x\ 100 \tag{4}$$

Where EF_{yield} = yield-based emission factor (g N_2O-N kg^{-1} grain DM), $Treatment\ emission$= emissions from soil treatment (g N_2O-N ha^{-2} d^{-1}), and $Grain\ yield$ = grain yield from soil treatment (kg ha^{-1}).

Cumulative anthropogenic GHG emissions were calculated by summing gas fluxes over the field trial (Dorich et al. 2020). The CO_2-eq GHG emissions were calculated using the GWP coefficients of 265 and 28 for N_2O and CH_4, respectively, over a 100-year time frame based on the mass of a gas emitted (IPCC 2014). Because plants were part of the experiment, dark respiration, also known as mitochondrial respiration as opposed to photorespiration, CO_2 emissions were not a true representation of the C cycle (Wang et al. 2001). Consequently, anthropogenic GHG emissions were calculated according to Kammann et al. (2012) in Eq. [5]:

$$Anthropogenic\ GHG\ flux = N_2O\ flux + CH_4\ flux \tag{5}$$

where $N_2O\ flux$ = GWP of N_2O from the soil (kg CO_2 eq ha^{-2} d^{-1}) and $CH_4\ flux$ = GWP of CH_4 from the soil (kg CO_2 eq ha^{-2} d^{-1}).

4.3.5 Statistical analyses

Shapiro and Bartlett/Levene tests confirmed assumptions of normality of distribution and homogeneity of variance of the residuals before analyses (Logan 2011). Levene's test was used instead of Bartlett's test if data was not normal. Log or sqrt transformations were applied to the response variables if the models did not meet these assumptions (de Klein et al. 2020). Transformed data were used for statistical analysis to assess treatment effects, but untransformed data were used to calculate mean values and graph results (de Klein et al. 2020).

To measure the properties of soil and manure treatments, a one-way analysis of variance (ANOVA, $P = 0.05$) was used. A blocked ANOVA, where treatment was a fixed factor and block was a random factor, was utilized if block was a significant factor ($P = 0.05$); if not, a linear one-way ANOVA was used for cumulative GHG data. If $P < 0.05$, differences between treatments were analyzed using a Tukey-Kramer test. Relationships between the soil properties (temperature, moisture, and biogeochemical values) and cumulative GHG emissions were examined using Spearman's rank correlations.

Soil temperature and moisture was collected and analyzed during the collection period (Apr 22 – Sept 27) and then totaled at chosen thresholds to represent an amended growing degree days. Soil temperature was correlated using total days greater than 15 °C, a key temperature that influences GHG emissions with biochar amendments (Cui et al. 2021). Soil moisture was correlated using total days greater than 30% volumetric water content due to the influence on soil water potential at this percentage (Atkinson 2018). These statistical calculations were performed using R v. 4.2.1 (R Core Team, 2020) (Logan 2011).

4.4 Results

4.4.1 Temperature and moisture

During the 2020 growing season (Apr-Sept), daily temperatures (11.0 °C) were 6.4% lower than the average (11.7 °C; Fig. 4.1a) The beginning of the experiment, from April to June, was variable in daily atmospheric average temperatures, with lows of 2.4 °C and highs of 12.9 °C. Soil temperatures peaked in late July at 23.5 °C but by the end of August, atmospheric temperatures began to decline and ranged from 6.8 °C to 16.8 °C. The total number of days greater than 15 °C ranged from 76-85 ($P > 0.05$; Table 4.2).

During the growing season, precipitation was very high, 35% higher than the long-term average (552.6 mm vs. 410.4 mm; Fig. 4.1b). The total number of days greater than 30% volumetric water content ranged from 56-71 ($P > 0.05$; Table 4.2). In April (25.3 mm), there was not much precipitation but greatly increased in May (146 mm). As a result, soil moisture declined from an average of 0.35 m^3m^{-3} to 0.20 m^3m^{-3} in late April, then sharply rose again to a high of 0.45 m^3m^{-3} in early May.

There were frequent soil wetting and drying cycles in late April, early and late May and early June due to high precipitation in May-July. By August, precipitation decreased from 69.1 mm to 41.5 mm in September. Soil moisture steadily declined during that period from 0.3 m^3m^{-3} with few peaks to 0.2 m^3m^{-3}. The CO_2 and CH_4 were negatively correlated ($P > 0.05$) and to soil temperature and moisture, respectively (Table 4.1). Everything else was positively ($P > 0.05$) correlated to soil temperature and moisture.

4.4.2 Greenhouse gas emissions

At the beginning of the field season in April, there were low CO_2 emissions (Fig. 4.4a) that increased mid-May but became almost negligible in early June across all treatments. The largest CO_2 peaks were present in mid-June from BM+BC10, mid and late-July from RM+BC10, with some fluxes until the end of sampling in September from BC5 (Fig. 4.4a). BM+BC10 remained low from the start of the experiment towards mid-Aug in which manures (RM and BM) were relatively lower than until the end of the experiment. Cumulative emissions over time (Fig. 4.5a) was generally steadily increased over time across all treatments, starting in mid-June and starting to plateau in September.

The N_2O fluxes (Fig. 4.4b) peaked in early and late May from RM+BC10, mid-June from BC5, and early July from BC10. The CT plots were lowest throughout the experiment and the manures (RM and BM) were again higher than other treatments around mid-Sept. The CH_4 fluxes (Fig. 4.4c) had little activity at the beginning of the sampling period, but increased fluctuations in mid-May from BM+BC10. Positive emissions occurred in late July, with peaks from BM+BC10, early Aug with peaks from BM+BC5, and late Sept from various treatments. Cumulative emissions over time for N_2O (Fig. 4.5b) and thus N_2O+ CH_4 (Fig. 4.5c) started earlier than CO_2, around early May. The CT had much lower cumulative emissions than the other treatments and two peaks are seen in mid-July and August (Fig. 4.5b & c).

The CO_2 emissions (Fig. 4.3a) ranged from 587.5 - 938.5 kg^{-1} ha^{-1} with the CT lowest (P = 0.024), followed by BM+BC5 (586.4 kg^{-1} ha^{-1}) and BM (683.7 kg^{-1} ha^{-1}). The highest CO_2 emissions (Fig. 4.3a) came from BM+BC10 (938.4 kg^{-1} ha^{-1}) followed by BC5 (892.3 kg^{-1} ha^{-1}). Cumulative emissions of N_2O (Fig. 4.3b) and anthropogenic (N_2O + CH_4; Fig. 4.3d), did not differ (P > 0.05) among treatments and followed a similar pattern. The N_2O emissions varied from 145.9 – 427.4 g^{-1} ha^{-1}, and anthropogenic emissions varied from 58.6 – 201.5 kg^{-1} ha^{-1}. All treatments had negative cumulative CH_4 emissions (Fig. 4.3c; P = 0.023), which were lowest (greatest sink potential) in RM (-78.0 g^{-1} ha^{-1}) and RM+BC10 (-68.9 g^{-1} ha^{-1}) and highest (greatest source potential) in BM+BC10 (-0.8 g^{-1} ha^{-1}) and BM+BC5 (30.2) (Fig. 4.3c).

Cumulative GHG emissions/area emission factor (EF_{area}; Fig. 4.5b) did not differ (P > 0.05) amongst treatments, but yield emission intensity/yield emission factor (EF_{yield}; Fig. 4.5a) (P = 0.022) was lowest EF_{yield} (0.12 g N_2O kg^{-1} grain) in the CT, and highest (1.35 g N_2O kg^{-1} grain; Fig. 4.6a) in the BM. BM+BC10 (0.31 g N_2O kg^{-1} grain) had higher EF_{yield} compared to BM+BC5 (0.19 g N_2O kg^{-1} grain) and RM+BC10 (0.10 g N_2O kg^{-1} grain). BM (0.15 % kg N_2O kg^{-1} N) had a lower EF_{area} than RM (0.25 % kg N_2O kg^{-1} N).

The exchangeable NPKS values were not statistically correlated any emissions except for CO_2 and S (Table 4.1). All emissions were positively correlated (P > 0.05) to EC, MBN and MBC and negatively correlated (P > 0.05) to pH. Additionally, all emissions were significantly and positively correlated (P < 0.05) TC and TN, except for CO_2 and TN (P > 0.05).

4.5 Discussion

4.5.1 Carbon emissions

At the start of the experiment, lower emissions and an increase in CO_2 (Fig. 4.4a) and CH_4 (Fig. 4.4c) came in early June due to wheat planting and the corresponding flush of microbial activity from soil disturbance (Ball 2013). Differences in C mineralization were also found in an incubation study in this same Luvisol (Weber et al. 2021a). Further emissions peaks in the summer result from frequent rain (Fig. 4.1b) and increased moisture in the soil (Fig. 4.2b). The CH_4 emissions were positively correlated, and the CO_2 emissions were negatively correlated, to soil moisture ($P > 0.05$; Table 4.1), due to frequent anaerobic conditions. After September, lower temperatures and precipitation rates (Fig. 4.1) reduced CO_2 (Fig. 4.4a) and CH_4 (Fig. 4.4c) emissions (Kim et al. 2021).

As predicted, the additions of RM and BM increased CO_2 emissions ($P = 0.024$; Fig. 4.3a) emissions compared to CT, as manure decreases soil bulk density and soil strength, which can enhance microbial activity and respiration (Lentz et al. 2014). However, the hypothesis that RM would have greater CO_2 (599.3 kg ha^{-1}; Fig. 4.3a) and CH_4 (-80.0 g ha^{-1}; Fig. 4.3c) emissions than BM (683.7 kg ha^{-1} and -46.8 g^{-1} ha^{-1}, respectively) was not supported. There were no differences in MBC between the two treatments by the end of the experiment (Table 3.5) and no significant ($P > 0.05$) correlations to microbial biomass (Table 4.1). The greatest CH_4 sink potential from RM and RM+BC10 likely came from the ability to moderate water content ($P > 0.05$; Table 4.2) in this wetter than normal season.

The importance of biochar's quantity and the synergistic effects of biochar's addition to manure are emphasized when comparing BM+BC5, which produced the lowest CO_2 emissions (586.5 kg ha^{-1}; P = 0.024), to BM+BC10, which produced the highest CO_2 (938.5 kg ha^{-1}; Fig. 4.3a) and CH_4 (0.89 g ha^{-1}; Fig. 4.3c) emissions. This result contradicts some findings that higher biochar rates may lead to aggregation, enhanced organomineral relations and dissolved organic C adsorption that aids biochar-induced negative priming (Guenet et al. 2021; Kammann et al. 2011). Treatments BM+BC10 and RM+BC10 had higher (938.5 and 861.2 kg ha^{-1}, respectively; P = 0.024) CO_2 emissions and grain yield (101.9 and 85.0 kg ha^{-1} respectively; data not shown) than BM+BC5 (586.5 and 61.5 kg ha^{-1}, respectively). This is the opposite of what was found in a meta-analysis by Liu et al. (2016), where soil CO_2 fluxes to biochar amendment decreased with biochar application rate.

At the beginning of the season, there were higher (P < 0.001) total C from RM+BC10 (35.7) and BM+BC10 (29.3) than BM+BC5 (26.7; Table 3.4), which may explain the greater grain yield as they are positively correlated (P > 0.05, Table 4.1). These statistical differences were no longer present a few months later (Table 3.4), and all treatments decreased in TC slightly. Because BC10 mixtures had higher TC than BC5 mixtures, inorganic C was likely released from the biochar over time (Jones et al. 2011), and more microbial substrates were available in the BC10 mixtures.

Lower emissions from BM+BC5 compared to CT (Fig. 4.3a), albiet a small difference, may rise from the increases in the hydrophobicity and aromaticity of biochar surfaces and organic matter mixtures (Jindo et al. 2016), which could lower the access of microbial substrates as there was higher MBC (P < 0.05; Table 3.4 and P > 0.05; Table 3.5) in BM+BC5 compared to BM+BC10. There is also an important soil-plant interface role in field trials, as an incubation experiment (Weber et al. 2021a) showed higher C mineralization in RM than in BM. An increased C use efficiency within the plants may have been stimulated from the biochar additions of BM+BC5 (Kammann et al. 2012; Kammann et al. 2011). Additionally, BM+BC10 may have had a shift toward a bacterial-dominated microbial decomposer community with lower C use efficiency than BM+BC5 (Jones et al. 2012).

Reductions in CO_2 emissions from pure biochar additions to soils are frequently found in other studies given biochar's recalcitrance (Abagandura et al. 2019; Lentz et al. 2014; Spokas and Reicosky 2009). The increased CO_2 emissions from BC5 (892.3 kg ha^{-1}) compared to the CT (587.5 kg ha^{-1}) found in this study (Fig. 4.3a) may have resulted from microorganism mineralization of labile C within the biochar and priming effect of the native SOM (Ameloot et al. 2013; Jones et al. 2011; Troy et al. 2013). Treatment BC10 had lower (646.3 kg ha^{-1}; P = 0.024) CO_2 emissions than BC5 (892.3 kg ha^{-1}) and higher than the CT (587.5 kg ha^{-1}; Fig. 4.3a) but was not different in CH_4 emissions. Therefore, greater amounts of biochar are needed in biochar-only applications for C sequestration, and do not pose concern for increases in CH_4. These results can vary depending on the type of biochar used, as higher temperature biochars are typically more recalcitrant (Ameloot et al. 2013; Gascó et al. 2016; Romero et al. 2021b).

4.5.2 The potential for biochar to be a methane sink

In a comparison of RM and BM, Romero et al. (2021a) found the only difference was the increased aromatic-C character of BM. One explanation for why RM and RM+BC10 (-68.9 g ha^{-1}) had greater ($P < 0.05$) CH_4 sink potential than BM+BC5 (-30.2 g ha^{-1}) and BM+BC10 (-0.89 g ha^{-1}; Fig. 4.3c) is that the biochar fed to cattle undergoes chemical reactions in acidic and alkaline environments within the rumen that act as a biocide to methanotrophs (Joseph et al.

2015b; Schmidt et al. 2019). Additionally, biochar that has passed through the rumen is suggested to adsorb signaling compounds that change gene expression and microbial populations (Joseph et al. 2015b). A potential change from *r* to *k* strategists (not measured) due to a higher MBC:MBN ratio in BM+BC10 compared to RM+BC10 ($P < 0.05$; Tables 3.4 & 3.5) can also retard CH_4 production (Yu et al. 2013). Biochar can thus aid long-distance electron exchange, helping cattle increase their feed intake efficiency, improving anoxic microbial respiration and CO_2 emissions (Schmidt et al. 2019).

Le Mer and Roger (2001) also found positive correlation methanogenic potential and the OM content in soils (0.405; $P < 0.05$; Table 4.1), explaining the greater CH_4 sink potential from RM+BC10 than BM+BC10 and BM+BC5 at the start of the season ($P < 0.05$; Table 4.1). Inhibition of CH_4 oxidation from BM+BC5 and BM+BC10 is also correlated to moisture, as BM+BC10 had the second lowest total number of days greater than 30% volumetric water content ($P > 0.05$; 60.5; Table 4.2) and the lowest CH_4 sink potential (Fig. 4.3c). One explanation is that while biochar did increase the soil porosity, the spaces were filled with water and increased overall anaerobic pockets (Yu et al. 2013). There was a negative correlation ($P < 0.05$; Table 4.1) between CH_4 emissions and pH, as sensitive methanogen populations increased as pH increased from biochar and manure additions at higher rates (Le Mer and Roger 2001). This inhibition may be important in environments that are more waterlogged throughout the year, such as paddy fields (Yu et al. 2013), as this study found that N_2O emissions were of greater impact when compared as cumulative GHG flux (Fig. 4.3d).

4.5.3 N_2O emissions, anthropogenic GHG emissions, and emission factors

Similar to a previous incubation by Weber et al. (2021a) and a field study by Jones et al. (2012), there were no significant differences ($P > 0.05$) in N_2O emissions (Fig. 4.3c), anthropogenic GHG emissions (represented by changes in N_2O, rather than CH_4, emissions; Fig. 4.3d), and EF_{area} (Fig. 4.6b) amongst treatments. The BC applied had no detectable levels of N input (Table 3.2), meaning BC's influence on N_2O emissions likely came from alternative changes in soil properties. Given that this plot has a history of urea use, residual NO_3-N may have been an influencing factor as well, as there was a positive correlation to TN ($P < 0.05$) across all emissions (Table 4.1); however, this is unlikely two years after the initial application (Grant et al. 2016).

Environmental conditions likely played a large role in emissions. The increase in N_2O emissions during the initial thaw in mid-April (Fig. 4.4b) was due to the release of organic substrates and the high moisture content of the soil (0.154 correlation; $P > 0.05$; Table 4.1), which causes microbes to use alternative electron acceptors, such as NO_3 (Russenes et al. 2016; Thilakarathna et al. 2021). As the field season progressed, N uptake by plants might have reduced the availability of NO_3 and NH_4 for N_2O emissions (Thilakarathna et al. 2021; Troy et al. 2013). Given the high N_2O fluxes in mid-June and July (Fig. 4.4b); however, this is likely overridden by high precipitation (Kim et al. 2021). Finer-textured soils require lower WFPS to induce denitrification, even at 50% WFPS for a silty loam (Lehmann and Joseph 2009), highlighting the increased emissions and MBN for rainy seasons at Breton.

