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Biochar and Manure from Cattle Fed Biochar as Agricultural Amendments Alter CH_4 Oxidation in a Gray Luvisol

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Abstract: Greenhouse gases (GHG) emissions from agricultural practices contribute 14% of anthropogenic emissions to the atmosphere, and novel practices to reduce these emissions, including feeding cattle a modified diet, are of interest. This study examines how additions of manure from cattle fed a regular diet or a diet supplemented with 2% biochar, and biochar at 5 or 10 Mg ha^{-1}, impact GHG emissions in a Gray Luvisol agricultural field experiment. Emissions of CH_4 and N_2O were monitored, and soil samples were collected to analyze exchangeable NPKS, microbial biomass, total C and N, electrical conductivity, and pH. Wheat (*Triticum aestivum*) was planted, and grain yield measured. We calculated the yield-based emission factor (EF_{yield}) and cumulative area-based GHG emissions emission factor (EF_{area}). The results showed an up to 98.5% change in the inhibition of CH_4 oxidation from biochar-manure + biochar at 5 and 10 Mg ha^{-1} compared to the CT. The biochar in biochar-manure may have acted as a biocide to methanotrophs, causing a reduction in the release of CH_4 over time. Yet, there were no significant differences in N_2O emissions amongst treatments. Therefore, biochar-manure + biochar at 5 and 10 Mg ha^{-1} applications may impact total GHG emissions and improve grain productivity and protein content compared to BM alone.

Keywords: greenhouse gases; cattle; manure; biochar

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Citation: Weber, T.L.; Hernandez-Ramirez, G.; Quideau, S.; MacKenzie, M.D. Biochar and Manure from Cattle Fed Biochar as Agricultural Amendments Alter CH_4 Oxidation in a Gray Luvisol. *Land* **2023**, *12*, 1353. https://doi.org/10.3390/land12071353

Academic Editor: Nir Krakauer

Received: 1 June 2023
Revised: 29 June 2023
Accepted: 30 June 2023
Published: 6 July 2023

1. Introduction

The last three decades have been warmer than any preceding decade, highlighting the global human impact on climate change [1]. 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 [2]. These GHGs trap heat at different rates, with CH_4 having 28× and N_2O having 265× the potential of CO_2 [1]. In the 1970s, atmospheric scientists realized N_2O's detrimental impact on the ozone layer [3]. Increasing livestock and thawing permafrost have increased CH_4 emissions by 150% since the 1750s [4]; however, sustainable strategies, such as organic fertilizer amendments and crop rotations, could reduce emissions up to 4500–6000 Mt CO_2-eq year^{-1} in agricultural operations by 2030 [5].

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 [6–8]. The first documented use of biochar was ~2500 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 [9]. 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 [10,11].

Biochar recalcitrance can offset CO_2 emissions and sequester C in the soil [12,13]. Through its recalcitrance, the addition of biochar can result in negative priming, defined as the decrease in mineralization of native organic C following the addition of organic matter [14]. While more scarce than short term studies (<1 year), longer studies (>3 years) have also shown promising results from biochar. Long-term biochar applications have been shown to increase organic C, microbial abundance [15,16], have no negative consequences for crop growth [17], and decrease CO_2 and N_2O fluxes [12].

In contrast, manure C mineralization is much more complete and rapid than biochar C, as seen in experiments by Weber et al. [18] and Troy et al. [19]. Increased CO_2 emissions can also occur with the mineralization of labile C, changes in microbial populations, or SOC priming [19]. Biochar's ability to reduce N_2O emissions through decreased denitrification is related to environmental conditions and soil properties [20–22]. 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 [3,19,23,24].

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 [25,26]. Biochar applications to acidic soils can increase porosity, raise pH, and decrease Al^{3+} solubility, reducing the populations of methanotrophic bacteria [25,27]. In summary, abiotic changes to the soil, changes to microbial communities, and direct absorption of various chemicals and gases in biochar pores can lead to potential GHG mitigation [25,28–30].

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 the immediate nutrients needed for crop production [6,29,31]. 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 [32–34]. In a study investing cattle-fed biochar, Romero et al. [35] discovered the presence of unchanged biochar in manure after being passed through the rumen, showing potential for biochar-manure applications. Little research, however, looks at the effect of manure from cattle fed with biochar on GHG emissions when such manure is applied to crops in temperate climates [16]. By studying the impact of biochar and biochar-loaded manure on field GHG emissions and soil properties in croplands, we can develop a clearer understanding of its role in sustainable agricultural practices.

The objectives of this experiment were to Investigate if (i) biochar (BC), biochar-manure (BM), or regular manure (RM) soil amendment, at different application rates, might reduce greenhouse gas emissions and (ii) if these responses are further influenced by environmental factors. We hypothesized 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. We further theorized that greater biochar application rates will lower denitrification and methanogenesis rates, reducing CH_4 and N_2O [21,25,28,29,36].

