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Complete List of Authors:	Weber, Tien; University of Alberta Faculty of Agricultural Life and Environmental Sciences, Department of Renewable Resources Romero, Carlos; University of Lethbridge, Department of Biological Sciences; Agriculture and Agri-Food Canada Lethbridge Research and Development Centre, Department of Biological Sciences MacKenzie, M. Derek; University of Alberta Faculty of Agricultural Life and Environmental Sciences, Department of Renewable Resources
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Biochar-manure changes soil C mineralization in a Gray Luvisol used for agricultural production

T.L. Weber¹, C.M. Romero^{2,3}, M.D. MacKenzie^{1*}

¹Department of Renewable Resources, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, 3-48 South Academic Building, Edmonton, AB, T6G 2E3, Canada.

²Department of Biological Sciences, Faculty of Arts & Science, University of Lethbridge, Lethbridge, AB, T1K 3M4, Canada

³Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, 5403–1st Ave. S., Lethbridge, AB, T1J 4B1, Canada

Corresponding Author: *Derek MacKenzie, Tel: 1-780-492-6388; Email: mdm7@ualberta.ca

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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 CO₂-C emission rates, with the highest cumulative CO₂-C emissions being observed for RM + BC. Treatments with BM halted 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 CO₂-C emissions over the long-term.

Keywords: biochar, animal manure, soil carbon, feedlot cattle, nitrogen mineralization

Short Title: Manure application from cattle fed biochar

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 O₂ limited thermal decomposition of organic matter (OM) at temperatures < 700 °C. Amending soil with biochar, *i.e.*, recalcitrant-C, has been shown to mitigate agricultural greenhouse gas (GHG) emissions by altering soil biochemical properties, *e.g.*, increasing cation exchange capacity and reducing microbial activity. 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). Applying biochar to croplands is also frequently associated with improved soil quality and crop productivity; biochar often increases soil pH and nutrient availability for plant uptake (Preston and Schmidt 2006).

Recently, findings that biochar use in animal feeding could lower enteric methane (CH₄) 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 (NH₃), nitrous oxide (N₂O) and CH₄ once applied to croplands (Kammann et al. 2017). Romero et al. (2021) 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

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presence or absence of biochar, on soil C and N mineralization over a 64-day incubation period. Due to pyrogenic-C's recalcitrant nature, we hypothesized that biochar would retain some of its properties once excreted by feedlot cattle (Romero et al. 2021), thereby limiting microbial activity and associated soil C and N mineralization (Whitman et al. 2015).

MATERIALS AND METHODS

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 Plots (53°07'N, 114°28'W) near Breton, AB, Canada. The soil, classified as a loamy Gray Luvisol (pH 6.3), was amended in four replications with (i) regular 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.

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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 (CO₂) 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 CO₂. 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.

CO₂-C fluxes and inorganic N, nitrate (NO₃-N) and ammonium (NH₄-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). CO₂-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. NO₃-N and NH₄-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

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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.

Statistical Analysis

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

$$A_m = A_0(1 - e^{-kt}) \quad (1)$$

where A_m is the cumulative amount of soil C or N mineralized, A_0 represents the labile pool of C or N, t is time, and k is the rate of mineralization constant (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's tests, respectively. Means were compared using Tukey's HSD, where F values were significant at $\alpha = 0.05$.

RESULTS & DISCUSSION

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 1.9 and 2.0 g kg⁻¹ for RM and BM,

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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).

Carbon Mineralization

Carbon mineralization (C_m ; $528 \text{ mg CO}_2\text{-C kg}^{-1}$) was increased ($P < 0.001$) with RM + BC relative to the other amendments ($> 354 \text{ mg CO}_2\text{-C kg}^{-1}$) (Table 1), while C_0 ($577 \text{ mg CO}_2\text{-C kg}^{-1}$) was only augmented ($P < 0.001$) with RM + BC compared to BM ($397 \text{ mg CO}_2\text{-C kg}^{-1}$). Mixing RM with BC stimulated a priming effect in soil while BM and BC alone did not (Fig. 1a), supporting our hypothesis that biochar-manure would have a lower soil C mineralization potential relative to manure-only treatments. C_m was reduced in BM ($217 \text{ mg CO}_2\text{-C kg}^{-1}$) compared to RM ($340 \text{ mg CO}_2\text{-C kg}^{-1}$) but was similar to CT ($215 \text{ mg CO}_2\text{-C kg}^{-1}$) (Table 1).