Surprisingly, BC5 (412.5 g ha^{-1}) and BC10 (427.4 g ha^{-1}) had higher, but not significantly different, N_2O emissions than the BM (303.2 g ha^{-1}) and RM (394.1 g ha^{-1}; Fig. 4.3b). Input of labile organic materials from manures usually act as electron donors in the denitrification process compared to the recalcitrant nature of biochar (Guenet et al. 2021; Troy et al. 2013). The labile organic matter is seen in the EF_{yield} /yield-based emission factor, where RM, BM, and BM+BC10 have some of the highest values ($P < 0.05$, Figure 4.5a). One explanation is that any aeration benefits from biochar in this study were likely overridden by inhibition of the *Nos* enzyme suppression of N_2O reduction to N_2 (Lehmann and Joseph 2009). Moreover, Liu et al. (2017) and Jones et al. (2011) found that biochar may not change soil aeration conditions sufficiently to change N mineralization, especially in soils lacking compaction as there was a positive correlation between N_2O and soil moisture (0.154; $P > 0.05$; Table 4.2) Denitrification enzyme activity in soils was found to increase with increasing biochar rates (Jones et al. 2012), supporting the findings of higher N_2O emissions from BC10 (427.4 g ha^{-1}) than from BC5 (412.5 g ha^{-1}).

All treatments decreased in EC between June ($P = 0.218$; Table 3.4) and October ($P = 0.013$; Table 3.4), with the BC10 and BM+BC10 treatments having the highest EC at 250 and 177 µS cm^{-1} respectively. Adviento-Borbe et al. (2006) found that if denitrification is the primary source of N_2O, the microbial community is more tolerant to salt stress than if nitrification and aerobic conditions are present. During the 2020 growing season (539 mm; Fig. 4.1b), precipitation was 169 mm higher than the long-term average, so it is likely denitrification predominated. The manure and control treatments had the lowest EC ($P = 0.013$; Table 3.4) compared to the biochar and biochar + manure treatments, so future research should investigate biochar's impact on the microbial community's salt tolerance in relation to N_2O emissions.

The RM alone resulted in higher N_2O emissions (394.1 g ha^{-1}; Fig. 4.3b) and EF_{area} (0.24 % kg N_2O kg^{-1} N; Fig. 4.6b) than BM (303.2 g ha^{-1} and 0.15 % kg N_2O kg^{-1} N, respectively). The biochar from the gut of the cattle that remained in BM likely interacted with rumen microbes, increasing the rate of complete denitrification to N_2 and lowering N_2O emissions (Troy et al. 2013). However, RM+BC10 (380.8 g ha^{-1}) had lower emissions ($P > 0.05$; Fig. 4.3b) than BM+BC10 (483.5 g ha^{-1}). Like CO_2, synergistic effects between manures and biochar are important in understanding N_2O emissions. The lower soil moisture from the RM and RM+BC10 than BM and BM+BC10, but higher N_2O emissions from RM than BM alone, ($P > 0.05$; Table 3.2) supports this hypothesis.

Joseph et al. (2015b) found that biochar adsorption of available N and P was retained when biochar fed cattle manure was incorporated into soils, allowing for greater substrate availability. Toxic effects from biochar on nitrifier and denitrifier communities have also been found; however, so restrictions to microbial N activity in the manures may have simultaneously occurred (Kammann et al. 2011; Lehmann and Joseph 2009; Lentz et al. 2014; Liu et al. 2017). The EF_{area} (Fig. 4.6a) showed significantly greater emissions from RM (0.658 g N_2O kg^{-1} grain) than RM+BC10 (0.098 g N_2O kg^{-1} grain). Additionally, MBN was lower ($P < 0.05$) in RM (180 mg kg^{-1}) than RM+BC10 (229 mg kg^{-1}), supporting possible microbial limitations.

Increases in pH from Summer 2020 (average 6.33; Table 3.4) to Fall 2020 (average 6.78; Table 3.4) likely led to lower N_2O/N_2 ratios (Kammann et al. 2012) because the addition of biochar enhances microbial *amoA* (ammonia-oxidizing bacteria) and *nosZ* (N_2O-reducing bacteria) genes from acidic soils (pH 5-6.5). The negative correlation between pH and N_2O (-0.119; $P > 0.05$) is also seen in Table 4.1. This change in microbial communities enhances the reduction of N_2O to N_2 and binds N_2O-N to metal ions (Liu et al. 2017; Romero et al. 2021b). This N_2O/N_2 ratio change is not as affected by nitrification in most agricultural soils (pH 5.5-7.0), however, explaining why there were no significant differences in N_2O emissions (Fig. 4.3b) or pH (Table 3.4 & 4.2) among treatments (Russenes et al. 2016).

4.6 Conclusion

In conclusion, the quantity of biochar and the synergistic effects of manure, biochar, and crop significantly affected greenhouse gas emissions when studying BM applications. The various treatments resulted in significant differences amongst treatments in CO_2 and CH_4 emissions, but not N_2O emissions, resulting in no differences in the anthropogenic GHG emissions (N_2O + CH_4). Although there was a key finding of inhibition of CH_4 oxidation from BM+BC10, the magnitude of CH_4-CO_2 eq was much smaller than N_2O-CO_2 eq, suggesting BM applications can be applied without making a difference for climate change relative to RM.

In summary, BM+BC5 may mitigate the greatest amount of anthropogenic GHG emissions (albeit not significant), while also improving protein content and grain biomass compared to BM alone. The difference in GHG emissions from BM compared to RM included potential alteration to microbial community functions in the manure-amended soil. Given the variety of results from different biochars from previous studies, further inquiries of various biochar properties (feedstock, temperature, etc.) on different soil types should be investigated.

Table 4.1. Spearman's rank correlation coefficients of relationships between the cumulative CO_2, N_2O, CH_4, and CO_2 eq emissions and soil properties (means ±SE; n = 4).

Variable	N	P	K	S	MBC	MBN	EC	pH†	TC	TN	Temp	Moisture
CO_2	-0.164	0.130	0.344	0.366*	0.064	0.195	0.047	-0.254	0.370*	0.279	0.019	-0.336
N_2O	-0.216	0.192	-0.017	0.194	0.104	0.184	0.269	-0.119	0.361*	0.440*	0.03	0.154
CH_4	0.140	-0.192	0.049	0.251	0.214	0.344	0.258	-0.26	0.405*	0.367*	-0.026	0.030
CO_2 eq	-0.321	0.208	0.038	0.087	0.083	0.124	0.097	-0.082	0.362*	0.381*	0.008	0.043

Note: Abbreviations: EC, electroconductivity; K, potassium; N, (organic) nitrogen; P, Phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; TC, total carbon; TN, total nitrogen; OM, organic matter.
†Soil pH was measured in an 1:5 soil:water (v:v) ratio. Means followed by a common letter within a column are not significantly different (P < 0.05). Significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 4.2. Soil temperature of total days greater than 15°C and soil moisture of total days greater than 30% volumetric water content for the respective growing season (Apr 22 - Sept 27 2020) (means ±SE; n = 4).

Treatment	Temperature	Moisture
	days	
CT	82.5±1.5	60.0±7.8
RM	77.0±2.5	57.3±4.4
BM	76.7±2.8	71.0±4.1
RM+BC10	82.7±1.3	56.5±6.9
BC5	80.3±0.6	59.0±6.8
BM+BC5	79.3±0.5	57.3±7.1
BC10	85.5±0.3	64.7±4.0
BM+BC10	78.0±2.9	60.5±5.6
***P* - Value**	0.310	0.703

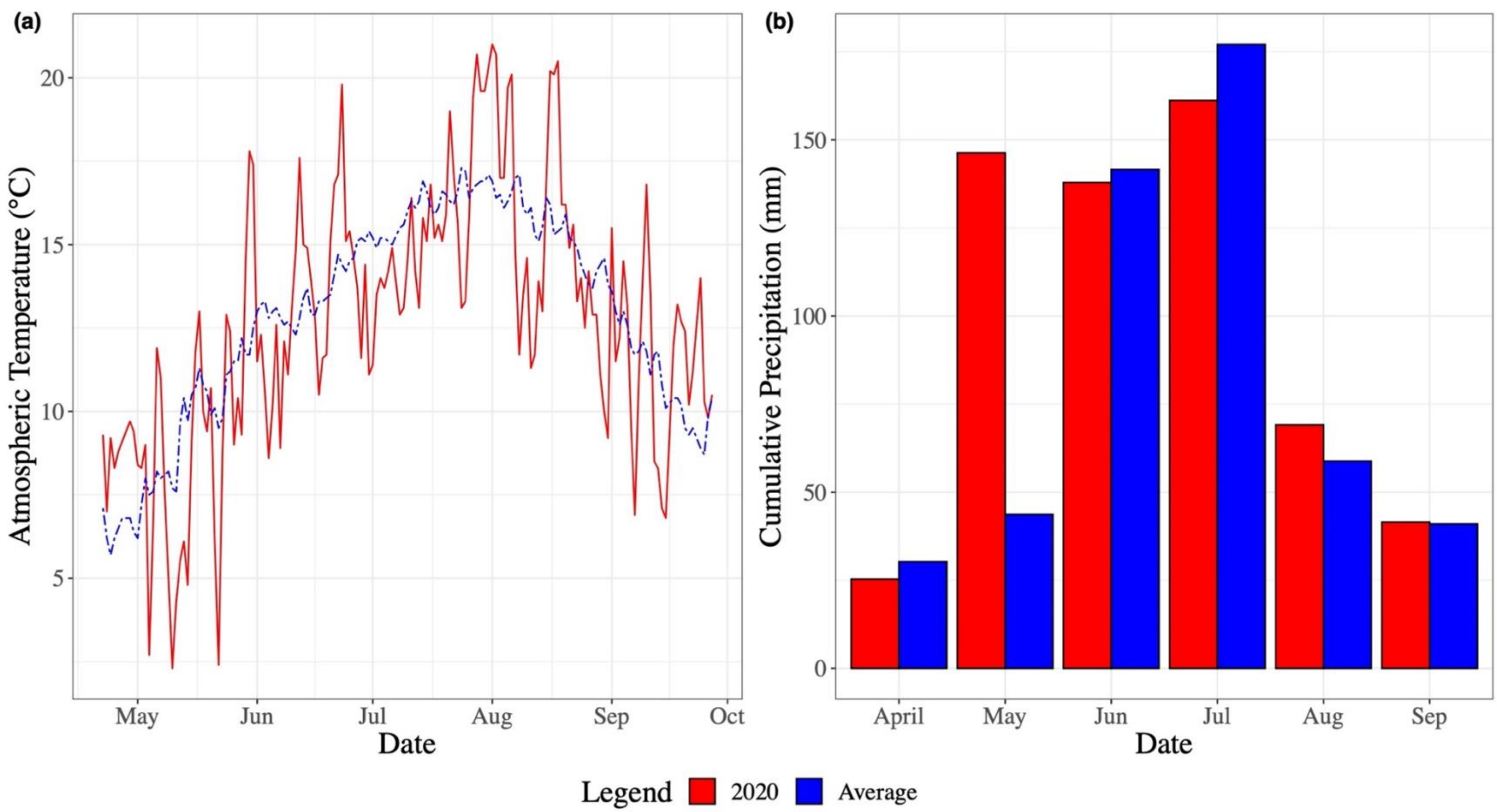

Figure 4.1. The a) average daily temperature and b) monthly precipitation during GHG sampling in 2020 compared to the average over the last decade.

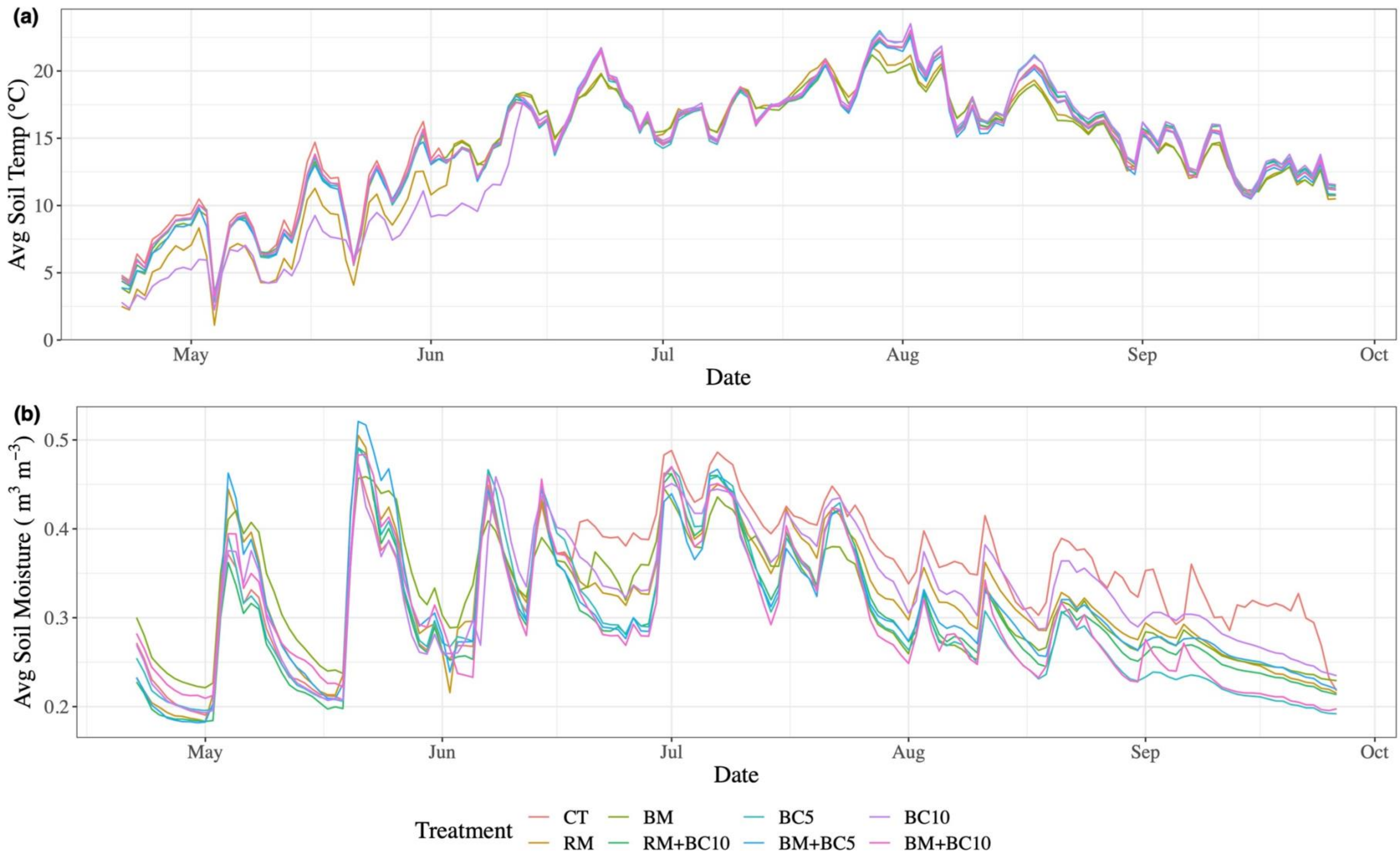

Figure 4.2. The soil a) temperature and b) moisture average (10 cm) per treatment during GHG sampling in 2020. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