2. Materials and Methods

2.1. 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 to 2011, oats and barley were harvested, followed by a fallow period. From 2013 to 2015, barley–canola rotations were fertilized with 80 kg N ha^{-1} urea in 2015. Wheat–barley–barley rotations were grown from 2016 to 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), as summarized in Table 1. Atmospheric data were 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).

Table 1. Treatment descriptions.

Abbreviation	Description
CT	Control (no amendments)
RM	Stockpiled manure from cattle on a typical western Canadian feedlot diet
BM	Stockpiled manure from the same feedlot diet as RM supplemented with 2% biochar (BM)
BC5	Biochar at a rate of 5 Mg ha^{-1}
BC10	Biochar a rate of 10 Mg ha^{-1}
RM + BC10	Manure from feedlot diet + biochar a rate of 10 Mg ha^{-1}
BM + BC5	Manure from feedlot diet supplemented with 2% biochar + biochar a rate of 5 Mg ha^{-1}
BM + BC10	Manure from feedlot diet supplemented with 2% biochar + biochar a rate of 10 Mg ha^{-1}

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 [37] One of the manures came from a regular western cattle diet consisting of 60% barley silage, 85% barley grain, and 5% mineral supplement [37], 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 [38], 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, USA) 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) [35].

2.2. Chemical and Biological Analyses of Soils and Crops

Soil and treatments were air-dried for 48 h, ground (<2 mm) with a Ball Mill MM200 (Brinkmann Retsch, Haan, Germany), and stored in 20 mL scintillation vials for total C and N and simultaneous thermal analysis (STA). A Thermo Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) measured total C and N values using dry combustion [39]. 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 [40]. The ratio of the heat of combustion of the recalcitrant region (410–725 °C) to labile region (150–410 °C) of organic matter was calculated as OM stability. Fresh soil samples from the field were analyzed for pH, EC, exchangeable NPKS, nitrogen, and microbial biomass. FE20 and FE30 m (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 h, vacuum filtered, and allowed to settle for 30 min. To measure exchangeable NPKS and microbial biomass, fresh subsamples from the field were incubated at 20 °C for 72 h [41]. 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 [42] at the end of the incubation. Ammonium concentrations were measured using the Salicylate-Hypochlorite method [43] and NO_3^-N and NO_2^-N were measured using the Hydrazine reduction method [44] 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 h and were compared to an unfumigated set [41]. 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 h, filtered using Whatman No. 42 filter paper [45] 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 1 × 1 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) [46]. Harvest index (HI) was calculated according to Thilakarathna et al. [47] as follows in Equation (1):

$$Harvest\ Index = \frac{Grain\ yield\ DM}{Aboveground\ DM} \quad (1)$$

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}).

2.3. 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 [48–50]. Chambers were custom-built at 10,000 cm^3 (64.1 cm length × 15.6 cm width × 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 min closure times [49]. 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 [49]. Chamber installation and measurement followed Roman-Perez et al.'s protocol [51], with one chamber in each plot for a total of 32 chambers [49]. A chisel and rubber mallet were used to push the chamber 5 cm into the soil [49,52]. 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 [52,53].

Gas samples were collected weekly and biweekly, from spring thaw to winter freeze, to capture potential differences between treatments throughout the field season [50]. Emissions were assumed to be negligible during the winter months with minimal biological activity [53]. 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 [49]. When plants began to grow, they were kept in the chamber to understand crop growth on GHG emissions [54]. Atmospheric conditions were recorded by a permanent on-site weather station adjacent to the research plots [55].

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 [49]. 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) [49,51]. 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) [56].

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, USA) were 1.52 and 2.01 ppm for CH_4 and 0.468 and 1.14 ppm for N_2O [56]. Standards were run for every 30 samples, and minimal detectable flux was approximately 0.94 and 0.27 g ha^{-1} d^{-1} for CH_4 and N_2O, respectively.

The N_2O (N_2O-N kg^{-1} h^{-1}) and CH_4 fluxes (CH_4-C kg^{-1} h^{-1}) were calculated using a modified ideal gas law in Equation (2) [52,53,57]:

$$F = \frac{S \times P \times V}{A \times R \times T} \quad (2)$$

where F = flux rate (g N_2O-N or CH_4-C ha^{-2} d^{-1}), S = slope of the linear or quadratic regression at time zero [57], 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 [58]. Area-based emission factor for N/cumulative GHG emissions, was calculated according to Thilakarathna, Hernandez-Ramirez, Puurveen, Kryzanowski, Lohstraeter, Powers, Quan, and Tenuta [47] in Equation (3):

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

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, Hernandez-Ramirez, Puurveen, Kryzanowski, Lohstraeter, Powers, Quan, and Tenuta [47] in Equation (4):

$$Yield\ emission\ intensity/EF_{yield} = \frac{Treatment\ emission}{Grain\ Yield} \times 100 \quad (4)$$

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 [58]. 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 [1]. 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 [59] and thus not included in the discussion or results. Consequently, anthropogenic GHG emissions were calculated according to Kammann, Ratering, Eckhard, and Müller [21] in Equation (5):

$$Anthropogenic\ GHG\ flux = N_2O\ flux + CH_4\ flux \quad (5)$$

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}).

