

Impact of amendment with hog, cattle manure, and biochar on N₂O, CO₂, and CH₄ fluxes of two contrasting temperate prairie agricultural soils

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Abstract

Liquid hog manure (LHM) and solid cattle manure (SCM) are valuable soil amendments for the nutrients and organic matter they augment. However, desire to mitigate the N_2O , CO_2 , and CH_4 fluxes associated with their use has led to the question of whether biochar co-applied with LHM and SCM could mitigate these greenhouse gas fluxes. A split-plot design was used at two agricultural field sites with contrasting soil types (Brown and Black) in Saskatchewan, Canada, to assess the effect of LHM and SCM (100 kg N ha^{-1}), alone and in combination with two different biochars applied at 8 Mg C ha^{-1} ; produced using either slow or fast pyrolysis of willow (*Salix* spp.) feedstock. Intact cores were collected from the plots and the N_2O , CO_2 , and CH_4 fluxes were measured during a six-week lab incubation. The cumulative N_2O emissions ($37.9\text{-}1956.8 \text{ mg m}^{-2}$), net CO_2 fluxes ($665.2\text{-}1233.2 \text{ g m}^{-2}$), and CH_4 consumption ($28.3\text{-}90.0 \text{ mg m}^{-2}$) were consistent with previously reported Canadian temperate agricultural soils, with and without LHM, SCM, or biochar addition. The impact of manure amendment on greenhouse gas fluxes was more apparent with LHM than SCM; reflecting higher inorganic N content, narrower C:N, and more easily mineralizable carbon in LHM. Overall, co-applying biochar with the manure sources reduced the manure-related N_2O emissions 31.5 to 43.1% and increased CH_4 consumption 94.1% to $2.1\times$ compared with manure alone. Regardless of soil type, neither of the biochars co-applied with the manures affected the net CO_2 fluxes compared with manure alone. The N_2O emissions were principally influenced by the impact of biochar addition on $\text{NO}_3\text{-N}$ supply and pH, while the net CO_2 fluxes were controlled by the opposing effects of heterotrophic (i.e., CO_2 production) and autotrophic (i.e., CO_2 consumption) respiration. The CH_4 consumption was related to the $\text{NH}_4\text{-N}$ supply and its influence on autotrophic methanotrophy. Co-application of biochar along with manure, particularly the combination of slow pyrolysis biochar and LHM, decreased N_2O emissions and increased CH_4 consumption in these young temperate prairie soils; presumably, through enhanced abiotic sorption and biotic N immobilization, in addition to promoting greater methanotrophic activity.

Introduction

Livestock manure has long been applied to arable crop, forage, and pasture lands as a soil amendment, to improve numerous soil physical, chemical, and biological properties that support increased plant productivity and economic returns [1,2]. Liquid hog manure (LHM) and solid cattle manure (SCM) continue to be applied on western Canadian arable fields, providing a low-cost alternative nutrient source to conventional fertilizers. Saskatchewan livestock producers currently manage roughly 1 million hogs and 2.2 million cattle (≈ 7 and 20% of Canada's inventory, respectively; [3,4]). Collectively, these animals produce an estimated 3 million tonnes of manure annually (Darren Stovin, Livestock Branch, Saskatchewan Ministry of Agriculture; personal communication). The Saskatchewan government's recent economic growth plan involves a 30% increase in provincial livestock cash receipts to \$3 billion annually by 2030 [5]; thus, necessitating the sustainable management of a corresponding enlarged manure stream going forward.

Gaseous losses of N_2O , CO_2 , and CH_4 , associated with LHM and SCM are well documented globally [e.g., 6,7,8] and in the western Canadian prairies [9-11]. Crop and livestock production currently contribute 8.4% (60 Mt CO_2e) of Canada's total annual greenhouse gas (GHG) emissions, of which manure management accounts for approximately 13% [8 Mt CO_2e ; 12]. With growing concern regarding the GHG emissions associated with the global agricultural sector [13] and the GHG legacy effects associated with LHM and SCM application [8], it is prudent to develop effective GHG mitigation practices aimed at reducing the environmental footprint of Saskatchewan's agri-food sector. Enhancing the environmental performance of an expanded livestock industry within the province will also help support Canada's GHG emissions reduction commitment to the Paris Agreement [14-16].