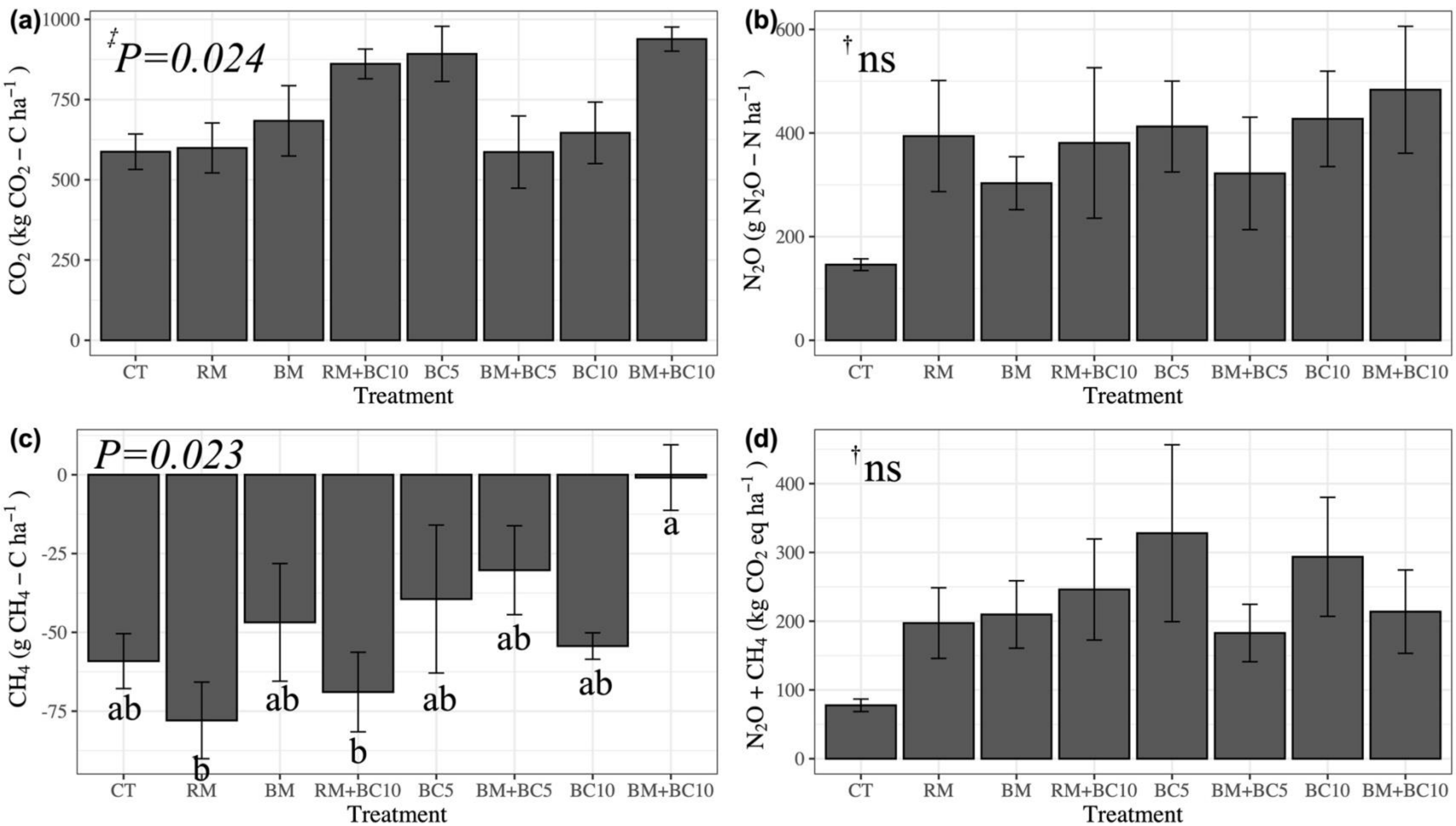

Figure 4.3. Effects of treatments on cumulative (a) soil respiration (CO_2), (b) N_2O, (c) CH_4, and (d) anthropogenic (N_2O + CH_4) GHG emissions. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Letters denote significant differences between treatments, P <0.05. †ns indicate not significant, P > 0.05. ‡Tukey's Honest Significant Difference (HSD) test did not show significant differences between treatments even the P-value was below 0.05.

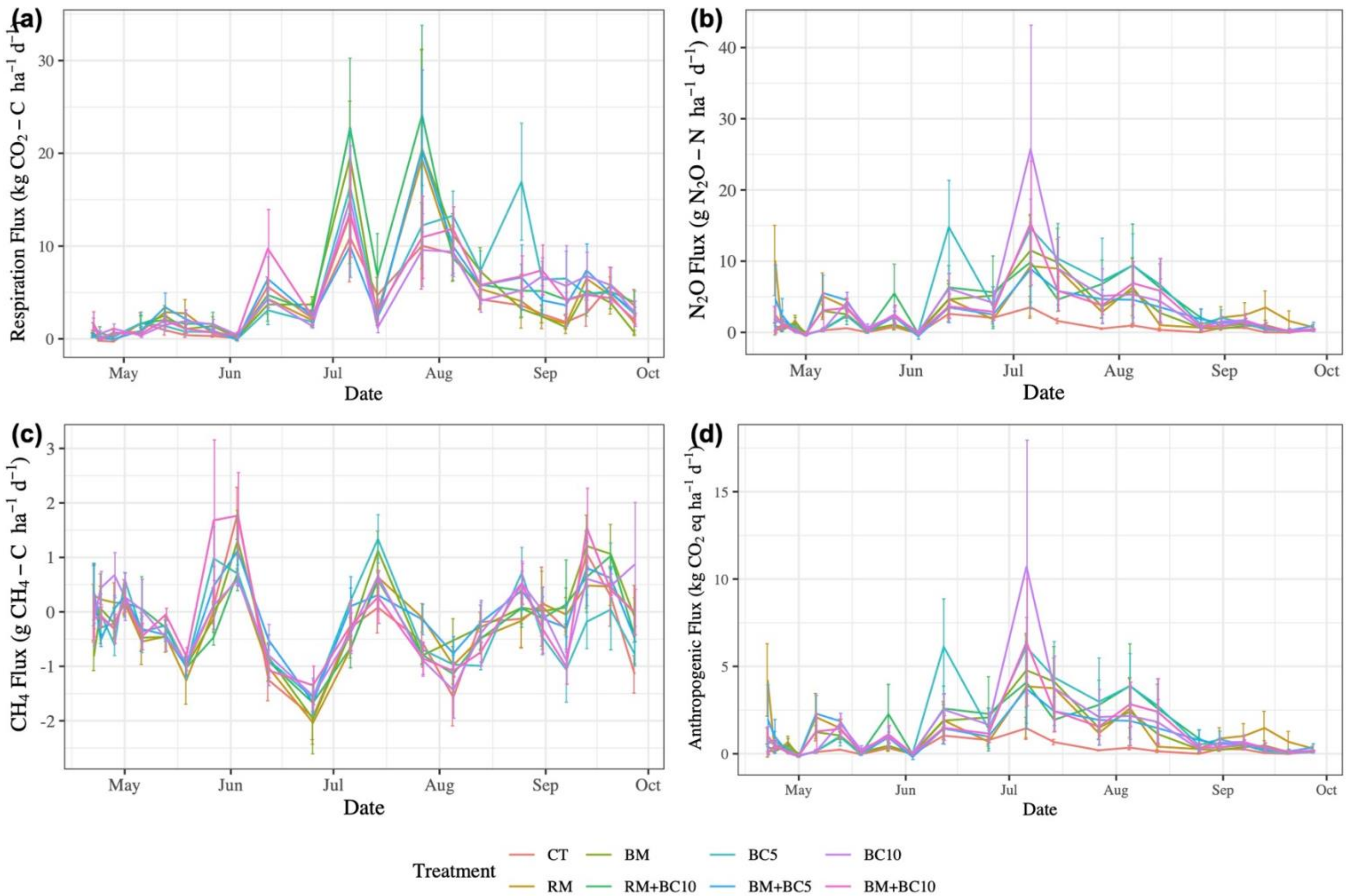

Figure 4.4. Effects of treatments on (a) soil respiration (CO_2), (b) N_2O, (c) CH_4, and (d) anthropogenic ($N_2O + CH_4$) GHG fluxes over time. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

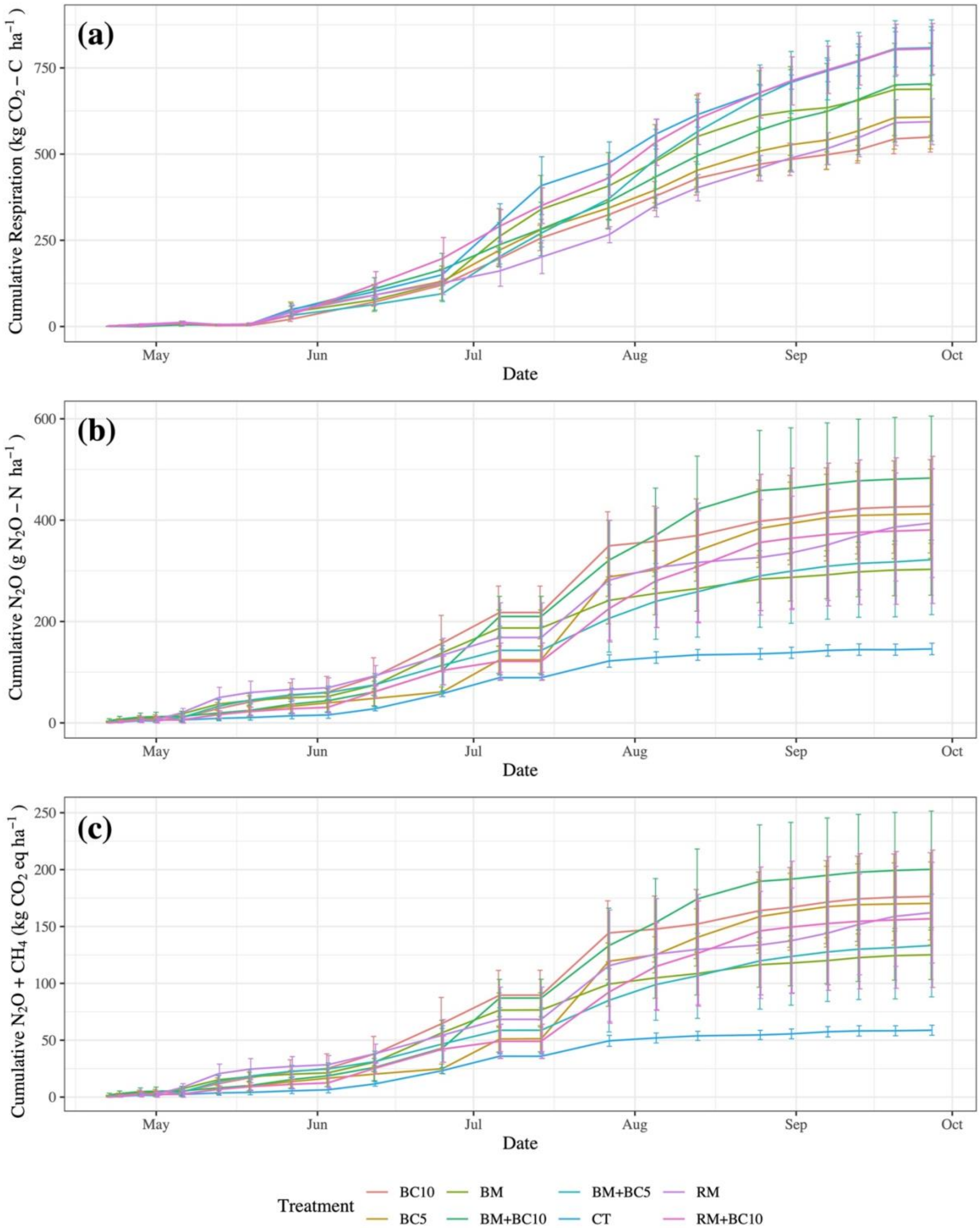

Figure 4.5. Effects of treatments on cumulative (a) CO_2, (b) N_2O, and (c) N_2O + CH_4 GHGs over time. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

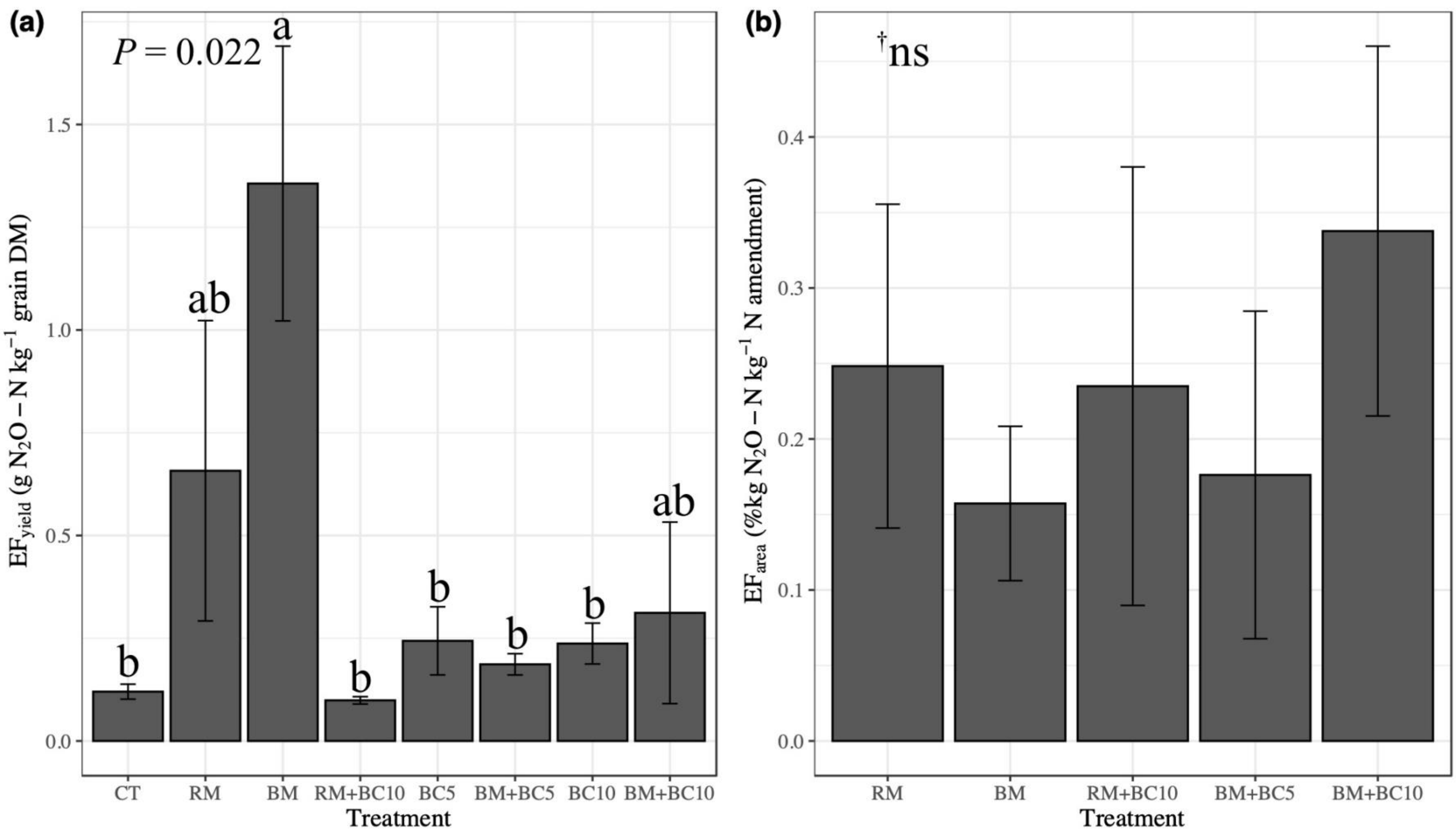

Figure 4.6. Effects of treatment combinations on cumulative (a) emission factor (EF) yield (yield emission intensity) and (b) area (cumulative GHG emissions). Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Letters denote significant differences between treatments, $P < 0.05$. †ns indicate not significant, $P > 0.05$.

Chapter 5: Manure from cattle-fed biochar and mixed with biochar increases organic matter stability in a Gray Luvisol field trial

5.1 Abstract

Conventional cultivation methods for crop production can reduce soil organic matter (OM) content and soil health for short-term gains. Utilizing regenerative agricultural practices, such as biochar, will stabilize carbon and can be combined with practices that will maintain crop productivity. This paper aims to fill in a knowledge gap on the impacts of biochar-supplemented feed and corresponding manure use as a field amendment. A field trial was conducted on a rainfed Gray Luvisol with plots containing; i) no amendments (control, CT), ii) biochar at 5 and 10 Mg ha^{-1} (BC5 and BC10), iii) raw manure (RM) and iv) biochar-manure (BM) (manure from cattle with biochar added at 2% of diet dry matter) at 100 kg total N ha^{-1}, and v) BC and RM (BC + RM) or BM (BC + BM). Soil samples were fractionated into particulate organic matter (POM) and aggregated and non-aggregated mineral-associated organic matter (MAOM) and analyzed with diffuse reflectance infrared fourrier transfom (DRIFT) spectroscopy. There was a notable separation between aggregated MAOM and POM in the PCA comparison of all treatments. Minimal differences between RM and BM (OC, TN, and C/N and PCA results) and similar PCA eclipses of the DRIFT wavelengths between BM+BC10 and BM+BC5 point to key differences in carbon stability when biochar is added to soil. In conclusion, RM+BC10 had the highest OC in the non-aggregated MAOM ($P < 0.05$; 7.38 g kg^{-1}) and POM ($P < 0.05$; 11.09 g kg^{-1}) and highest short-term stability. The BM+BC10 had the highest ($P > 0.05$) OC (Table 5.1 & 5.6) and C/N in the aggregated MAOM fractions and highest long-term stability.

5.2 Introduction

As human populations increase worldwide, conventional cultivation methods for crop production, such as fertilizers and tillage, can reduce soil organic matter (SOM) (Cambardella and Elliott 1992). Soil organic matter stability is defined as the overall physical resistance to decay, while recalcitrance is specifically the resistance of SOM to microbial and enzymatic degradation due to chemical structure (Dynarski et al. 2020). Both are important in determining how easily SOM can be mineralized (Fernández et al. 2011). To maintain and improve the regenerative capacity of soil functions for crop production, it is necessary to understand SOM stability (Sherwood and Uphoff 2000).