2.4. Statistical Analyses

Shapiro and Bartlett/Levene tests confirmed assumptions of normality of distribution and homogeneity of variance of the residuals before analyses [60]. Levene's test was used instead of Bartlett's test if data were not normal. Log, sin, or sqrt transformations were applied to the microbial biomass, total C, OM stability, electroconductivity, and NPKS response variables as these models did not meet the assumptions [50]. Transformed data were used for statistical analysis to assess treatment effects, but untransformed data were used to calculate mean values and graph results [50].

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 were collected and analyzed during the collection period (22 April–27 September) 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 [61]. Soil moisture was correlated using total days greater than 30% volumetric water content, due to the influence on soil water potential at this percentage [62]. These statistical calculations were performed using R v. 4.2.1 (R Core Team, 2020) [60].

3. Results

3.1. Temperature and Moisture

During the 2020 growing season (April–September), daily temperatures (11.0 °C) were 6.4% lower than the average (11.7 °C; Figure 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 (Figure 2a). The total number of days greater than 15 °C ranged from 76 to 85 ($p > 0.05$; Table 2).

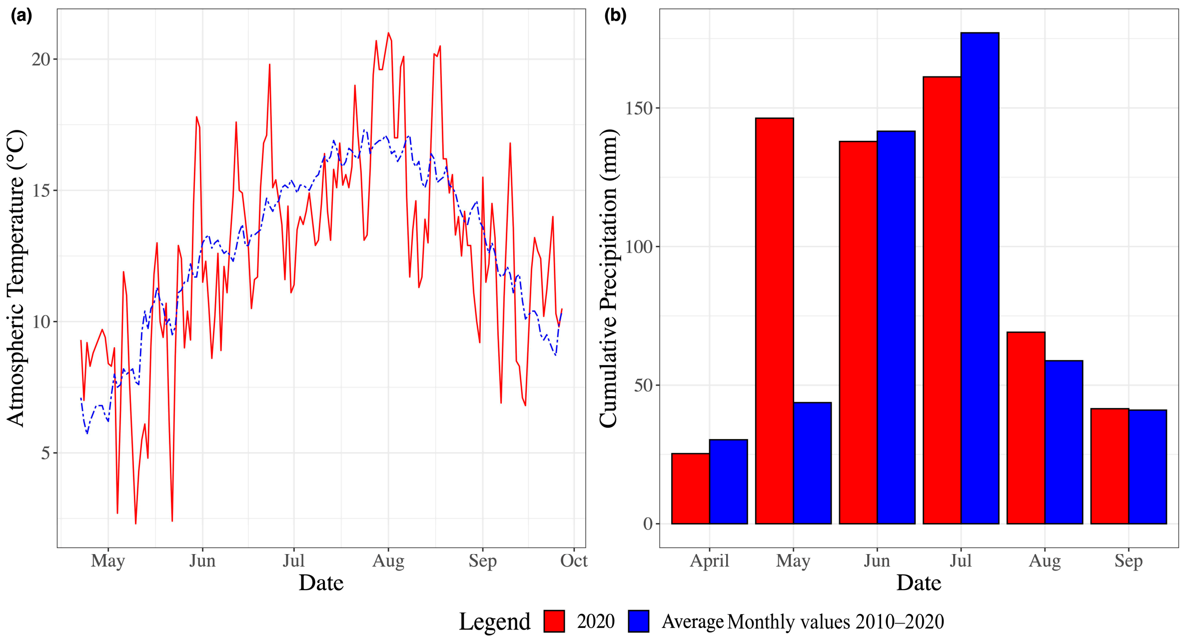

Figure 1. The (**a**) average daily temperature and (**b**) monthly precipitation during GHG sampling in 2020 compared to the average over the last decade (2010–2020).

During the growing season, precipitation was very high, 35% higher than the long-term average (552.6 mm vs. 410.4 mm; Figure 1b). The total number of days greater than 30% volumetric water content ranged from 56 to 71 ($p > 0.05$; Table 2). In April (25.3 mm), there was not much precipitation, but this greatly increased in May (146 mm). As a result, soil moisture declined from an average of 0.35 $m^3 m^{-3}$ to 0.20 $m^3 m^{-3}$ in late April, then sharply rose again to a high of 0.45 m^3 m^{-3} in early May (Figure 2b).