Biochar is produced via thermochemical decomposition of biomass, under oxygen-limited conditions, at temperatures between 300 and 700°C [17]. Biochar is generally produced using either slow or fast pyrolysis, with the slow pyrolysis process involving higher maximum temperature, slower heating rate, and longer residence time compared with fast pyrolysis (Basu, 2013). Although biochar is the least economically desirable pyrolysis co-product, its utility as an effective soil amendment for providing both agronomic and environmental benefits is well known [18-20]. For example, using biochar as a soil conditioner to reduce GHG emissions has been extensively reported [e.g., 21,22,23]. Likewise, biochar has been incorporated at different stages within livestock production systems, to reduce GHG emissions associated with LHM lagoons [24,25], stockpiled SCM [26,27], and supplementing animal diets, to lessen GHG emissions from enteric fermentation and manure [28,29]. Numerous reviews have reported on soil GHG dynamics following biochar addition, alone or in combination with conventional fertilizers [e.g., 30,31,32]. The reported variable effects of biochar addition to soil on GHG fluxes, with and without fertilizers, have been primarily attributed to varying soil type, feedstock, and pyrolysis conditions [33-35]. Many previous biochar studies have been conducted on highly weathered, acidic tropical soils. However, there is a dearth of knowledge regarding the relatively young and fertile temperate prairie soils. Recently, Hangs et al. [36] reported the porous structure, large surface area, and surface chemistry of biochar to provide sorptive capacity that enhanced the soil retention of manure-nitrogen (N), increased the manure-N use efficiency by annual crops, and mitigated inorganic fertilizer ^{15}N movement beyond the application zone during a four-year field study in the Canadian prairies. Nonetheless, few studies have looked at biochar's impact on GHG fluxes associated with manure amendments in Canadian temperate soils [i.e., 37,38,29,39] and to our knowledge, the combination of LHM and biochar remains to be examined.

As interest in the nutrient cycling legacy effects associated with LHM and SCM applications continue to increase [40-43], it is timely to investigate whether biochar co-applied with LHM or SCM may mitigate GHG fluxes from prairie agricultural soils. The objective of this six-week incubation study

was to assess the effect of willow (*Salix* spp.) biochar, produced by either slow or fast pyrolysis, on the N₂O, CO₂, and CH₄ fluxes in two contrasting prairie soil types, alone or in combination with LHM and SCM. We hypothesized the biochars would have a greater impact on the short-term GHG fluxes following the addition of LHM, as it predominantly contains NH₄-N, which contributes to N₂O through nitrification and subsequent denitrification, and that could be easily sorbed by the biochar. Solid cattle manure, with dominantly organic N forms of wide carbon (C):N ratio, would be less influenced by the sorption properties of biochar. Additionally, we expected to see different effects from slow versus fast pyrolysis biochars on GHG fluxes, because of their contrasting physical and chemical properties. Quantifying the impact of co-applying contrasting biochars with animal manures on manure-related GHG fluxes should add to our current understanding of biochar-mediated soil N and C dynamics.