Not only does soil serve as a necessary part of agricultural production, with 1500–2000 Pg of C globally, but soil also serves as the third-largest global C pool (Fernández et al. 2011; Post and Kwon 2000). Although soil C accumulates at an average rate of 2.4 g C m^{-2} y^{-1} in most ecosystems, the rate of C loss from cultivation (up to 50% in the top 20 cm after 30-50 years of cultivation) outweighs this gain (Post and Kwon 2000). Thus, finding ways to decrease the loss of SOM and soil organic carbon (SOC) has gained traction (Fernández et al. 2011; Post and Kwon 2000) and regenerative agriculture seeks to find ways to simultaneously improve productivity and environmental management of soil quality (Sherwood and Uphoff 2000).

Biological, chemical, and physical processes activity, and environmental conditions control the rate and degree of SOM stabilization and soil quality (Kimetu and Lehmann 2010; Post and Kwon 2000; Schuman et al. 2002). Degradation rates range from rapid catabolism with simple sugars to slow mineralization rates with lignin and humic acids (Riffaldi et al. 1996). Aggregation, where organic matter and minerals bind to each other (Lehmann and Joseph 2009), plays an important role in microbial degradation rates (Dong et al. 2016; Pituello et al. 2018).

Soil texture, clay mineral, SOM content, cations, oxides, and carbonates are the most important factors in determining soil aggregation formation and stability (Dong et al. 2016). Finally, the characteristics of the material being amended also regulate decomposition rates and end products.

One agricultural amendment that may contribute to SOM stabilization is biochar (Chen et al. 2020), a surrogate for the black carbon found in most natural ecosystems produced as a result of a fire which may improve C sequestration in agroecosystems (Certini 2005). Biochar plays an important role in organic carbon transformation due to its negatively charged surfaces, amorphous structures and turbostratic (layers are not aligned) crystallites (stacks of flat aromatic sheets) (Lehmann and Joseph 2009), adsorption of hydrophilic organic matter (OM), physical protection from microbial activity (Du et al. 2017; Hagemann et al. 2017; Lehmann and Joseph 2009), and ability to reside in soil aggregates (Pituello et al. 2018) which all contribute to its overall OM recalcitrance to any changes. A FTIR study of *Terra Preta de Indio* molecular markers found to have increased recalcitrant carbon (aliphatic and aromatic) due to historical additions of biochar (Solomon et al. 2007)

A complex interface of factors influence the ability of biochar to enhance OM stability. The chemical and physical properties of the mineral matrix and pre-existing SOM can influence biochar stability rates (Kimetu and Lehmann 2010). For example, soils with low fertility will see a greater mineralization rate in native SOM from biochar's stimulation of microbial communities (de la Rosa et al. 2018; Plaza et al. 2016). A considerable amount of biochar can be lost through erosion, and freeze-thaw, tillage, and wet-dry cycles can expose SOM to physical and microbial degradation (de la Rosa et al. 2018; Dynarski et al. 2020; Lehmann and Joseph 2009; Post and Kwon 2000). Due to numerous aspects that influence aggregation, both increases and decreases in soil aggregation (Dong et al. 2016; Du et al. 2017; Pituello et al. 2018; Zhang et al. 2015) have been found with biochar additions.

Unfortunately, the sole application of biochar is costly (Hagemann et al. 2017; Shahzad et al. 2018) and can potentially limit crop production (Pokharel and Chang 2019) given its high C/N ratio and lack of immediate plant-available nutrients. Hence, recent studies have investigated the use of enhanced biochar (EB), biochar which has a post-pyrolysis treatment with mineral salts or weak acids, being added to cattle feedstock (Tamayao et al. 2021; Terry et al. 2020). Terry et al. (2020) found that adding 1-2% biochar to cattle feed (dry wt) was unsuccessful in promoting growth but did not see any adverse effects on health. Furthermore, feeding cattle with enhanced biochar means that biochar is still present in the corresponding manure (Romero et al. 2021a; Tamayao et al. 2021; Terry et al. 2020), and the stability mechanisms for MAOM may be present in biochar-loaded manure without any harm to the cattle. Application of manure is another mechanism to potentially improve SOM stabilization through increased aggregate stability (Haynes and Naidu 1998) while also providing readily available plant nutrients (Cimò et al. 2014). Thus, the co-application of manure and biochar show promise in sustainable agricultural practice.

To study decomposition rates, particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions (aggregated and non-aggregated) was investigated. Particulate organic matter is partly decomposed plant residues, whereas MAOM is microbial processed OM stabilized within fine-sized fractions (Lavallee et al. 2020). Increases in MAOM can indicate increased soil structure and stability (Keesstra et al. 2016), allowing for further understanding of aggregation impacts from amendments (Parikh et al. 2014). Meanwhile, POM is sensitive to changes in soil management and serves as a short-term soil quality monitor (Christensen 2001;

Guan et al. 2019). Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) was used to study the molecular resolution of mineral and organic functional groups (Margenot et al. 2016; Singh et al. 2016), helping to explore recalcitrance differences between various agricultural amendments (Tivet et al. 2013).

The objectives of this experiment were to i) investigate how biochar (BC), biochar-manure (BM), and regular manure (RM) additions influence OM stability when added to a Gray Luvisol and ii) how these changes influence OM pools and distribution. First, it was hypothesized that biochar and biochar-manure would result in higher OM stability due to their amorphous structures and turbostratic crystallites protecting it from microbial activity and abiotic oxidation (Lehmann and Joseph 2009) as seen through increased aromatic and aliphatic C (Lehmann et al. 2007; Solomon et al. 2007). Second, it was hypothesized that there would be greater potential for C sequestration from biochar additions with increased C/N ratios (Cotrufo et al. 2019; Paetsch et al. 2016). Finally, it was hypothesized that biochar and manure promote aggregate stability, with more aggregated fractions in manure, biochar, and biochar-manure treatments (Du et al. 2017; Pituello et al. 2018).

5.3 Methods

5.3.1 Experimental design and treatments

The study used a randomized complete block design with four replicates per treatment. Treatments were incorporated into the top 5 cm of the soil at the Breton Research Station (53°07′N, 114°28′W) on September 13, 2019, that had 5.1% OM. From 2010-2011, oats and barley were harvested, followed by a fallow period. From 2013-2015, barley-canola rotations were fertilized with 80 kg N ha^{-1} urea in 2015. Wheat-barley-barley were grown from 2016-2018 with an application of 50 kg N ha^{-1} urea in 2017 and 2018. CDC Go wheat was planted on 3 June 2020 with a field plot seeder and harvested in fall. Wheat yielded 2512.5 - 3215.0 kg ha^{-1} of biomass and 579 – 1485 kg ha^{-1} of grain, with 9.5 - 12.1 % of protein. No further fertilization treatments occurred in 2020.

Treatments included; (1) stockpiled manure from cattle on western feedlot diet (RM; 60% barley silage, 85% barley grain, and 5% mineral supplement) at a rate of 5.4 Mg ha^{-1} (target of 100 kg total N ha^{-1}), (2) stockpiled manure from RM with 2% biochar (BM) at a rate of 4.9 Mg ha^{-1} (target of 100 kg total N ha^{-1}), (3) biochar a rate of 10 Mg ha^{-1} (BC10), (4) biochar at a rate of 5 Mg ha^{-1} (20BC), (5) a combination of (1) and (4), (6) a combination of (2) and (3), (7) a combination of (2) and (4), and (8) a control (soil without manure) (CT). The soil was a Gray Luvisol with a loamy sand texture (Dyck et al. 2012; Li et al. 2018). Inorganic N of cattle feedlot manure is around 40% of TN (Eghball et al. 2002), so RM and BM had approximately 40 kg available N ha^{-1} with no available N in the BC (under detection limit) and CT (<0.1 kg available N ha^{-1}) applications.

A feedlot study conducted at Lethbridge Research and Development Centre of Agriculture and Agri-Food Canada (AAFC) near Lethbridge, AB, provided the various manures. Eighty yearling steers were used in a 235-day feeding trial (Terry et al. 2020). The RM came from a regular western cattle diet consisting of 60% barley silage, 85% barley grain, and 5% mineral supplement (Terry et al. 2020), and the BM came from the western diet supplemented with 2% biochar (dry-matter basis). Southern yellow pine (*Pinus echinata*) biochar was used in the AAFC trials (BM manure) as well as the BC plots (BC5, BC10, RM + BC5, BM + BC10, and RM + BC10), with the properties presented in Table 1. Natural Carbon, Inc. (Greenwood Village, CO) recommended and provided this biochar for the feedlot and field trials for its patented post-pyrolysis treatment step in a front-end biomass pyrolysis (<650 ◦C) (Romero et al. 2021a).

5.3.2 Soil and treatment analyses

Surface soil samples (0−15 cm) were collected from the Breton Research Station in Fall 2020, one year after amendment. Soil temperature and moisture content data were collected using RT1 and EC5 sensors, respectively, with EM50 data loggers (METER, Pullman, WA, USA). Samples were air-dried for 48 hours, sieved <2 mm, then ground with a Ball Mill MM200 (Brinkmann Retsch, Haan, Germany) to be stored in labelled 20-mL scintillation vials for Total C and N analysis with a dry combustion technique using a Thermo Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (Sparks et al. 2020).

Thermal stability was measured using Differential Scanning Calorimetry (DSC; STA 6000, Perkin Elmer, Waltham, MA USA) on approximately 20 mg of dried soil. The overall soil organic matter stability was calculated by integrating the heat of combustion curves and creating a ratio of values for the thermally labile region (150 °C-410 °C) and the thermally recalcitrant region (410 °C-725 °C) (Fernández et al. 2011). The soil pH and EC protocols utilized a 1:2 (w:v) soil to water extract ratio that was shaken for 1 hour, filtered, and then measured with FE20 and FE30 Meters (Mettler Toledo Columbus, OH, USA).

Microbial biomass utilized 500 g soil samples were incubated at 20 °C for 72 hours (Solaiman 2007). Post-incubation, 20 g samples (dry wt eq) fumigated with 30 ml chloroform to a 50 ml glass beaker were compared to another set of 20 g samples (dry wt eq). These sets were extracted with a 0.5 M K_2SO_4 solution in a 1:2 soil: extract ratio (w:v), shaken at 250 rpm for 1 hour, filtered using Whatman No. 42 filter paper, and then analyzed using a Shimadzu Total Organic Carbon Analyzer (Shimadzu Corporation Kyoto, Japan).

NPKS and microbial biomass were measured using sub-samples incubated at 20 °C for 72 hours (Solaiman 2007). Ion-exchange membranes were extracted with 15 mL of 0.5 M HCl and analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Thermo iCAP6300 Duo, Thermo Fisher Scientific, Waltham, MA, USA) to measure P, K, and S values (Qian et al. 1992). The Salicylate-Hypochlorite method (Bower and Holm-Hansen 1980) measured NH_4^+ and NO_3-N and NO_2-N were measured using the Hydrazine reduction method (Kamphake et al. 1967) on a colorimetric autoanalyzer (Gallery Plus, Thermo Fisher Scientific, Waltham, MA, USA).

5.3.3 Soil Fractionation

Samples from each treatment were divided into particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions, modifying Moni et al. (2012) and Elliott et al. (1984) protocols (outlined in Fig. 5.1). Soil samples were sieved at 2 mm, and 5 g of this sieved soil was oven-dried at 60 °C for 24 h to account for moisture content.

For wet aggregate stability, 20 g (dry wt) of soil was placed in a 53 µm sieve and soaked in DI water for 10 minutes. Then, by raising and lowering by hand, the aggregates were broken apart at a rate of 50x/minutes for 2 minutes. The fraction < 53 µm that passed through the sieve represented the non-aggregated MAOM, and the other > 53 µm was backwashed into a beaker for size fractionation.

For size fractionation, 200 ml deionized water was added to the aggregated MAOM and shaken overnight with eight glass beads (5 mm diameter) in a 250 mL bottle to disrupt the aggregates. Samples were wet-sieved the next day with 53 μm sieves. The fraction < 53 μm represented the aggregated MAOM, and > 53 μm represented POM. These POM, aggregated MAOM, and non-aggregated MAOM fractions were dried at 50 °C for 48 hours and further weighed to calculate their relative distribution. We subtracted the POM weights from the bulk samples to compare the non-aggregated MAOM fraction to the aggregated MAOM fraction.

The three fractions were then ground with a Ball Mill MM200 (Brinkmann Retsch, Haan, Germany) and stored in a 20 mL scintillation vial for analysis. To measure total C and N, a Thermo Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (Sparks et al. 2020) was utilized.

5.3.4 Diffuse Reflectance Infrared Fourier Transform Spectroscopy Analysis

Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) was used to analyze the soil organic matter quality of bulk soil and all fractions. The samples were placed in aluminum sample cups on an AutoDiff autosampler (PIKE Technologies, Madison, WI) and scanned for DRIFT spectra. The spectra were collected at 0.4 cm^{-1} resolution from 4000-450 cm^{-1} wavelengths, with 24 scans per sample being averaged to reduce background noise (Agilent Cary 660 FTIR Analyzer (Agilent Technologies, USA). The spectra were then baseline corrected and smoothed to identify the peaks (Kimetu and Lehmann 2010; Waldrip et al. 2014). An organic-free mineral matrix spectra was subtracted from the total soil spectra following a modified protocol by James et al. (2019) and Kaiser et al. (2002). To remove organic matter, samples were shaken in 6% sodium hypochlorite (NaClO) solution, adjusted to a pH of 8 with concentrated hydrochloric acid (HCl) overnight, and rinsed ten times with ultra-pure water (Milli-Q; Labcono Water Pro PS).

5.3.5 Statistical analyses

Prior to statistical analysis, Shapiro and Bartlett/Levene tests confirmed assumptions of normality of distribution and homogeneity of variance of the residuals (Logan 2011). Then, log or sqrt transformations were applied to the response variables if models did not meet these assumptions (Logan 2011). If transformations were not able to meet the assumptions, non-parametric analyses were used (Kruskal–Wallis test for violation of normality). The resulting transformed data were used for statistical analysis to assess treatment effects, but the untransformed data were used to calculate mean values and standard errors.

To measure the soil and manure treatment properties, a one-way analysis of variance (ANOVA, $P = 0.05$) was used. A blocked ANOVA, where treatment is a fixed factor and block is a random factor, was used if block was a significant factor ($P = 0.05$). If not, a linear one-way ANOVA was used instead for POM and MAOM OC, TN, and C/N. Differences were detected among treatments at $P < 0.05$. If $P < 0.05$, differences between treatments were analyzed using a Tukey-Kramer test. Relationships between the soil properties (temperature, moisture, and biogeochemical values) and fraction organic carbon, nitrogen, and C/N ratios were examined using Spearman's rank correlations. Soil temperature was correlated using total days greater than 15 °C and soil moisture was correlated using total days greater than 30% volumetric water content during growing season (Apr – Sept) as described in 4.3.5.

Absorbance peaks of 3400 cm^{-1} was selected to represent O-H stretching and amide and amine groups, 2930 cm^{-1} to represent aliphatic, 1650 cm^{-1} to represent C=C aromatics, and 1515 cm^{-1} to represent the polysaccharide group as they had the highest loadings (James et al. 2019; Peng et al. 2011). Principal component analysis (PCA) was used to assess the DRIFT spectra wavelengths to soil treatments (Fig. 5.3a) and fractions (Fig. 5.3b), which was then separated to compare manure (Fig 5.4), biochar (Fig. 5.5), and biochar manure combinations (Fig. 5.6) respective to the CT. These statistical calculations are performed using R v. 4.2.1 (R Core Team, 2020) (Logan 2011).

5.4 Results

5.4.1 Fractions

The percent of C in aggregated MAOM decreased from 73.9 to 63.7% with increasing biochar while it increased in non-aggregated MAOM from 26.1 to 37.6% with increasing biochar (data not shown). The RM and BM addition with BC10 resulted in the highest OC in all fractions, with CT, RM, and BM having the lowest OC content across all fractions (Table 5.1). RM+BC10 had the highest OC in the non-aggregated MAOM and POM when compared per kg of soil ($P < 0.001$; Table 5.2). BM+BC10 resulted in the highest OC in aggregated (29.4 g kg^{-1}) and non-aggregated MAOM (35.5 g kg^{-1}), yet RM+BC10 had the highest OC in the POM (30.8 g kg^{-1}). There were some differences ($P < 0.05$) amongst treatments for TN in the respective fractions (Table 5.1), but not per kg of soil (Table 5.2; $P < 0.05$). Non-aggregated MAOM had the highest ($P < 0.05$) values in the BM+BC10 (2.81 g kg^{-1}) and aggregated MAM had the highest ($P < 0.05$) values in RM+BC10 (2.17 g kg^{-1}) in the respective fractions. The C/N ratios were highest ($P < 0.05$) in RM+BC10 for aggregated MAOM & POM (Table 5.1), but not different in non-aggregated MAOM (Table 5.1). When comparing per kg of soil, RM+BC10 had the highest POM C/N ratios ($P < 0.05$) but were not different ($P > 0.05$) in the MAOM fractions (Table 5.2).