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^3 m^{-3} with few peaks to 0.2 m^3 m^{-3} (Figure 2b). The $CO_{2\ eq}$ and CH_4 were negatively correlated ($p > 0.05$) to soil temperature and moisture, respectively (Supplemental Table S1). Everything else was positively ($p > 0.05$) correlated to soil temperature and moisture.

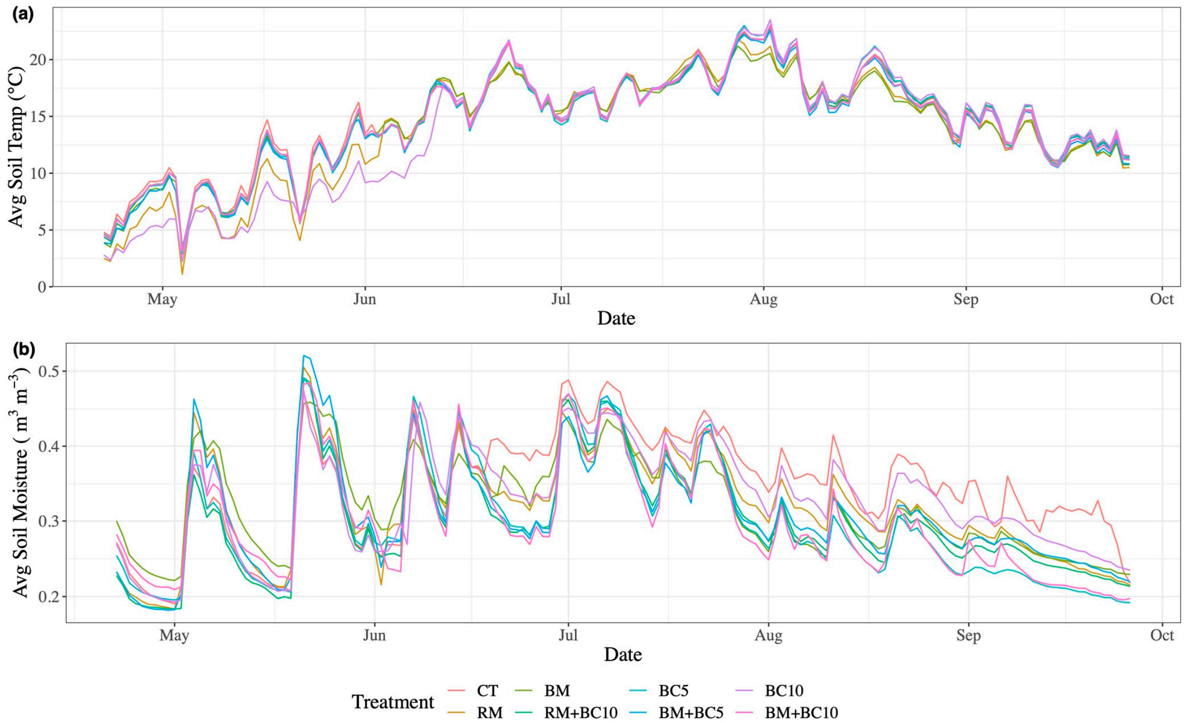

Figure 2. The soil (**a**) temperature and (**b**) moisture average (10 cm) per treatment during GHG sampling in 2020. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10,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

Table 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 (22 April–27 September 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

3.2. Greenhouse Gas Emissions

Cumulative emissions of N_2O (Figure 3a) and anthropogenic (N_2O + CH_4; Figure 3c), did not differ ($p > 0.05$) among treatments and followed a similar pattern. The N_2O emissions varied from 145.9 to 427.4 g ha^{-1}, and anthropogenic emissions varied from 58.6 to 201.5 kg ha^{-1}. All treatments had negative cumulative CH_4 emissions (Figure 3b; $p = 0.023$), which were lowest (greatest sink potential) in RM (−78.0 g ha^{-1}) and RM + BC10 (−68.9 g ha^{-1}) and highest (greatest source potential) in BM + BC10 (−0.9 g ha^{-1}) and BM + BC5 (−30.3 g ha^{-1}).