Materials And Methods

Study Sites

The intact soil cores used in this study were collected from the Ap horizon (0-15 cm), from research plots of a companion four-year field study [36], within two agricultural fields located in south-central and north-east Saskatchewan, Canada. The fields are managed as a cereal-legume-oilseed rotation as originally reported in Hangs et al. [36]. The two sites are located within contrasting Soil Climatic Zones and encompass the range of SOC contents typically encountered within the province. The fields are located near Central Butte (UTM Co-ordinates: 13U 399696 5620689) in the southern Mixed Grassland ecoregion and Melfort (13U 532910 5851674) in the northern Boreal Transition ecoregion of Saskatchewan. Both are relatively young and fertile temperate soils, developed in glacial parent material deposited ca. 13,000 years ago [44]. The Central Butte loam soil is an Orthic Brown Chernozem [Aridic Haplocryoll; 45] of the Ardiff Association [46], developed in a moderately stony and moderately calcareous glacial till deposit. The Melfort clay soil is an Orthic Humic Vertisol [Typic Humicryert; 45] of the Melfort Association [47], developed in a non-stony and moderately calcareous glacio-lacustrine deposit. The Central Butte soil (labelled "Brown soil" hereafter) has an Agriculture Capability Classification Rating of 4, with severe limitations that restrict annual crop growth; primarily, resulting from its low organic matter content, which limits its water-holding capacity. Conversely, the Class 1 Melfort "Black" soil has no significant limitations for growing annual crops. These contrasting soils are representative of the diverse arable soil types (e.g., texture, pH, fertility, etc.) encountered in the province (Table 1).

Table 1

Selected physical, chemical, and nutrient properties (0-15 cm) of two contrasting Saskatchewan agricultural field soils used to examine the effects of animal manures and biochars, alone or in combination, on the soil N₂O, CO₂, and CH₄ fluxes during a six-week incubation.

Site	Soil Type ^a	Texture (% sand/clay)	pH _{1:2} ^b	EC _{1:2} ^c	ACCR ^d	SOC ^e	TN ^f	Extractable Nutrients (mg kg ⁻¹)				
								NH ₄ -N	NO ₃ -N	P	K	S
Central Butte	Brown ^g	loam (49/20)	7.0	0.34	4	1.7	0.12	6.1	5.1	14.0	484.8	5.9
Melfort	Black ^h	clay (7/62)	6.8	0.12	1	6.3	0.38	11.5	10.4	18.0	369.8	7.8
^a Taxonomy based on the Canadian [160] and US [45] soil classification systems.												
^b 1:2 (soil:water; on a weight basis) extraction.												
^c Electrical conductivity; dS m ⁻¹ .												
^d Agriculture Capability Classification Rating; unlimited (Class 1) and severe (Class 4) limitations to arable crop production.												
^e Soil organic carbon; %.												
^f Total nitrogen; %.												
^g Orthic Brown Chernozem (Aridic Haplocryoll); Ardiff Association; for a complete description (e.g. map unit, parent material, stoniness, drainage, etc.) see SCSR [46].												
^h Orthic Humic Vertisol (Typic Humicryert); Melfort Association; for a complete description see SCSR [47].												

Experimental Design, Animal Manures, and Willow Biochars

A two-factor (manure and biochar) field experiment was set up in a split-plot design and replicated four times at each site. The three whole plot factors (3×6 m; n=4) were no manure, LHM, or SCM (100 kg N ha⁻¹). The three split-plot factors (1×2 m; n=12) were no biochar or biochar (8 Mg C ha⁻¹) produced from willow using either slow or fast pyrolysis (detailed below). The manure and biochar were broadcast applied at the beginning of the study and incorporated into the soil to a depth of 15 cm using a roto-tiller. For the combined treatments, the manure was applied first, followed by the biochar, and then the plot was roto-tilled. The LHM was collected from an earthen manure lagoon, used to store effluent from a commercial hog barn (Big Sky Farms Ltd., Humbolt, SK, Canada), and thoroughly mixed prior to subsampling for nutrient analyses and field application. The SCM was from a private feedlot pen containing straw bedding that had been piled and passively composted for one-year. The SCM was air-dried and thoroughly mixed prior to analysis and application. The willow biomass feedstock used to produce the biochars was provided by Agriculture and Agri-Food Canada Agroforestry Development Centre at Indian Head, SK, Canada. The biomass was sourced from their wetland vegetation management program that involves harvesting native “willow rings”, on a four- to five-year rotation, which surround thousands of small wetlands throughout the northern glaciated plains of North America [48,49]. The harvested biomass consisted of five willow species (*Salix bebbiana* Sarg., *Salix discolor* Muhl, *Salix eriocephala* Michx., *Salix interior* Rowlee, and *Salix petiolaris* Sm.) and provides a renewable bioenergy feedstock for building space heating. The willow was mechanically harvested using a Bio-Baler™ (Anderson Group Co. Chesterville, QC, Canada), air-dried (ca. 30% moisture content), and shredded using a Haybuster® hammer mill (DuraTech Industries International, Inc. Jamestown, ND, USA). The willow feedstock was sent to Biochar Solutions Inc. (Carbondale, CO, USA) for production of the slow pyrolysis (“Slow”) biochar and the Saskatchewan Research Council (Saskatoon, SK, Canada) for production of the fast pyrolysis (“Fast”) biochar. The proximate and ultimate analyses of the willow-based Slow and Fast pyrolysis biochars used in this study (Tables 2 and 3), along with their corresponding pyrolysis conditions were originally reported in Hangs et al. [50] and Szmigielski et al. [51], respectively. Given the dependence of biochar-C concentration on feedstock and pyrolysis conditions [20], consistently reporting biochar application rates in the literature, in terms of actual C added per hectare, would support meta-analysis efforts to synthesize biochar amendment effects, for developing reliable prescriptions [50].