The reaction rate coefficient (K_c) was increased with RM + BC, BM and BM + BC relative to CT and BC treatments (Table 1). Apparently, there was a synergistic effect between RM and BC, considering that RM- and BC-only resulted in lower K_c values (Table 1). Lentz et al. (2014) found that applying manure with biochar improves the ability of heterotrophs to degrade biochar-C, agreeing with our findings of greater soil C mineralization under RM + BC. Adding biochar to manure may also improve aeration, further increasing microbial activity over labile-C (Whitman et al. 2015). Our observation supports this potential interaction as cumulative $\text{CO}_2\text{-C}$ emissions in RM + BC did not plateau as quickly as with the other treatments (Fig. 1a).

Interestingly, BM + BC and RM + BC exhibited distinct $\text{CO}_2\text{-C}$ emission patterns (Fig. 1a), despite soil C mineralization rates being similar between RM and BM treatments (Table 1). Romero et al. (2021) demonstrated that biochar passes through the gastrointestinal tract of

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feedlot cattle mostly unaltered. Based on this observation, we speculate that manure OM in BM is as labile as in RM, given that manure-C does not interact with biochar-C (Romero et al. 2021). However, biochar-manure is expected to be more aromatic than RM when considering the whole OM mixture (Romero et al. 2021). The latter supports our findings that adding biochar to BM does not increase CO₂-C emissions as much as adding biochar to RM (Table 1).

Nitrogen Mineralization

N_m and N_o were not affected ($P = 0.130$) by amendment type, even though K_n was increased ($P < 0.05$) with BM + BC relative to CT and BC treatments (Table 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 NO₃-N + NH₄-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 1); cattle-ingested biochar was presumably more reactive than its raw counterpart (Joseph et al. 2015).

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 CO₂-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

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sequestration by lowering CO₂-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.

For Review Only

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Conflicts of Interest

The authors declare there are no competing interests.

Contributions

Investigation: T.L.W; Writing and editing: C.M.R, M.D.M, T.L.W; Resources and supervision, M.D.M. All authors have read and agreed to the published version of the manuscript.

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Table 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 \pm 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 \pm 20 ^d	397 \pm 63 ^{ab}	18.3 \pm 1.9 ^c	141 \pm 10 ^a	267 \pm 10 ^a	0.012 \pm 0.001 ^b
BC	260 \pm 3 ^{cd}	533 \pm 65 ^{ab}	16.0 \pm 2.8 ^c	143 \pm 4 ^a	276 \pm 2 ^a	0.012 \pm 0.001 ^b
BM	217 \pm 18 ^{bc}	397 \pm 20 ^b	54.9 \pm 1.5 ^a	169 \pm 9 ^a	229 \pm 9 ^a	0.021 \pm 0.001 ^{ab}
RM	340 \pm 8 ^{bc}	403 \pm 9 ^{ab}	41.8 \pm 1.2 ^b	155 \pm 6 ^a	246 \pm 6 ^a	0.017 \pm 0.003 ^{ab}
BM + BC	354 \pm 22 ^b	406 \pm 31 ^{ab}	47.9 \pm 4.6 ^{ab}	146 \pm 2 ^a	190 \pm 2 ^a	0.023 \pm 0.002 ^a
RM + BC	528 \pm 25 ^a	577 \pm 28 ^a	55.6 \pm 1.5 ^a	156 \pm 10 ^a	247 \pm 10 ^a	0.017 \pm 0.003 ^{ab}
<i>P</i>-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 ($P < 0.05$).