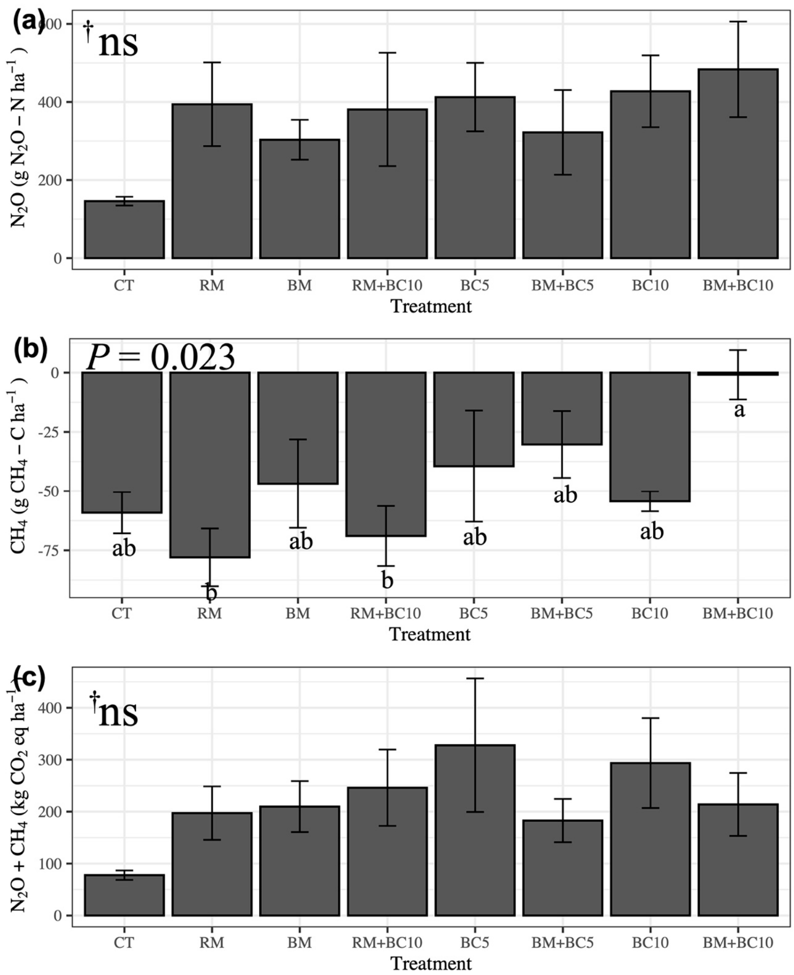

Figure 3. Effects of treatments on cumulative (**a**) N_2O, (**b**) CH_4, and (**c**) anthropogenic ($N_2O + CH_4$) GHG emissions. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}. Letters denote significant differences between treatments, $p < 0.05$. † ns indicate not significant, $p > 0.05$.

The N_2O fluxes (Figure 4a) 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 (Figure 4b) 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 August with peaks from BM + BC5, and late September from various treatments.

Cumulative GHG emissions/area emission factor (EF_{area}) did not differ ($p > 0.05$) amongst treatments, but yield emission intensity/yield emission factor (EF_{yield}) ($p = 0.022$) 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; Figure 5a) 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).