The NPKS, temperature, and TN values were not significantly correlated to the fractions except for S for aggregated MAOM OC and TN (Table 5.3). Both MBC and MBN were strongly correlated ($P < 0.001$) to aggregated MAOM OC and TN. The TC was strongly correlated ($P < 0.001$) to POM and non-aggregated MAOM OC and C/N. The pH was negatively, but mostly significantly ($P < 0.05$), correlated to all fractions, particularly POM. Temperature was positively corelated ($P > 0.05$), to all fractions except for aggregated and non-aggregated MAOM OC and C/N ratio. Moisture was negatively correlated ($P > 0.05$) to all fractions except for non-aggregated MAOM TN and was not strongly correlated (0.055).

5.4.2 Diffuse Reflectance Infrared Fourier Transform Spectroscopy

The results of the DRIFT analysis are presented in Fig. 5.2 and corresponding ANOVAs of selected wavelengths in Tables 5.8-5.11. The region 2500–2000 cm^{-1} did not show any peaks and was omitted from the discussion. The 3400 cm^{-1} wavelength is attributed to labile N-H and O-H stretching in amine and amide groups (Parikh et al. 2014; Tatzber et al. 2007), which was greatest in BM+BC5 in the bulk soil ($P < 0.001$; Table 5.4), CT in the POM ($P < 0.001$; Table 5.5), and BC5 in the aggregated ($P < 0.01$; Table 5.6) and non-aggregated MAOM ($P < 0.001$; Table 5.7). Less aromatic aliphatic H and N-H stretching around 2930 cm^{-1} (Waldrip et al. 2014) was greatest in BM+BC5 in the bulk soil and non-aggregated MAOM ($P < 0.001$; Table 5.4 & 5.11), CT in the POM fractions ($P < 0.001$; Table 5.5), and BC5 in the aggregated MAOM ($P < 0.01$; Table 5.5). Recalcitrant aromatic C=C (1650 cm^{-1}) was greatest in the RM+BC10 in the bulk fraction ($P < 0.001$; Table 5.4), the CT treatment in the POM fraction ($P < 0.01$; Table 5.5), BC5 in the aggregated MAOM ($P < 0.01$; Table 5.6), and BC5 in the non-aggregated MAOM ($P < 0.001$; Table 5.7). Finally, the labile 1515 cm^{-1} region that corresponds to ester C-O-C and polysaccharides (Parikh et al. 2014) showed minimal peaks in all treatments and fractions (Fig. 5.2). Polysaccharides wavelengths were greatest RM+BC10 for bulk ($P < 0.001$; Table 5.4) fraction, CT for POM ($P < 0.01$; Table 5.5) and non-aggregated fractions ($P < 0.001$; 5.11), and BC5 for the aggregated MAOM (Table 5.6).

The 899 cm^{-1} wavelength was assigned to the C-O-C stretching of labile amorphous cellulose (Bekiaris et al. 2015), where there are small peaks in non-aggregated MAOM and some bulk treatments (Fig. 5.2). The 900-750 cm^{-1} related to C-H bending aromatic CH out-of-plane deformation (Fultz et al. 2014; Wu et al. 2012) varied greatly between treatments for all fractions except the aggregated MAOM (Fig. 5.2). Similar to Dong et al. (2016), one noticeable trend is that as size decreased, so did differences amongst treatments from 3600 to 1280 cm^{-1} (Fig. 5.2).

5.4.3 Principal Component Analysis

When comparing all treatments, The PC1 axis explained 41.11% and the PC2 axis explained 26.7% of the variation (Fig. 5.3a) and three fractions (Fig 5.3b). The greatest clustering occurred in the RM, BC10, and BM+BC10 which were influenced by the polysaccharide wavelength. The BC5 and RM+BC10 were influenced by the C/N ratio and nitrogen, and the CT with the aromatic and aliphatic wavelengths. Finally, the BM had the most variation, primarily contributed by aromatic, aliphatic, and carboxyl wavelengths. There is a separation between aggregated MAOM and POM, with the aggregated grouped by carbon and nitrogen, and the POM by the C/N ratio and aromatic wavelengths. The non-aggregated MAOM was the least varied and was dictated by all wavelengths and variables, but mostly the aromatic.

When examining the manure treatment only, PC1 axis explained 47.21% and the PC2 axis explained 30.84% of the variation (Fig. 5.4). The BM had the greatest variation and was directed by its C/N, while RM had the smallest variation and was influenced by its elemental carbon and nitrogen content (Fig. 5.4a). The aggregated MAOM was influenced by elemental carbon and nitrogen content, the POM by the C/N and polysaccharide and amine and amide wavelengths, and the non-aggregated MAOM was in between the two (Fig. 5.5b).

In examining the biochar treatments only, PC1 axis explained 47.72% and the PC2 axis explained 20.86% of the variation (Fig. 5.5). The BC5 was strongly influenced by C/N ratio and amine and amide wavelengths and the BC10 was mostly influenced by the polysaccharide wavelength, C/N ratio, and nitrogen (Fig. 5.5a). The BC5 had the largest variation and CT had the smallest variation, primarily pulled by carbon and amine and amide wavelengths (Fig. 5.5a). The aggregated MAOM distribution was mostly dictated by carbon and nitrogen, POM by C/N and amine and amide wavelengths, and the non-aggregated MAOM between these (Fig. 5.5b).

Finally, PCA of the biochar and manure treatments only revealed that PC1 axis explained 46.04% and the PC2 axis explained 25.18% of the variation (Fig. 5.6). There were similar eclipses between BM+BC10 and BM+BC5, pulling towards the nitrogen and polysaccharide wavelength and RM+BC10 pulling towards the C/N (Fig. 5.6a). The aggregated MAOM was strongly clustered and influenced by nitrogen, POM by C/N and aromatic wavelengths, and the non-aggregated MAOM was in the middle of the two (Fig. 5.6b).

5.5 Discussion

5.5.1 Fractions

There were higher amounts of soil OC (Table 5.1) from the biochar (BC5 and BC10) and manure (BM and RM) treatments compared to the CT, supporting the hypothesis of C accumulation due to biochar and manure additions. This finding matches Guan et al. (2019) who noted greater OC in charred vs. uncharred maize applications due to pyrogenic C in charred amendments. Likewise, the higher C/N ratio from the BC treatments (Table 3.5; mainly seen in BC10 & Table 5.1; mainly seen in RM+BC10) from the biochar amendment would indicate greater recalcitrance than manures or CT.

There were no statistical differences in OC, TN, and C/N fractions between RM and BM (Table 5.2), except for the OC of the non-aggregated MAOM (Table 5.2). A lack of statistical differences in the bulk soil microbial biomass and physio-chemical properties between the manures (Table 3.5) was present. Finally, manure accumulated in aggregated MAOM, yet BM+BC10 and RM+BC10 had more OC in the non-aggregated MAOM (Table 5.1). This finding differed from the hypothesis of biochar accumulation in the aggregated fractions. All amendments, nonetheless, resulted in an increase in TC (Table 3.5) compared to the CT. Similarly, TN was greatest in the MAOM fractions compared to POM in all treatments, but did not vary amongst treatments, except for aggregated fractions (Table 5.1 & 5.6). TN was not significantly correlated to any variable (OC, TN, or C/N) for any fractions (Table 5.3) and matched a previous incubation's finding of no statistical changes in N mineralization (Weber et al. 2021a).

There was a general lack of differences between RM and BM that suggests that the two manures are equally labile and should result in similar changes when applied to the soil, rejecting the hypothesis of greater stability from biochar-manure. Although there were statistical differences within the pH and TC of manures (Table 5.2), they were not different enough to result in changes in thermal stability, as seen in the OM ratio (0.18 and 9.21; Table 5.2). Results from a greenhouse gas emission incubation experiment by Romero et al. (2021b) found similar emission rates between BM and RM. Finally, an incubation (Weber et al. 2021a) found similar N and C mineralization rates between BM and RM.

Synergistic effects between manure and biochar include the binding of BC to manure pores (Lehmann and Joseph 2009), explain the highest amounts of OC in the aggregated fraction in the manures (RM or BM) + BC treatments followed by RM and BC alone ($P < 0.05$; Table 5.1 and 5.6). The OM coating on the biochar has been found to influence co-composted biochar (Hagemann et al. 2017; Lehmann and Joseph 2009). Although this coating influence may be limited as there was a lack of differences amongst treatments in the OC aggregated fraction in terms of kg of soil ($P > 0.05$; Table 5.2). One reason for this difference in per kg of the fraction (Table 5.1) is that the total mineral surfaces for SOM adsorption are a confining factor for MAOM contribution, suggesting that BC applications alone may be limited to the spaces within the soil (Dynarski et al. 2020).

The limitation to biochar mineral surfaces is demonstrated in treatments with BC5 (BM+BC5 and BC5 had greater aggregated compared to non-aggregated MAOM) compared to treatments with BC10 (BM+BC10, RM+BC10, and BC10 had higher non-aggregated compared to aggregated-MAOM) ($P < 0.05$; Table 5.1 & Table 5.2). Since BM+BC5 had greater absorbance than BM+BC10 in aggregated-MAOM fractions ($P < 0.05$, Table 5.6, Fig. 5.2c) at 1650 cm^{-1} and pull towards the aromatic wavelength (Fig. 5.6a), the amendments mixed at a higher BC rate in the aggregated fraction may be limited by pore size for long-term stability. Additionally, TC was highly statistically significant in the correlation between POM and non-aggregated OC and C/N ratios (Table 5.3).

Furthermore, the addition of biochar did not change the aggregation of MAOM (data not shown), which did not match the hypothesis of greater aggregation within the biochar and biochar-manure treatments. This lack of change was also seen when comparing the OC, TN, and C/N as kg of soil, where there were no significant differences amongst treatments in aggregated MAOM (Table 5.2). Because manure contains microorganisms and plant or animal-derived polysaccharides, it was hypothesized to increase aggregation (Chen et al. 2020; Li et al. 2018), especially in clay-dominated soils (Dong et al. 2016). The results differed from various studies (Du et al. 2017; Pituello et al. 2018) that suggest that biochar surface hydrophobic–hydrophilic interfaces with clay minerals increase aggregation. This lack of aggregate differences instead brings us to a similar conclusion as Lehmann et al. (2001), that organic matter and aggregation were not strongly related at the MAOM level.

The results were consistent with other studies (Dong et al. 2016; Zhang et al. 2015) that found little or no change in aggregates. In the aggregated MAOM fraction (Table 5.6 & Fig. 5.2c) across the entire spectra (4000 to 450 cm^{-1}) there were fewer differences amongst treatments compared to other fractions. Additionally, Demyan et al. (2013) found in a FTIR-EGA (evolved gas analysis via Fourier transform infrared spectroscopy) study, NaOCl treatment can alter the nature of the MAOM, and labile peaks evolved at relatively low temperature, suggesting a focus on C/N results when analyzing the MAOM fractions.

Although increased root activity from biochar additions has been found to improve aggregation (Du et al. 2017), this was not observed likely due to the below-average yield across the region (Alberta Agriculture 2020) as a result of higher than normal precipitation in 2020 – ranging from 56-71 total days greater than 30% volumetric water content ($P > 0.05$; Table 5.12). The rainy season may have negated the ability of biochar to improve water retention capacity and OM crosslinking, which would have improved aggregation (Dong et al. 2016). This is seen in the correlation where moisture was negatively correlated ($P > 0.05$; Table 5.3) to all fractions except for non-aggregated MAOM TN. Furthermore, although there were no significant changes in microbial biomass (Table 3.5), there was a positive and statistically significant correlation between microbial biomass and aggregated MAOM (Table 5.3), emphasizing the importance of microbial activity in aggregated fractions. Conversely, the POM and non-aggregated MAOM fractions were correlated to chemical and environmental properties, such as TC and pH (P <0.05; Table 5.3).

5.5.2 Soil organic matter stability

The DRIFT analysis showed higher (P <0.001) absorbance in the aromatic region (1650 cm^{-1}) from the BM in the bulk soils (Table 5.4 & Fig. 5.2a) compared to the RM, suggesting that differences, albeit small, are present. Because biochar was only applied at 2% dry weight to the cattle, Terry et al. (2019) reported no differences in rumen fermentation or digestibility between the biochar and regular diet; therefore, these differences do not impact overall stability when applied given their large and overlapping eclipses (Fig. 5.4a).

There were differences between the two manure treatments in the corresponding PCA (Fig. 5.4a), where BM had the greatest variation with a contribution from all wavelengths, and the RM had the smallest variation with a contribution from carbon, nitrogen, and C/N Ratio. This difference in wavelengths came from the increased aromatic-C characteristic of BM (Romero et al. 2021a), as the amine and amine group (3400 cm^{-1}) that is easily available for microorganisms (Tatzber et al. 2007) primarily influenced BC5 (Fig. 5.5a). there was a greater pull towards labile oxygen and energy-rich polysaccharides and amine/amide (James et al. 2019) from the CT and RM compared to BM, which had a greater pull towards C/N and recalcitrant aromatic and aliphatics (Fig. 5.4a). One possibility is that electroconductivity has been found to increase aggregate stability at higher rates (Dong et al. 2016). While RM and BM electroconductivity (EC) did not statistically differ (Romero et al. 2021a), there were significant correlations between EC and POM and non-aggregated MAOM C/N (Table 5.3).

Another important consideration is the RM vs. BM additions to biochar, given their difference in eclipse locations (Fig. 5.6a). The manure (RM and BM) and biochars (BC5 and BC10) suggested synergistic effects on one another as there are changes in OC, TN, and C/N between the RM+BC10 and BM+BC10 ($P < 0.05$; Table 5.1 & 5.6) and large differences in PCA eclipses from BM+biochar and RM+biochar treatments (Fig. 5.6a). The similar eclipses between BM+BC10 and BM+BC5, pulling towards the polysaccharide wavelength (which is preferentially utilized by soil microbes (Parikh et al. 2014); Fig. 5.6a), suggest that the differences between the different biochar rates are minimal.

The RM+BC10 has a greater absorbance at aromatic 1650 cm^{-1} in the aggregated MAOM than BM+BC10 ($P < 0.05$, Table 5.6, Fig. 5.2c), which may be linked to temporary aggregate stability (Chen et al. 2020; Christensen 2001). This means RM+BC10 (30.8 g kg^{-1} OC) had the highest lability, followed by the BC5 (20.8 g kg^{-1} OC) and BC10 (21.4 g kg^{-1} OC; $P < 0.05$; Table 5.1) in the POM fraction. The RM+BC10 also demonstrated the highest OC in the non-aggregated MAOM ($P < 0.05$; 7.38 g kg^{-1}) and POM ($P < 0.05$; 11.09 g kg^{-1}) in the per kg of soil comparisons (Table 5.2) and BM+BC10 had the highest (albeit not significantly different; $P > 0.05$) OC (Table 5.1 & 5.6) and C/N in the aggregated MAOM fractions (Table 5.2). The RM+BC10 had a greater absorbance in the POM and non-aggregated MAOM OC and C/N (Table 5.2), supporting greater recalcitrance in those fractions.

While there is no difference between MAOM fractions, there was a notable separation between aggregated MAOM and POM in the PCA comparison of all treatments, with the non-aggregated MAOM between the other two fractions. (Fig. 5.3-5.6b). The carbon and nitrogen contributed to the aggregated MAOM eclipse in all treatments except when comparing the biochar and manure combinations (Fig. 5.6b), where it is only nitrogen. The POM is contributed to amine and amide groups in the manure (Fig. 5.4a) and biochar (Fig. 5.5a) comparisons, suggesting its labile nature is stronger (Tatzber et al. 2007) when the biochar or manure are applied alone. Polysaccharide-C is a result of direct deposit of plant, animal, and microbial deposits (Solomon et al. 2007) and a strong contributor to POM and non-aggregated MAOM (Fig. 5.3-5.6b). This relationship between fraction and polysaccharide presence shows a clear occurrence of labile partly decomposed plant residues (Lavallee et al. 2020).

These differences in the POM fraction were minimal and are hypothesized to change over time. A study by Singh et al. (2016) found that aromatic to aliphatic ratios changed after a 6 month incubation, but does depend on biochar and soil type. Similar to the observations of Lavallee et al. (2020); Rumpel et al. (2000); Zhang et al. (2015), POM had 2-3x higher C/N compared to MAOM (Table 5.1 & 5.6), and MAOM and is therefore hypothesized to be utilized more efficiently (less CO_2 respiration due to its lower C/N ratio and chemical complexity).

Finally, the type of biochar utilized (Chen et al. 2020), soil texture (Parikh et al. 2014), and ecosystem (Cotrufo et al. 2019) can change stability rates, so future studies should investigate various biochars in different environments. In addition, possible changes between the type of biochar, time, temperature, and type of soil has been seen in FTIR analyses by Singh et al. (2016) comparison of aromatic (3050 cm^{-1}) to aliphatic (2950–2750 cm^{-1}) in the light fractions. Organo-mineral exchanges are reversible from external factors, such as roots or tillage, so future amendments and agricultural practices should consider their impact on SOM dynamics (Dynarski et al. 2020).