Table 2

Selected physical and chemical properties of biochars, produced using either slow or fast pyrolysis, derived from willow (*Salix* spp.) feedstock harvested from natural willow rings encompassing wetlands in Saskatchewan, Canada^{a,b}.

	MST ^c	VM ^d	FM ^e	Ash	FC ^f	TC ^g	H ^h	O ⁱ	N ^j	S ^k	PH	PSA ^l	SSA _{BET} ^m	CEC ⁿ	Db ^o	HHV ^p
Biochar	%											µm	m ² g ⁻¹	Meq 100g ⁻¹	g cm ⁻³	MJ kg ⁻¹
Slow	1.6	13.6	87.0	10.6	72.8	81.3	1.9	3.9	0.7	0.04	9.7	500	175	20	0.7	28.9
Fast	2.1	31.2	83.3	10.9	56.5	70.7	3.6	12.0	1.4	0.1	9.5	60	3	26	1.4	26.9
^a These Slow and Fast pyrolysis biochar data were originally published by Hangs et al. [50] and Szmigielski et al. [51], respectively.																
^b Sub-samples were analyzed in triplicate per ASTM D2013, D3172, D3173, D3174, D3175, D4239, D5373, and D5865 by Loring Laboratories (Calgary, AB, Canada) unless otherwise noted.																
^c Gravimetric moisture content.																
^d Volatile matter.																
^e Fixed matter; material remaining after the elimination of volatiles (including ash and organic matter).																
^f Fixed carbon.																
^g Total carbon.																
^h Hydrogen; does not include H from sample moisture.																
ⁱ Oxygen; does not include O from sample moisture.																
^j Nitrogen.																
^k Sulfur.																
^l Particle size analysis; samples were analyzed on a Beckman-Coulter LS Particle Size Analyzer by Core Laboratories Canada (Calgary, AB, Canada).																
^m Specific surface area derived using N ₂ and the Brunauer, Emmett and Teller equation; by Pore Science Labs (Bristol, PA, USA).																
ⁿ Cation-exchange capacity.																
^o Bulk density.																
^p Higher heating value.																

Table 3

Ash element content (%) of biochars, produced using either slow or fast pyrolysis, derived from willow (*Salix* spp.) feedstock harvested from natural willow rings encompassing wetlands in Saskatchewan, Canada^{a,b}.

Biochar	Si	Al	Ti	Fe	Ca	Mg	Na	K	P	S	Undetermined
Slow	28.8	7.3	0.2	3.6	33.7	6.0	1.2	11.1	4.0	1.2	3.0
Fast	35.0	6.0	0.2	3.5	22.3	7.7	1.4	15.5	4.4	1.5	2.7
^a These Slow and Fast pyrolysis biochar data were originally published by Hangs et al. [50] and Szmigielski et al. [51], respectively.											
^b Sub-samples were analyzed in triplicate per ASTM D3682 by Loring Laboratories (Calgary, AB, Canada).											