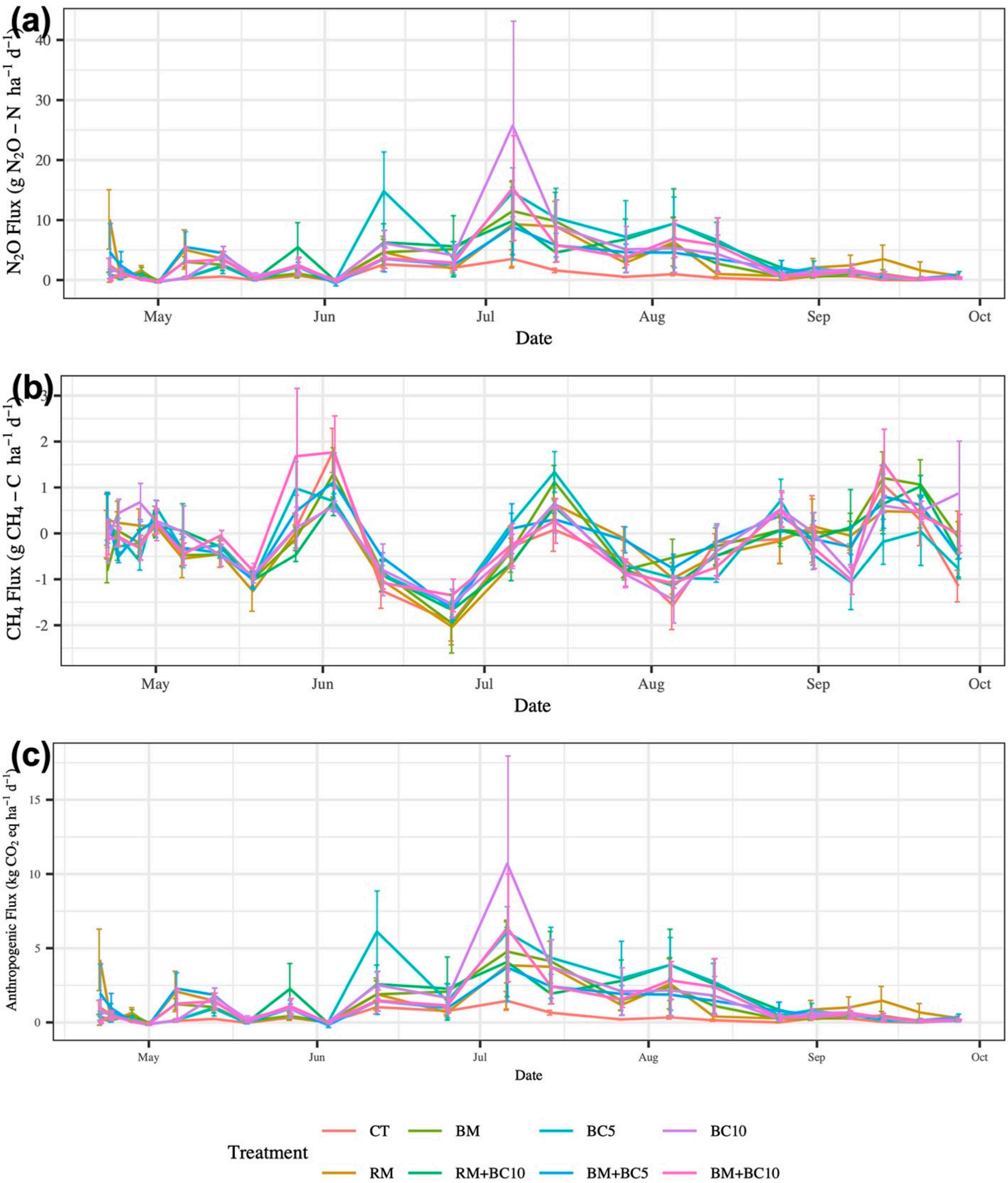

Figure 4. Effects of treatments on (**a**) N_2O, (**b**) CH_4, and (**c**) anthropogenic (N_2O + CH_4) GHG fluxes over time. Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; BC5, biochar applied at 5 Mg ha^{-1}; BC10, biochar applied at 10 Mg ha^{-1}.