5.6 Conclusion

In conclusion, while there were minimal differences between RM and BM when applied alone, additions to BC showed greater differences in agricultural sustainability. The BM+BC10 was found to increase the long-term stability of sequestered carbon, resulting in the highest ($P < 0.05$) organic C in aggregated MAOM (29.4 g kg^{-1} fraction and 12.30 g kg^{-1} soil) and stronger pull towards to aromatic and aliphatic than RM+BC10. On the contrary, RM+BC10 had the highest ($P < 0.05$) OC allocation in the POM (30.8 g kg^{-1} fraction and 11.09 g kg^{-1} soil) than BM+BC10, suggesting its shorter-term stability. The BM+BC10 treatment combinations had greater aromatic C peaks than BM+BC5 as higher biochar rates retained greater aromatic C concentrations; however, but this additional BC is not essential based on PCA comparisons. There were no differences between aggregated and non-aggregated MAOM, but a clear separation between POM and aggregated MAOM, amongst treatments, showing the need to explore OM fractions in determining aromaticity. In summary, combinations of biochar-manure and biochar show retention of aromatic C, supporting long-term C sequestration potential in agricultural applications.

Table 5.1. Organic carbon, total nitrogen, and C/N ratio per kg of the respective fraction (means ±SE; n = 4).

Treatments	Aggregated MAOM			Non-Aggregated MAOM			POM		
	OC	**TN**	**C/N**	**OC**	**TN**	**C/N**	**OC**	**TN**	**C/N**
	(g kg^{-1} fraction)			**(g kg^{-1} fraction)**			**(g kg^{-1})**		
CT	21.8± 0.5^{c}	2.20 ±0.04^{b}	10.0±0.0^{ab}	17.6± 0.5^{c}	1.99±0.05	9.0± 0.1	10.3±1.2^{c}	0.90±0.10	12.7±0.2^{c}
RM	22.8± 0.6^{bc}	2.29±0.07^{ab}	10.0±0.1^{ab}	18.9± 0.6^{bc}	2.11±0.07	9.1± 0.1	11.1±0.8^{bc}	1.00±0.10	11.7±0.8^{c}
BM	23.0± 0.6^{bc}	2.25±0.06^{ab}	10.3±0.0^{ab}	18.3± 0.0^{c}	1.99±0.10	9.4± 0.2	11.3±1.2^{bc}	0.83±0.10	14.1±0.5^{c}
RM+BC10	29.2± 0.8^{a}	2.31 ±0.13^{ab}	12.9±0.6^{a}	31.0± 0.4^{ab}	2.17±0.11	14.8± 2.4	30.8±3.3^{a}	0.87±0.10	37.2±6.4^{a}
BC5	25.1± 1.8^{abc}	2.68±0.02^{ab}	9.5±0.2^{b}	23.3± 0.5^{abc}	1.91±0.13	12.2 ± 0.5	20.8±1.2^{abc}	0.85±0.10	25.7±2.2^{ab}
BM+BC5	27.3± 0.7^{ab}	2.61±0.06^{ab}	10.6±0.1^{ab}	22.8± 0.9^{abc}	2.14±0.11	10.8± 0.3	19.0±3.9^{bc}	0.82± 0.12	23.9±2.2^{b}
BC10	25.8± 1.6^{abc}	2.52±0.15^{ab}	11.0±0.2^{b}	26.0± 0.9^{abc}	1.96±0.09	13.2± 0.9	21.4±4.0^{abc}	0.84± 0.12	26.1±2.4^{ab}
BM+BC10	29.4± 2.2^{a}	2.81±0.11^{a}	10.7±0.8^{ab}	35.5± 1.0^{a}	2.15±0.08	16.4± 4.0	23.1±0.15^{ab}	1.01± 0.13	27.4±1.1^{ab}
***P*-Value**	<0.001***	0.012*	0.007**	0.003*	0.019*†	0.135	<0.001***	0.324	<0.001***

Note: Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.
Abbreviations: BC, black C; OM, organic matter; TC, total carbon; TN, total nitrogen. Significance: *P < 0.05; **P < 0.01; ***P < 0.001. Means followed by a different letter within a column are significantly different (P < 0.05). †Tukey's Honest Significant Difference (HSD) test did not show significant differences between treatments even though P<0.05.

Table 5.2. Organic carbon, total nitrogen, and C/N ratio per kg of soil (means ±SE; $n = 4$).

	Aggregated MAOM			Non-Aggregated MAOM			POM		
	OC	**TN**	**C/N**	**OC**	**TN**	**C/N**	**OC**	**TN**	**C/N**
Treatments	**(g kg^{-1} soil)**			**(g kg^{-1} soil)**			**(g kg^{-1} soil)**		
CT	10.31±0.77	1.04±0.08	9.91±0.02	2.93±0.39^{c}	0.33±0.04	8.84±0.12	3.72±0.31^{b}	0.32±0.02	11.7±0.30^{c}
RM	10.20±0.47	1.03±0.05	9.91±0.11	4.12±0.38^{bc}	0.46±0.04	8.96±0.09	3.70±0.33^{b}	0.34±0.04	11.0±0.74^{c}
BM	9.76±0.09	0.96±0.01	10.2±0.08	4.15±0.56^{bc}	0.45±0.06	9.17±0.18	3.93±0.28^{b}	0.29±0.01	13.6±0.41^{c}
RM+BC10	11.81±1.47	0.94±0.15	9.42±0.14	7.38±1.17^{a}	0.52±0.08	14.6±2.36	11.09±1.80^{a}	0.30±0.02	37.2±6.2^{a}
BC5	10.57±1.52	1.13±0.17	9.42±0.14	5.16±0.19^{ab}	0.42±0.01	12.2±0.49	7.49±0.37^{ab}	0.30±0.02	25.0±2.05^{ab}
BM+BC5	11.83±1.36	1.13±0.13	10.5±0.17	5.14±0.52^{ab}	0.48±0.05	10.6±0.35	6.31±1.05^{b}	0.27±0.03	22.5±1.83^{b}
BC10	11.05±1.08	1.08±0.11	10.2±0.28	6.14±0.72^{ab}	0.46±0.03	13.1±0.88	7.11±1.09^{ab}	0.28±0.03	25.2±2.14^{ab}
BM+BC10	12.30±1.45	1.17±0.07	10.5±0.75	6.33±0.19^{ab}	0.52±0.02	12.2±0.75	8.08±0.32^{ab}	0.31±0.02	26.2±1.08^{ab}
***P*-Value**	0.378	0.479	0.029*†	<0.001***	0.185	0.096	<0.001***	0.575	<0.001***

Note: Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.
Abbreviations: BC, black C; OM, organic matter; TC, total carbon; TN, total nitrogen. Significance: *P < 0.05; ***P < 0.001. Means followed by a different letter within a column are significantly different ($P < 0.05$). †Tukey's Honest Significant Difference (HSD) test did not show significant differences between treatments even though $P < 0.05$.

Table 5.3 Spearman's rank correlation coefficients of relationships between carbon, nitrogen, and C/N per kg of soil (Table 5.2) and soil properties (Table 3.5) and soil temperature and moisture (means ±SE; n = 4).

Variable		N	P	K	S	MBC	MBN	EC	pH†	TC	TN	Temp	Moisture
POM	OC	0.015	0.134	0.322	0.339	0.065	0.271	0.403	-0.586***	0.572***	0.132	0.260	-0.023
	TN	0.055	0.248	0.438	0.228	0.323	0.338	0.132	-0.211	-0.053	0.145	0.163	0.051
	C/N	-0.022	0.027	0.161	0.23	-0.007	0.204	0.357	-0.533**	0.602***	0.123	0.215	-0.051
Agg-MAOM	OC	0.206	-0.004	0.27	0.449*	0.600***	0.589***	0.283	-0.255	0.255	0.253	-0.041	-0.162
	TN	0.006	-0.048	0.231	0.363*	0.553**	0.559***	0.33	-0.187	0.22	0.326	0.018	-0.164
	C/N	0.375*	0.142	0.046	0.111	0.18	0.139	-0.059	-0.197	0.08	-0.058	-0.004	-0.119
Non-agg-MAOM	OC	0.036	-0.120	0.105	0.312	0.038	0.226	0.31	-0.546**	0.546**	0.073	-0.139	-0.003
	TN	0.027	-0.192	0.026	0.11	0.124	0.178	-0.11	-0.360*	0.328	0.182	0.044	0.055
	C/N	0.004	0.025	0.099	0.303	0.034	0.255	0.520**	-0.460**	0.558**	0.083	-0.277	-0.117

Note: Abbreviations: EC, electroconductivity; K, exchangeable potassium; N, exchangeable nitrogen; P, exchangeable phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; S, exchangeable sulfur; TC, total carbon; TN, total nitrogen; OM, organic matter.

†Soil pH was measured in an 1:5 soil:water (v:v) ratio. Means followed by a common letter within a column are not significantly different (P < 0.05). Significance: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Table 5.4. Key bulk DRIFT wavelengths result of one-way ANOVAs (means ±SE; n = 3).

Wavelength	3400 Amine/Amide	2930 Aliphatic	1650 Aromatic	1515 Polysaccharide
Treatments	(a.u.)			
CT	0.146 ± 0.001^{g}	0.058 ± 0.001^{h}	0.355 ± 0.001^{c}	0.341 ± 0.001^{b}
RM	0.196 ± 0.001^{d}	0.147 ± 0.001^{b}	0.314 ± 0.001^{f}	0.217 ± 0.001^{f}
BM	0.223 ± 0.001^{b}	0.121 ± 0.001^{d}	0.395 ± 0.001^{b}	0.323 ± 0.001^{c}
RM+BC10	0.221 ± 0.001^{c}	0.133 ± 0.001^{c}	0.435 ± 0.001^{a}	0.432 ± 0.001^{a}
BC5	0.177 ± 0.001^{e}	0.089 ± 0.001^{f}	0.343 ± 0.001^{d}	0.275 ± 0.001^{d}
BM+BC5	0.263 ± 0.001^{a}	0.217 ± 0.001^{a}	0.316 ± 0.001^{f}	0.152 ± 0.001^{g}
BC10	0.167 ± 0.001^{f}	0.101 ± 0.001^{e}	0.325 ± 0.001^{e}	0.264 ± 0.001^{e}
BM+BC10	0.134 ± 0.001^{h}	0.070 ± 0.001^{g}	0.241 ± 0.001^{g}	0.143 ± 0.001^{h}
***P*-Value**	<.0001***	<.0001***	<.0001***	<.0001***

Note: Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Means followed by a common letter within a column are not significantly different (P < 0.05). Significance: ***, P < 0.001.

Table 5.5. Key POM DRIFT wavelengths result of one-way ANOVAs (means ±SE; n = 3).

Wavelength	3400 Amine/Amide	2930 Aliphatic	1650 Aromatic	1515 Polysaccharide
Treatments	(a.u.)			
CT	0.326±0.001^{a}	0.342±0.001^{a}	0.361±0.001^{a}	0.317±0.001^{a}
RM	0.227±0.001^{d}	0.251±0.001^{e}	0.260±0.001^{e}	0.209±0.001^{c}
BM	0.122±0.001^{f}	0.141±0.001^{g}	0.156±0.001^{g}	0.167±0.001^{e}
RM+BC10	0.190±0.001^{e}	0.198±0.001^{f}	0.215±0.001^{f}	0.161±0.001^{f}
BC5	0.227±0.001^{d}	0.254±0.001^{d}	0.260±0.001^{e}	0.218±0.001^{b}
BM+BC5	0.275±0.001^{b}	0.319±0.001^{b}	0.294±0.001^{b}	0.204±0.001^{c}
BC10	0.268±0.001^{c}	0.304±0.001^{c}	0.291±0.001^{c}	0.206±0.001^{c}
BM+BC10	0.267±0.001^{c}	0.303±0.001^{c}	0.286±0.001^{d}	0.198±0.001^{d}
***P*-Value**	<.0001***	<.0001***	0.002**	<.0001***

Note: Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Means followed by a common letter within a column are not significantly different ($P < 0.05$). Significance: **, $P < 0.01$; ***, $P < 0.001$.

Table 5.6. Key aggregated MAOM DRIFT wavelengths result of one-way ANOVAs (means ±SE; *n* = 3).

Wavelength	3400 Amine/Amide	2930 Aliphatic	1650 Aromatic	1515 Polysaccharide
Treatments	(a.u.)			
CT	0.204±0.001^{d}	0.253±0.001^{e}	0.244±0.001^{d}	0.186±0.001^{g}
RM	0.227±0.001^{b}	0.262±0.001^{d}	0.282±0.001^{b}	0.203±0.001^{d}
BM	0.202±0.001^{e}	0.273±0.001^{c}	0.247±0.001^{d}	0.191±0.001^{ef}
RM+BC10	0.204±0.001^{d}	0.264±0.001^{d}	0.285±0.001^{b}	0.246±0.001^{b}
BC5	0.251±0.001^{a}	0.311±0.001^{a}	0.303±0.001^{a}	0.255±0.001^{a}
BM+BC5	0.220±0.001^{c}	0.294±0.001^{b}	0.273±0.001^{c}	0.226±0.001^{c}
BC10	0.181±0.001^{g}	0.231±0.001^{g}	0.244±0.001^{d}	0.192±0.001^{e}
BM+BC10	0.185±0.001^{f}	0.249±0.001^{f}	0.227±0.001^{e}	0.190±0.001^{f}
***P*-Value**	0.002**	0.002**	<.0001***	<.0001***

Note: Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Means followed by a common letter within a column are not significantly different ($P < 0.05$). Significance: **, $P < 0.01$; ***, $P < 0.001$.

Table 5.7. Key non-aggregated MAOM DRIFT wavelengths result of one-way ANOVAs (means ±SE; n = 3).

Wavelength	3400 Amine/Amide	2930 Aliphatic	1650 Aromatic	1515 Polysaccharide
Treatments	(a.u.)			
CT	0.251 ± 0.001^{f}	0.202 ± 0.001^{g}	0.333 ± 0.001^{d}	0.228 ± 0.001^{a}
RM	0.252 ± 0.001^{e}	0.239 ± 0.001^{f}	0.308 ± 0.001^{g}	0.189 ± 0.001^{d}
BM	0.295 ± 0.001^{c}	0.285 ± 0.001^{c}	0.354 ± 0.001^{b}	0.228 ± 0.001^{a}
RM+BC10	0.238 ± 0.001^{h}	0.200 ± 0.001^{g}	0.295 ± 0.001^{h}	0.183 ± 0.001^{e}
BC5	0.338 ± 0.001^{a}	0.300 ± 0.001^{b}	0.372 ± 0.001^{a}	0.205 ± 0.001^{c}
BM+BC5	0.319 ± 0.001^{b}	0.321 ± 0.001^{a}	0.345 ± 0.001^{c}	0.212 ± 0.001^{b}
BC10	0.270 ± 0.001^{d}	0.269 ± 0.001^{d}	0.314 ± 0.001^{f}	0.179 ± 0.001^{f}
BM+BC10	0.247 ± 0.001^{g}	0.245 ± 0.001^{e}	0.328 ± 0.001^{e}	0.205 ± 0.001^{c}
***P*-Value**	<.0001***	<.0001***	<.0001***	<.0001***

Note: Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Means followed by a common letter within a column are not significantly different (P < 0.05). Significance: **, P < 0.01; ***, P < 0.001.