Greenhouse Gas Fluxes

Prior to seeding the field plots, an intact soil core was collected from the center of each plot using sectioned PVC pipe (10 x 15 cm) inserted into the soil and excavated. Subsequently, the intact soil cores were randomly placed on laboratory benches and incubated (20 °C) for six weeks. The field capacity (FC) for each soil type and treatment combination was determined using a pressure plate [52]. Deionized water was added to bring the soil

to 100% FC (24-36% w w⁻¹; depending on the soil type and treatment) and this moisture level was maintained by monitoring the soil moisture content every two days. Without growing plants, water loss over the incubation period was minimal and gas samples were never collected within 24 hours after watering. Measurements of soil N₂O, CO₂, and CH₄ fluxes during the six-week incubation followed the protocols of Hangs et al. [53]. Briefly, at the end of each week, each core was placed inside of an incubation chamber (16 × 37 cm diameter and height, respectively; Fig. S1), to estimate the N₂O, CO₂, and CH₄ concentration change in the chamber headspace over a 1-h period. The chamber headspace (≈ 5141 cm³) air was continually mixed by an internal fan (0.037 m³ min⁻¹, Sunon Inc., Brea, CA, USA), to prevent gas stratification during the sampling period. Gas samples were collected after 30 and 60 min (C₃₀ and C₆₀, respectively) with a 20-mL syringe (via a rubber septum on the chamber lid) and transferred to pre-evacuated 12-mL Exetainer™ vials (Labco Ltd., High Wycombe, UK) containing silica gel desiccant. Several ambient air samples were collected at the beginning of each sampling time, and the mean value was used as the time zero concentration [C₀; 54]. Gas concentrations were measured using a Varian CP3800 GC (Varian Canada Inc., Mississauga, ON) as detailed in Yates et al. [55] and Agnew et al. [10]. Fluxes of N₂O, CO₂, and CH₄ were calculated as the change in gas concentration during each sampling period as described by Ginting et al. [56] and Agnew et al. [10]. Briefly, if the ratio of (C₃₀-C₀)/(C₆₀-C₃₀) > 1, then fluxes were calculated according to Hutchinson and Mosier [57]; however, if the ratio was ≤ 1, then fluxes were calculated using linear or quadratic regression, depending on which model had a lower *P* value. Cumulative gas production over the six-week incubation study was calculated by interpolating between data points and integrating over time, assuming a constant flux [54].

Soil Analyses

Prior to establishing the field research plots, ten soil samples (0-15 cm) were collected from each site, composited, and a sub-sample analysed to characterize the soils at each site. Details of the following soil analyses were originally reported in Hangs et al. [58]. Briefly, the samples were analyzed for extractable nutrient levels (N, P, K, S), organic C, pH, and EC. Total inorganic N [ammonium (NH₄⁺-N) + nitrate (NO₃⁻-N)] and P were extracted using 2.0 M KCl [59] and modified Kelowna [60] extractions, respectively, with the extracts analyzed colorimetrically (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA). Extractable S was determined using 0.01M CaCl₂ [61] and analyzed using microwave plasma-atomic emission spectrometry (4100 MP-AES; Agilent technologies, Melbourne, Australia). Extractable K was determined using 1.0 M NH₄OAc [62] and analyzed using atomic emission spectroscopy (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Total N was determined using a H₂SO₄/H₂O₂ digest [63] and analyzed colorimetrically as well. Soil organic C was measured using a LECO C632 Carbon Analyzer (LECO Corporation, St. Joseph, MI, USA), after a sulfurous acid pre-treatment, to remove inorganic C [64]. Soil pH and EC [1:2 soil (g):water (g) suspension; 65] were analyzed using an Accumet AP85 pH/EC meter (Accumet, Hudson, MA, USA). Particle size distribution was determined using the modified pipette method [66], after pre-treatment with 30% H₂O₂ to remove organic matter, followed by a 10% solution of sodium hexametaphosphate to disaggregate the soil.