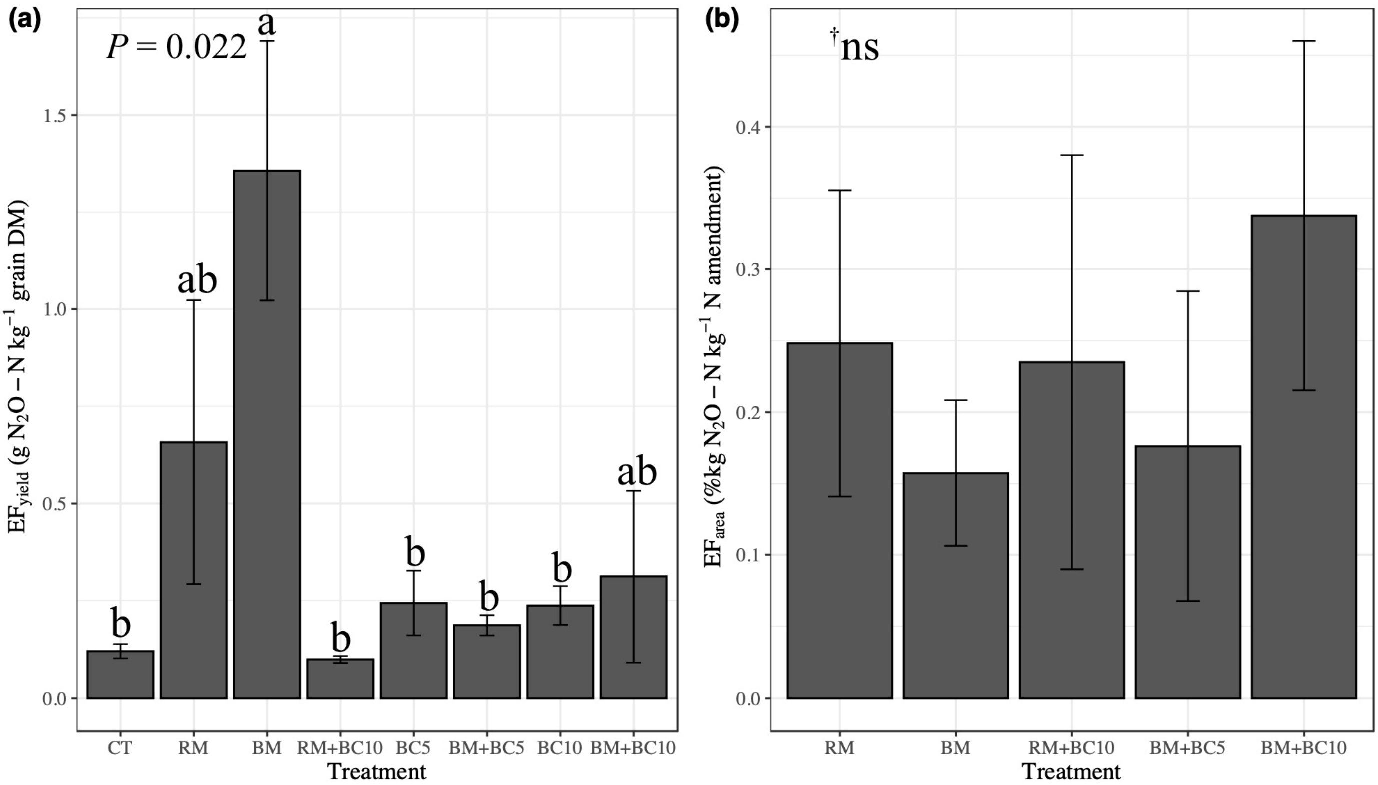

Figure 5. Effects of treatment combinations on cumulative (**a**) emission factor (EF) yield (yield emission intensity) and (**b**) area (cumulative GHG emissions). Treatments: CT, control, RM, manure from cattle fed a traditional barley diet; BM, manure from cattle fed RM supplemented with 2% biochar; 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, $p > 0.05$.

The exchangeable NPKS values were not statistically correlated with any emissions except for S (Supplemental Table S1). 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 ($p < 0.05$) to TC and TN, except for TN ($p > 0.05$).

4. Discussion

4.1. The Potential for Biochar to Be a Methane Sink

Differences in C mineralization were found in an incubation study in this same Luvisol [18]. Emissions peaks in the summer result from frequent rain (Figure 1b) and increased moisture in the soil (Figure 2b). The CH_4 emissions were positively correlated to soil moisture ($p > 0.05$; Supplemental Table S1), due to frequent anaerobic conditions. After September, lower temperatures and precipitation rates (Figure 1) reduced CH_4 (Figure 4b) emissions [52].

The hypothesis that RM would have greater CH_4 emissions than BM was not supported, as BM had 52% more source potential than RM (Figure 3b). There were no differences in MBC between the two treatments by the end of the experiment (data in review in another manuscript) and no significant ($p > 0.05$) correlations to microbial biomass (Supplemental Table S1). The greatest CH_4 sink potential from RM and RM + BC10 likely came from the ability to moderate water content ($p > 0.05$; Table 2) in this wetter-than-normal season.

At the beginning of the season, there was up to 29% higher ($p < 0.001$) total C from RM + BC10 (35.7 g kg^{-1}) and BM + BC10 (29.3 g kg^{-1}) than BM + BC5 (26.7 g kg^{-1}; data in review in another manuscript), which may explain the greater grain yield as they were positively correlated ($p > 0.05$, Supplemental Table S1). These statistical differences were no longer present a few months later (data in review in another manuscript), and all treatments decreased in TC slightly. Because the BC10 mixtures had higher TC than the BC5 mixtures, inorganic C was likely released from the biochar over time [63], and more microbial substrates were available in the BC10 mixtures. Treatment BC10 and BC5 did not differ in CH_4 emissions (Figure 3b). 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 [20,24,64].

In a comparison of RM and BM, Romero, Redman, Terry, Hazendonk, Hao, McAllister, and Okine [35] 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 78 and 195% greater ($p < 0.05$) CH_4 sink potential than BM + BC5 (-30.2 g ha^{-1}) and BM + BC10 (-0.89 g ha^{-1}; Figure 3b), respectively, 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 [32,33]. Additionally, biochar that has passed through the rumen is suggested to adsorb signaling compounds that change gene expression and microbial populations [32]. As such, biochar can aid long-distance electron exchange, helping cattle increase their feed intake efficiency, improving anoxic microbial respiration and CO_2 emissions [33].

Le Mer and Roger [65] also found positive correlation between methanogenic potential and the OM content in soils (0.405; $p < 0.05$; Supplemental Table S1), explaining the greater CH_4 sink potential from RM + BC10 than BM + BC10 and BM + BC5 at the start of the season ($p < 0.05$; Supplemental Table S1). The 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 2) and the lowest CH_4 sink potential (Figure 3b). One explanation is that while biochar did increase the soil porosity, the spaces were filled with water and increased overall anaerobic pockets [27]. There was a negative correlation ($p < 0.05$; Supplemental Table S1) between CH_4 emissions and pH, as sensitive methanogen populations increased as pH increased from biochar and manure additions at higher rates [65]. This inhibition may be important in environments that are more waterlogged throughout the year, such as paddy fields [27], as this study found that N_2O emissions were of greater impact when compared as cumulative GHG flux (Figure 3c).

4.2. N_2O Emissions, Anthropogenic GHG Emissions, and Emission Factors

Similar to a previous incubation by Weber, Romero, MacKenzie, and Naeth [18], and a field study by Jones, Rousk, Edwards-Jones, DeLuca, and Murphy [17], there were no significant differences ($p > 0.05$) in N_2O emissions (Figure 3a), anthropogenic GHG emissions (represented by changes in N_2O, rather than CH_4, emissions; Figure 3c), and EF_{area} (Figure 5b) amongst treatments. The BC applied had no detectable levels of N input (data in review in another manuscript), 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 (Supplemental Table S1); however, this is unlikely two years after the initial application [66].