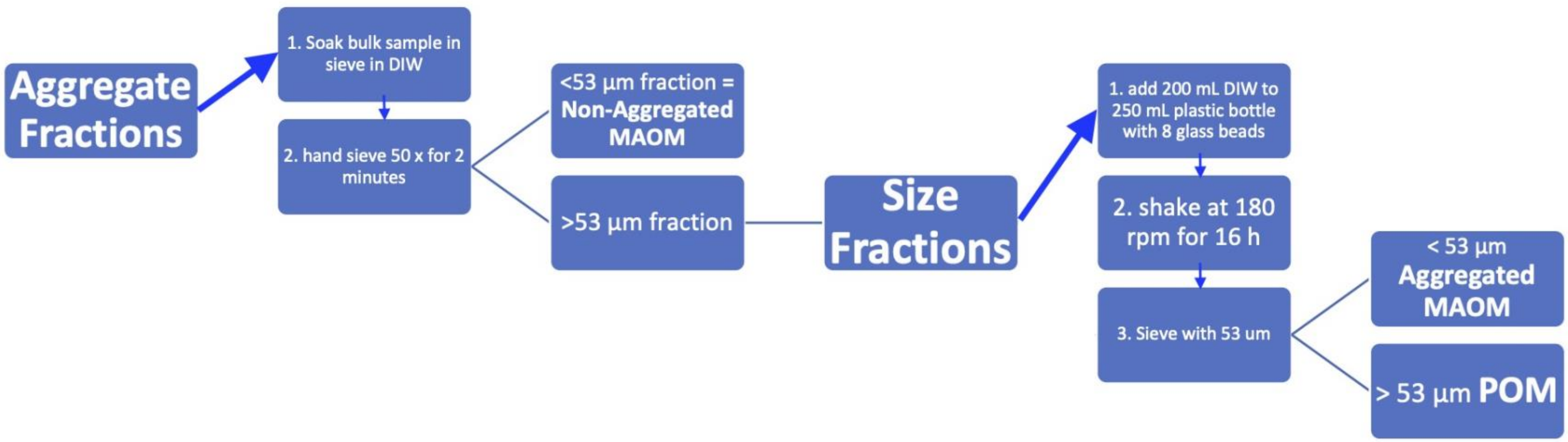

Figure 5.1 Organic matter fractionation schematic

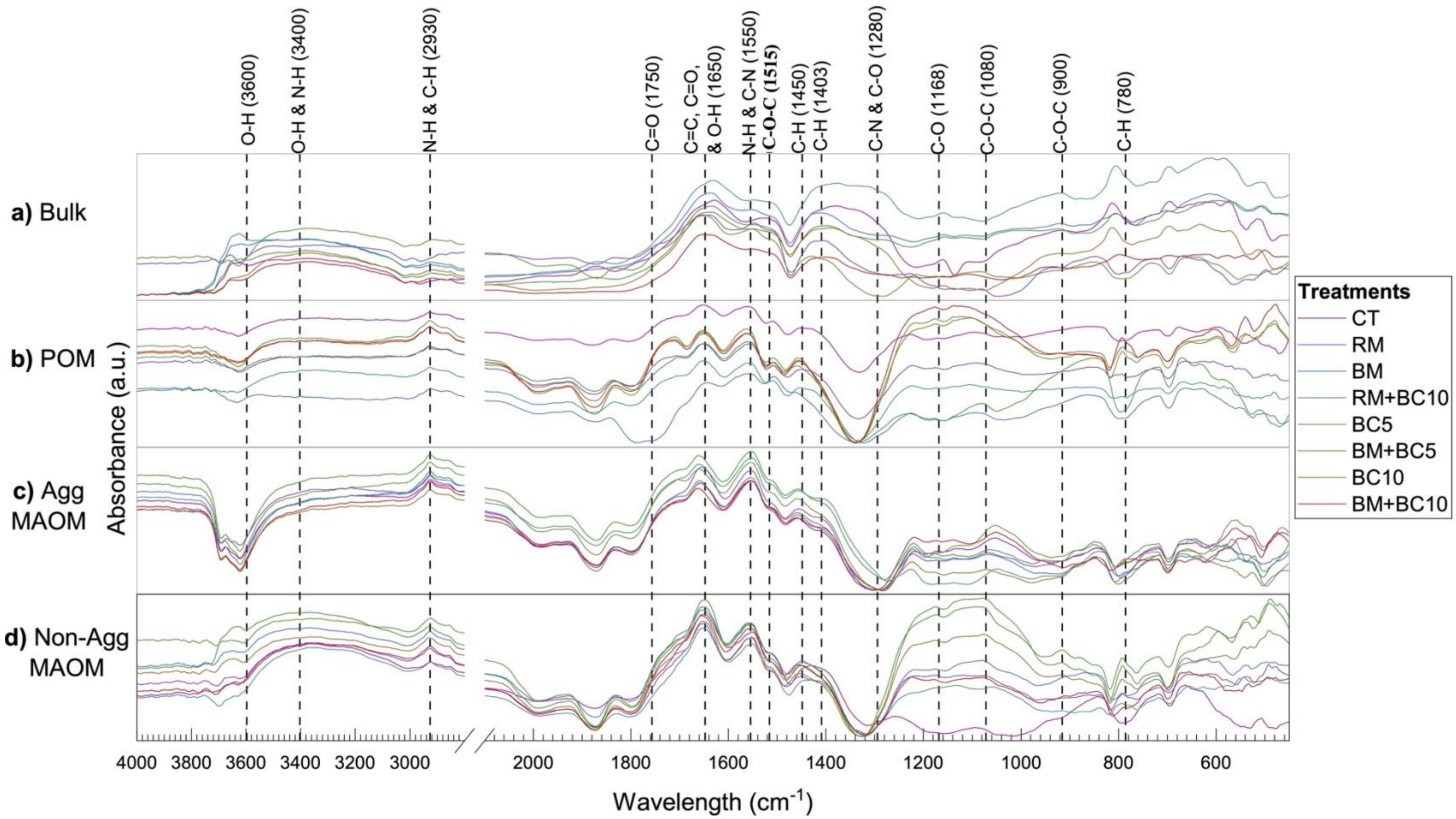

Figure 5.2. Diffuse reflectance infrared Fourier transform (DRIFT) spectra of soil a) bulk, b) particulate organic matter, c) aggregated mineral-associated organic matter, and d) non-aggregated mineral-associated organic matter fractions as affected by CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

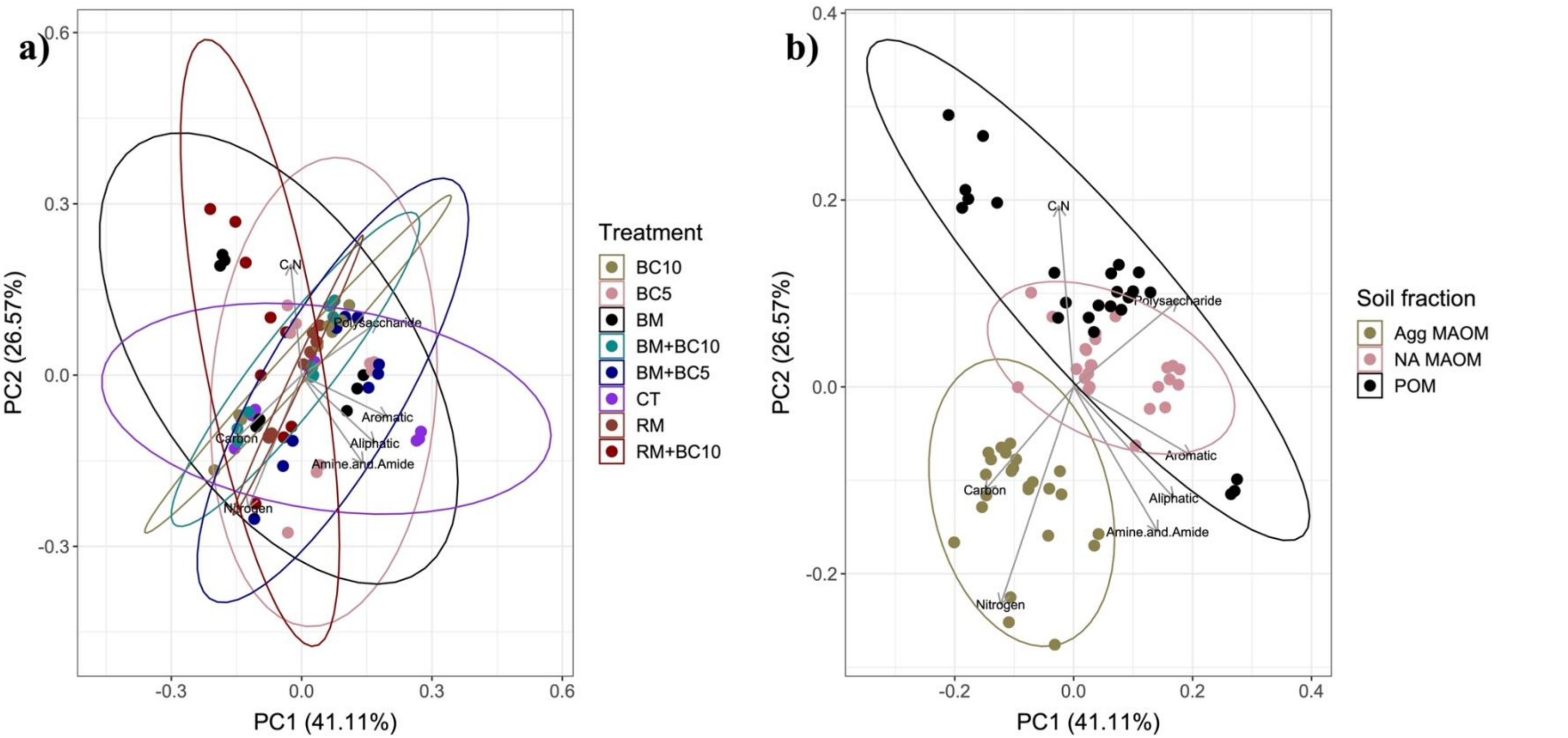

Figure 5.3. PCA biplot showing the influence of all (a) treatments and (b) fractions on wavelength and C and N as assessed by DRIFT spectroscopy. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

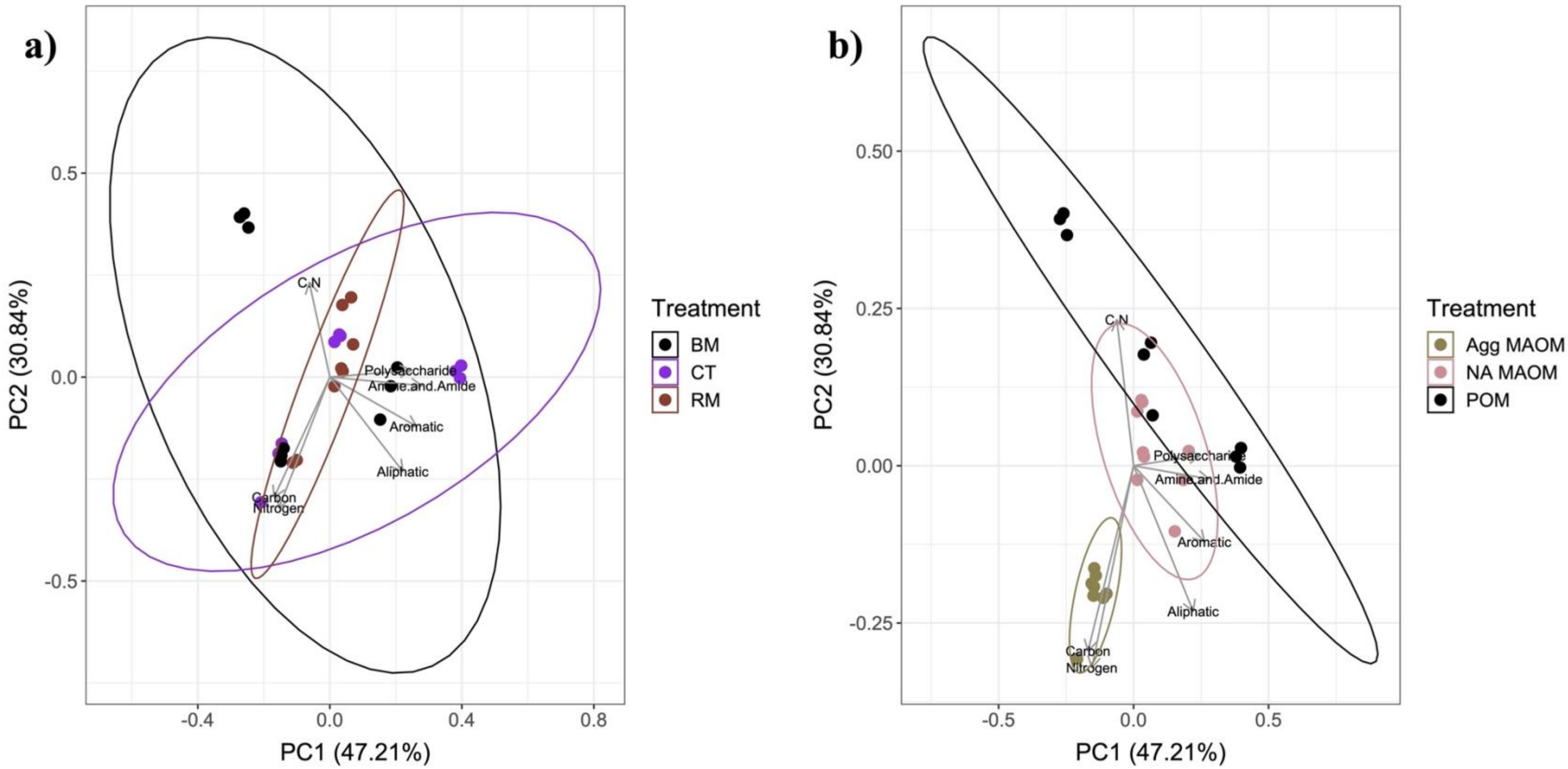

Figure 5.4. PCA biplot showing the influence of manure (a) treatments and (b) fractions on wavelength and C and N as assessed by DRIFT spectroscopy. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar.

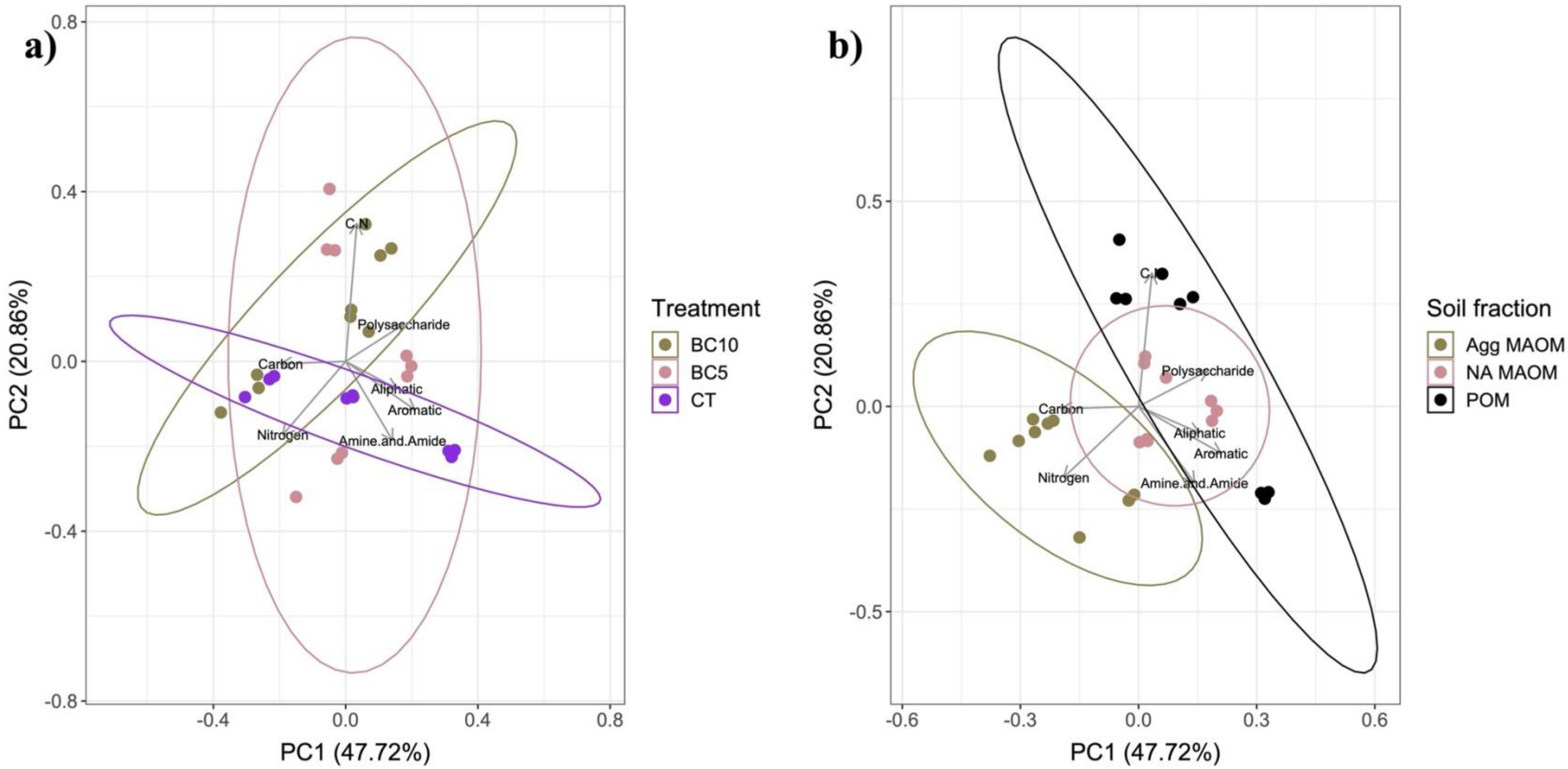

Figure 5.5. PCA biplot showing the influence of biochar (a) treatments and (b) fractions on wavelength and C and N as assessed by DRIFT spectroscopy. Treatments: CT, control; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