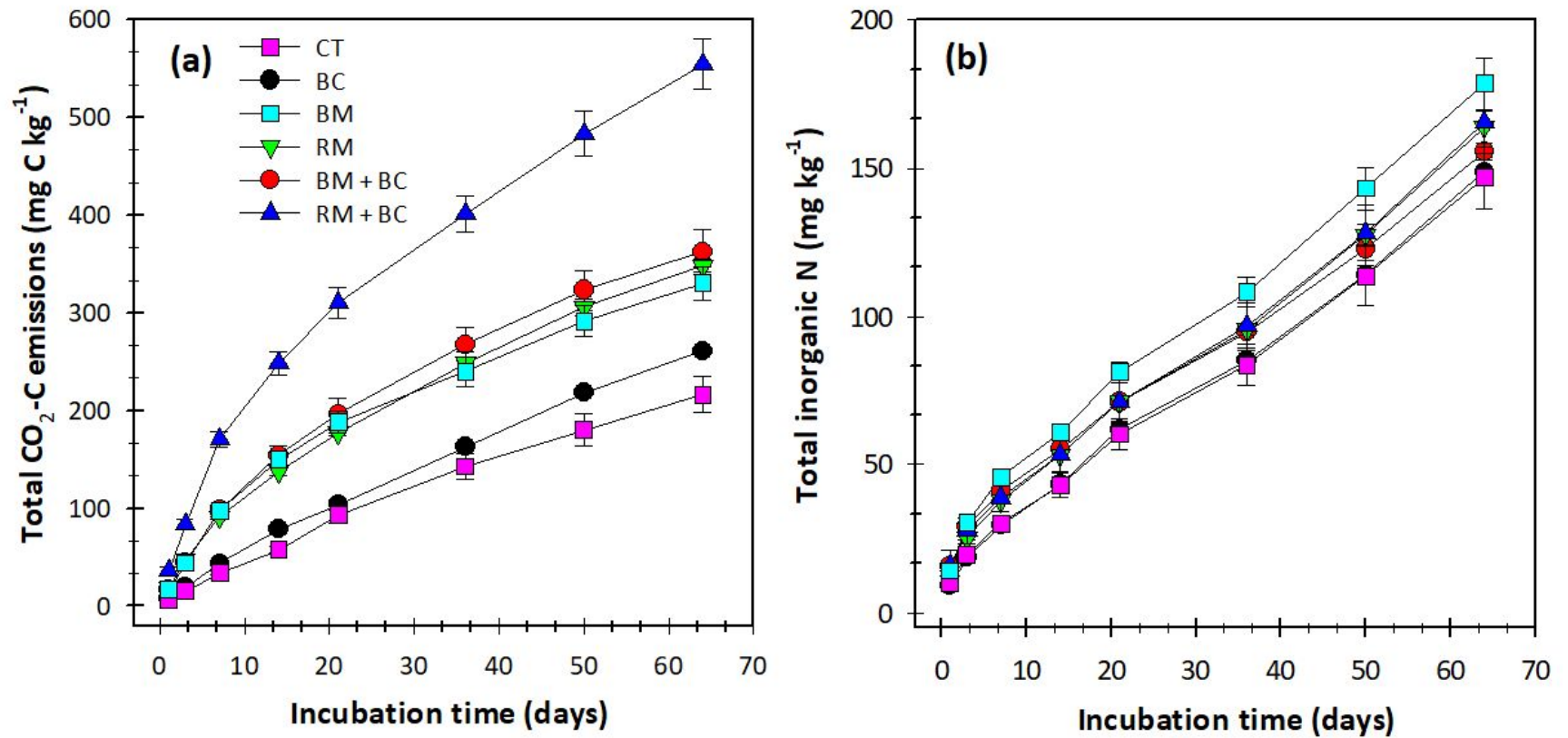


Fig. 1. Respired soil C (a) and mineralized soil N (b) as affected by manure/biochar treatments. Amendments: BC, biochar (10 Mg ha⁻¹); RM, manure from feedlot cattle on a control diet (160 Mg ha⁻¹); CT, control (no amendments); BM, manure from feedlot cattle on a control diet with the addition of BC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