Environmental conditions likely played a large role in emissions. The increase in N_2O emissions during the initial thaw in mid-April (Figure 4a) was due to the release of organic substrates and the high moisture content of the soil (0.154 correlation; $p > 0.05$; Supplemental Table S1), which causes microbes to use alternative electron acceptors, such as NO_3 [47,67]. As the field season progressed, N uptake by plants might have reduced the availability of NO_3 and NH_4 for N_2O emissions [19,47]. Given the high N_2O fluxes in mid-June and July (Figure 4a); however, this was likely overridden by high precipitation (Figure 1b) [52]. Finer-textured soils require lower WFPS to induce denitrification, even at 50% WFPS for a silty loam [68], 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 19% higher, but not significantly different, N_2O emissions than the BM (303.2 g ha^{-1}) and RM (394.1 g ha^{-1}; Figure 3a), on average. Labile organic materials from manures usually act as electron donors in the denitrification process compared to the recalcitrant nature of biochar [19,69]. 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 5a). One explanation is that any aeration benefits from biochar in this study were likely overridden by an inhibition of the *Nos* enzyme suppression of N_2O reduction to N_2 [68]. Moreover, Liu et al. [70], and Jones, Murphy, Khalid, Ahmad, Edwards-Jones, and DeLuca [63], 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 2). Denitrification enzyme activity in soils was found to increase with increasing biochar rates [17], 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 ($p = 0.218$) and October ($p = 0.013$; data in review in another manuscript), with the BC10 and BM + BC10 treatments having the highest EC at 250 and 177 µS cm^{-1}, respectively. Adviento-Borbe et al. [71] 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; Figure 1b), precipitation was 169 mm higher than the long-term average, so it is likely that denitrification predominated. The manure and control treatments had the lowest EC ($p = 0.013$; data in review in another manuscript) 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 resulted in 26% higher N_2O emissions (394.1 g ha^{-1}; Figure 3a) and 4% higher EF_{area} (0.24% kg N_2O kg^{-1} N; Figure 5b) 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 [19]. However, RM + BC10 (380.8 g ha^{-1}) had 24% lower emissions ($p > 0.05$; Figure 3a) than BM + BC10 (483.5 g ha^{-1}). The 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, ($p > 0.05$; data in review in another manuscript) supports this hypothesis.

Joseph, Pow, Dawson, Mitchell, Rawal, Hook, Taherymoosavi, Van Zwieten, Rust, Donne, Munroe, Pace, Graber, Thomas, Nielsen, Ye, Lin, Pan, Li, and Solaiman [32] 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. The toxic effects from biochar on nitrifier and denitrifier communities have also been found; however, some restrictions to microbial N activity in the manures may have simultaneously occurred [16,68,70,72]. The EF_{area} (Figure 5a) showed 148% 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) to Fall 2020 (average 6.78; data in review in another manuscript) likely led to lower N_2O/N_2 ratios [21], 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 Supplemental Table S1. This change in microbial communities enhances the reduction of N_2O to N_2 and binds N_2O-N to metal ions [20,70]. This N_2O/N_2 ratio change is not as affected by nitrification in most agricultural soils (pH 5.5–7.0), however, thus explaining why there were no significant differences in N_2O emissions (Figure 3a) or pH (data in review in another manuscript) among treatments [67].

5. Conclusions

In conclusion, the quantity of biochar and the synergistic effects of manure, biochar, and crop significantly affect CH_4 emissions when studying BM applications. Although there was a key finding of the 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.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/land12071353/s1, Table S1: Spearman's rank correlation coefficients of relationships between the cumulative N_2O, CH_4, and CO_2 eq emissions and soil properties (means ± SE; $n = 4$).

Author Contributions: Conceptualization and methodology: M.D.M.; investigation: T.L.W.; writing and editing: T.L.W., S.Q., G.H.-R. and M.D.M.; resources and supervision, M.D.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported by Agriculture and Agri-Food Canada (AGGP2-037), Alberta Agriculture and Forestry (Project #2017R036R), Blue Rock Animal Nutrition, National Carbon, and the University of Alberta.

Data Availability Statement: Data are contained within the article.

Acknowledgments: We acknowledge the Natural Resources and Analytical Laboratory (NRAL), for their assistance with chemical analyses; the staff and volunteers of the Soil Plant Relations (SPR) lab for their help with laboratory and field work, and the folks at the Sustainable Land Ecosystems Research Group for their aid with the GHG analyses.

Conflicts of Interest: The authors declare no conflict of interest.

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