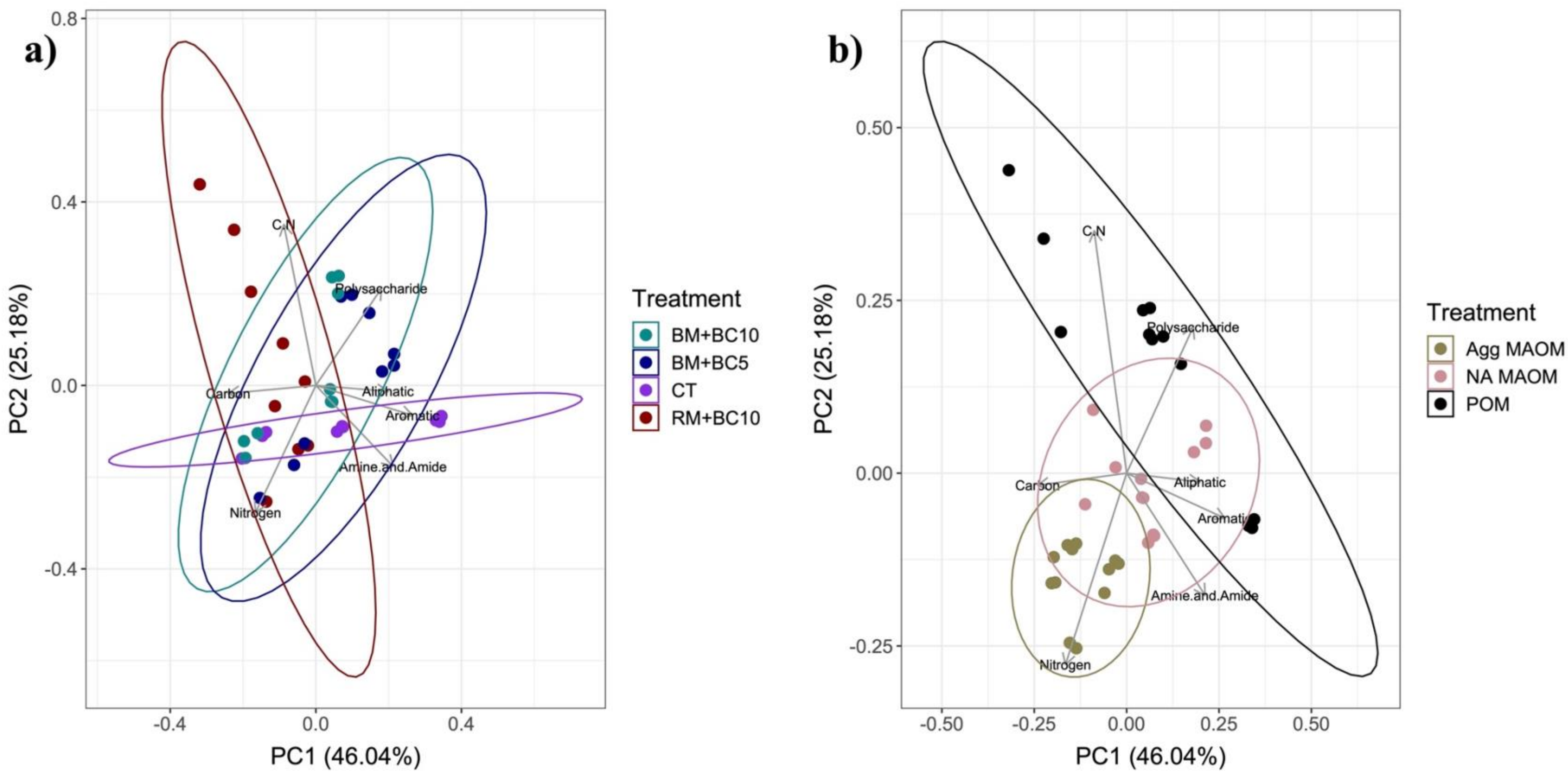

Figure 5.6. PCA biplot showing the influence of biochar and manure (a) treatments and (b) fractions on wavelength and C and N as assessed by DRIFT spectroscopy. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

Chapter 6. Synthesis, conclusions, and future work

6.1 Synthesis of research findings

6.1.1 Objectives

Chapter two investigated a laboratory incubation of biochar-manure, manure, and biochar amendments in different combinations to see the possible effects when applied to soil in a controlled environment. Chapters three to five took the treatments to the field at the Breton Research Station, looking at biochar-manure and manure combinations with biochar at different rates to see the influence of environmental conditions and practical applications of these amendments. There are many types of biochar and soils, and thus their relations may change results under different situations.

The main research objectives included: How do changes to cattle fed biochar affect the resulting manure properties, and how does this affect soil properties (chemical, physical, and biological) when applied as an organic amendment? What is the influence of environmental conditions (specifically temperature and precipitation) on these field practices? Finally, do these changes from the amendments alter soil properties to potentially improve agricultural yields?

6.1.2 C and N dynamics

A 64-day incubation experiment was conducted to quantify the short-term effect of biochar-manure on soil carbon (C) and nitrogen (N) mineralization in a Gray Luvisol. Carbon mineralization (Cm; 528 mg CO_2-C kg^{-1}) was increased (P < 0.001) with regular manure (RM) + biochar (BC) (> 354 mg CO_2-C kg^{-1}) relative to biochar manure (BM) + BC amendments. Treatments with BM inhibited soil C mineralization, indicating manure-C stabilization. Nitrogen mineralization (Nm and No) was not affected (P = 0.130) by amendment type. In conclusion, BM+BC amendments were shown to benefit soil C sequestration without limiting soil N availability, supporting further work in the field.

6.1.3 Crop production

A two-year study was conducted to determine the effect of biochar-manure on spring wheat (*Triticum aestivum* L.) followed by canola (*Brassica napus* L.) crop in a Gray Luvisol field. A correlation analysis found that BM+BC5 performed better under warmer and drier conditions while BM+BC10 performed better under wetter-colder conditions. Irrespective of the biochar option, implementing biochar-manure application became widely more important in wet and cold years. Microbial communities shifted toward higher amino acid utilization associated with biochar additions in both crops, potentially influencing N use efficiency which describes the higher wheat protein content found with BC treatments (11.6 and 10.8 % for BC10 and BC5, respectively). In conclusion, BM+BC10 performed best in the study during the first season after amendment application with a wheat crop and under cold and rainy conditions.

6.1.4 GHG emissions

Emissions of CO_2, CH_4 and N_2O were monitored over a wheat growing season in a Gray Luvisol agricultural field experiment. The highest ($P < 0.05$) soil respiration was from BM+BC10 (939 kg ha^{-1}) and RM+BC10 (861 kg ha^{-1}) treatments, suggesting that inorganic C released from biochar treatment stimulated microbial activity. A key finding was the greater inhibition of CH_4 oxidation ($P < 0.05$) from BM+BC5 (-30 g ha^{-1}) and BM+BC10 (-0.9 g ha^{-1}) than RM (-78 g ha^{-1}) and RM+BC10 (-69 g ha^{-1}). In conclusion, anthropogenic emissions (N_2O +

CH_4) of BM+BC5 (133 kg ha^{-1}) may improve grain productivity and protein content, compared to BM alone, while not significantly impacting GHG emissions.

6.1.5 OM stability

Soils from biochar-manure treatments on a Gray Luvisol field after a growing season with spring wheat, were fractionated into particulate organic matter (POM) and mineral-associated organic matter (MAOM) and analyzed with DRIFT to better understand organic matter stability. The RM+BC10 had the highest ($P < 0.05$) lability (greatest amount of particulate organic matter (POM; 30.8 g kg^{-1} fraction and 11.09 g kg^{-1} soil)), and BM+BC10 had the highest ($P < 0.05$) stability, or in other words, the greatest amount of mineral-associated organic matter (MAOM; 29.4 g kg^{-1} fraction and 12.30 g kg^{-1} soil) as biochar was initially limited to spaces within the soil matrix. The percentage of aggregated vs. non-aggregated MAOM was not different among treatments ($P > 0.05$), but there was a clear separation between POM and aggregated MAOM amongst treatments in a PCA comparison. In conclusion, combinations of biochar-manure and biochar show retention of aromatic C, supporting long-term C sequestration potential in agricultural applications.

6.2 Implications for future research

Soil properties can control C storage (Cotrufo et al. 2019; Weber et al. 2022) and further studies should investigate other soil types to estimate storage potential across different farms in Alberta and around the world. For example, soil organic matter mineralizes 9-10x more rapidly in tropical soils than in temperate soils due to its less degraded state and greater microbial energy potential (Grisi et al. 1998). Since grasslands have more MAOM and less POM than forests, this

POM fraction has a large impact on OM stability and C sequestration potential (Cotrufo et al. 2019). Soils with low fertility will also see a greater mineralization rate from biochar's stimulation of microbial communities (de la Rosa et al. 2018; Plaza et al. 2016).

Soil physio-chemical properties may also play a role. Soil texture is also important, as short-term mineralization can be 8-78% lower in SOC in coarse- than fine-clay fractions (Lehmann and Joseph 2009) due to limited available mineral surfaces for OM adsorption (Dynarski et al. 2020). The optimal pH range for methanogens is 6-8, while methanotrophs tolerate a slightly more acidic pH, but are more sensitive to pH increases (Jeffery et al. 2016; Yu et al. 2013). Additionally, low pH can increase Al^{3+} solubility, which is toxic for methanotrophic bacteria (Jeffery et al. 2016). Therefore, soils with a pH <5 have significantly lower CH_4 emissions than soils with a neutral-basic pH of 6-8 (Jeffery et al. 2016). Soils with a higher clay content have a greater ability to stabilize biochar C and lower CO_2 emissions (Abagandura et al. 2019). Conversely, soils with coarse texture significantly increased MBC content and CO_2 emissions when amended with biochar (Liu et al. 2016). Six times greater release of CO_2 and N_2O was found in a Luvisolic compared to a Vertisol from five and seven times higher initial C and N values, respectively (Singh et al. 2010). As such, various soil types should also be investigated to better understand soil, plant, biochar, and manure relations.

Most studies have reported a strong negative relationship between soil pH and N_2O since protein folding of enzyme *N_2OR* is inhibited at low pHs (Bergaust et al. 2010; Chapuis-Lardy et al. 2007; Russenes et al. 2016). N_2O production during nitrification does not appear to be significantly affected by the wide pH range typical of agricultural soils (pH 5.5-7), however, so pH will likely only have a significant effect when dentification is a dominant process (Russenes et al. 2016). Future studies can benefit from isotopic analyses to see the impact of biochar-manure on these cycles.

Finally, the type of biochar amended (and likely fed to the cattle) can influence the soil properties when amended. For example, greater N mineralization and lower leaching rates were found with the lower highest treatment temperature (HTT) biochars (440 °C vs. 550 °C) as higher pyrolysis temperatures produced a greater percentage of aromatic structures (Ameloot et al. 2013; Clough et al. 2013; Keith et al. 2011). Furthermore, biochar that underwent fast pyrolysis (greater labile OM from incompletely pyrolyzed fractions) resulted in N immobilization and slow pyrolysis (greater recalcitrant OM) resulted in N mineralization (Bruun et al. 2011; Clough et al. 2013).

In comparing four biochar types, Singh et al. (2010) noted a greater N_2O emission from a poultry manure biochar than a stem-wood biochar due to labile N sources, emphasizing the importance of biochar's biomass properties. Likewise, aged biochar may provide greater protection to labile OM and lower CH_4 emissions (Keith et al. 2011). Spokas and Reicosky (2009) note that peanut-hull biochar stockpiled outside for a year lost 67% of the N and gained 286-times in surface area compared to un-weathered peanut-hull biochar, so different types of biochars and pyrolysis methods should be investigated in future work.

Additional emissions management of manure and biochar should also be considered. Many different biochar pyrolysis conditions can impact the structure and gasification reactivity of biomass chars (Cetin et al. 2004). The longevity of the effects of biochar on soil properties depends on the initial biomass and pyrolysis techniques (Cimò et al. 2014; Merino et al. 2015), so finding the right pyrolysis process for the job means calculating the potential greenhouse gas emissions (GHG) released from the pyrolysis technique in the mitigation equation.

Alterations such as composting before pyrolysis can decrease biochar's surface area, lowering its methane (CH_4) mitigation potential (Clough et al. 2013). While biochar may not have a large liming effect on the Breton plots, different soil types might benefit from this surface area reduction. The avoided emissions can add up to \$0.22–\$6.82/MT lime (Galinato et al. 2011), which should be included in biochar inclusion considerations. Moreover, because manure excreted was not calculated, net GHG emissions cannot be determined. Management of manure can also release varying amounts of GHG emissions (Weber et al. 2021b), so various manure practices (such as stockpiled vs. composted) in combination with biochar-feeding regimes should be investigated.

Soil can be lost in all forms of erosion (wind, water and tillage), and various crop rotations and amendments can influence this rate of loss (Dyck et al. 2012). Seasonal freeze-thaw, tillage, and wet-dry cycles can increase the rate of biochar degradation (Dynarski et al. 2020; Lehmann and Joseph 2009; Post and Kwon 2000). Izaurralde et al. (2001) calculated 70% of the variation in soil organic carbon (SOC) content comes from carbon (C) inputs alone and 85% from rotations, highlighting the importance of comparing different inputs (i.e. composted manure and composted biochar-manure) with various rotations to find the optimal combinations for increased yield.

Studies should investigate the effect of biochar-manure on soil compaction from agricultural machinery as compaction inhibits the growth of nitrifiers but favors the colonization of denitrifiers (Liu et al. 2017). These compaction sites can alter bulk density, aeration porosity, and hydraulic conductivity, increasing nitrous oxide (N_2O) emissions by up to 40 times compared with a non-compacted control (Kiani et al. 2020; Teepe et al. 2004).

6.3 Life cycle analysis and a carbon economy

A literature review that looks at various studies examining each stage of the life cycle analysis (LCA) could give better insight to the future of biochar applications. Amonette et al. (2021) calls for systematic and mechanistic research on biochar looking at standardized and local feedstocks, respectively, given the mixture of results from scientific studies. Amendments with other treatments should also be considered, such as changes in stability (greater increases in POM than MAOM fractions) when biochar is amended with solid waste compost and sewage sludge (Plaza et al. 2016).

Treatments were compared to the control to determine percentage of increase in GHG emissions and C stability using data from chapter 4 and 5 (Table 6.1). In this experiment, the manures, RM and BM had 1% increase in emissions and stored 8% more, on average, compared to the control. The biochars, BC5 and BC10, increased GHG emissions 4% and stored 26% more, on average, compared to the control. Meanwhile, RM+BC10 and BM+BC10 emitted the most, 7% increase, on average; but also sequestered 43% more compared to the control. Finally, BM+BC5, increased emissions 3% and sequestered 20% more than the control, which is about half of the manure+BC10 rates. Given their benefit for crop production and long-term C storage, mixing biochar and manure shows promise.

There is no denying that the number of farms is growing in Western Canada. Alberta alone reported a total farm area of 10.36 million ha, primarily consisting of beef and feedlots (Statistics Canada 2022). If farmers are applying biochar at a rate of 10 Mg ha^{-1}, nearly 103 million Mg of biochar could be added. Even at 5 Mg ha^{-1}, application of 52 million Mg of biochar could be greatly beneficial long-term. Total sequestration was calculated at 12.7 Mg MAOM C ha^{-1} for BM+BC10 (Table 6.1) and sequestration across all farms would total 132 million Mg MAOM C. If biochar was applied at 5 MT ha^{-1} the sequestration would be calculated at 10.8 Mg MAOM C ha^{-1} for BM+BC5 (Table 6.1) and sequestration across all farms would still total 112 million Mg MAOM C. Compared to the CT, this 1.8 and 3.8 Mg MAOM C ha^{-1} sequestration increase for BM+BC5 and BM+BC10 (Table 6.1) results in 18.6 and 39.3 million, respectively, more Mg MAOM C sequestered in the province. In a comparison of RM applications, this results in 1.4 and 3.3 Mg MAOM C ha^{-1} sequestration increases for BM+BC5 and BM+BC10 (Table 6.1) 14.5 and 34.2 million, respectively, more Mg MAOM C sequestered provincially.

Projecting that a Mg of CO_2 is worth \$50, farmers that are able to gain C sequestration under these treatments will inject around \$5 billion into the industry. This preliminary data, while valuable, needs more economical and scientific analyses to ensure a broad scale application, specifically looking at the area of farms in Alberta that are only Gray Luvisols. Approximately 3.0-4.3 million ha of farm land were classified as Gray Luvisols in 1991, with an 60,700 ha expansion each year (Dyck et al. 2022; Hamley 1992). This means around 6.2 million ha of farms are currently classified as Gray Luvisols. Applying 10 Mg ha^{-1} of biochar on these Gray Luvisols alone means \$3 billion from C sequestration! Policies that develop from this research that encourage persistent soil C stocks will be important for future sustainable agricultural initiatives (Dynarski et al. 2020).

Table 6.1. Summary of carbon emitted (GHG) and stored (MAOM fractions).

Treatment	GHG Emitted	Increase in Emissions	Stable Carbon	Increase in Stability	Total increase in Stability	Total increase in Emissions
	CO_2 eq kg ha^{-1}		MAOM C kg ha^{-1}		%	
CT	646.09	-	8979.38	-	-	-
RM	760.52	114.42	9390.11	410.74	4.57	0.71
BM	808.24	162.15	9977.90	998.52	11.12	1.72
RM+BC10	1017.25	371.16	12939.70	3960.32	44.10	6.83
BC5	1062.71	416.62	10617.16	1637.79	18.24	2.82
BM+BC5	719.45	73.36	10774.43	1795.05	19.99	3.09
BC10	822.33	176.24	11997.00	3017.63	33.61	5.20
BM+BC10	1139.93	493.84	12742.85	3763.47	41.91	6.49

Note: Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

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