At the end of the incubation period, the soil within each PVC core was removed, dried to a constant weight at 60°C, weighed, and its mass divided by the core volume it occupied, to determine its bulk density (Db). The soil samples were ground using a flail-type grinder (< 2 mm fraction retained) and sub-samples were analysed for cation-exchange [CEC; 62], water-holding capacity [WHC; 67], water filled pore space [WFPS; 67], along with water-extractable organic C (WEOC) and N (WEON) according to Chantigny et al. [68]. Microbial biomass-C (MB-C) and -N (MB-N) were estimated using the fumigation-extraction method [69], with MB-C and MB-N concentrations calculated using *k*_{EC} values of 0.45 and 0.54, respectively [70]. The organic C and N concentrations within filtrates associated with the WEOC, WEON, MB-C, and MB-N analyses were analyzed using a Shimadzu TOC-VCPN analyzer (Shimadzu Corporation, Kyoto, Japan). The microbial metabolic quotient (MeQ; qCO₂; mg CO₂-C hour⁻¹ mg⁻¹ MB-C) was estimated for each core by dividing the hourly microbial CO₂-C respiration measurements from the incubation study by the corresponding MB-C content [71].

Plant Root Simulator (PRS)™-probes (Western Ag Innovations Inc., Saskatoon, SK, Canada) were used to measure the soil N supply rate within each core during the incubation period. The PRS™-probes were inserted at the beginning of the incubation and replaced with fresh PRS™-probes weekly for six weeks. Replacing fresh PRS™-probes in the same soil slot provides an accurate *in situ* measure of soil inorganic N availability and a reliable index of inorganic N supply during the burial period [72]. The PRS™-probe regeneration and analysis followed the protocols of Hangs et al. [73]. Briefly, after removal, the PRS™-probes were washed free of soil with deionized water to ensure complete removal of any residual soil. The PRS™-probes were eluted with 0.5M HCl with the eluate analyzed for NH₄-N and NO₃-N colorimetrically. Prior to re-use, the cation- and anion-exchange PRS™-probes were saturated with Na⁺ and HCO₃⁻, respectively, by being shaken three successive times in 0.5 M NaHCO₃ for four hours.

Statistical analyses

The measurement variable data were subject to an analysis of variance, via a split-plot design model, using the GLIMMIX procedure of SAS [version 9.4; SAS Institute, Inc. Cary, NC; 74]. The statistical approach was consistent with that used for the corresponding field study from which the intact soil cores for this study were collected [36]. Specifically, the whole plot factor was manure, and the split-plot factor was biochar. Soil type, manure, biochar, and year were considered as fixed effects and replicate as a random effect. Exploratory analyses were conducted to find the best covariance structure to model the repeated measurements across time (i.e., week), along with examining residual variances between locations. Ten different covariance structures were assessed and depending on the measurement variable, either a Ante(1), CS, HF, UN, SIMPLE, TOEP, or VC covariance

structure was used (based on the smallest AICc value). Both the covariance structure and a “_residual_” effect were included in a random statement, to model repeated measurements. Any variance heterogeneity between the sites was modeled for all analyses, using the random statement with group option set to site and the subject option set to site×replicate×manure×biochar. The SLICE statement was used to facilitate comparisons for interactions. The lines option for the LSMMeans statement was used to generate letter-based pair-wise means comparisons using least significant differences (LSD; equivalent to Fisher’s protected LSD) at a significance level of 0.05. A principal component analysis (PCA) was also performed using JMP 10 (Version 10; SAS Institute, Cary, NC, USA), to investigate the relationships among the measured variables examined in this study. The cosine of the angle between variable vectors indicates the correlation strength among variables, with variable groupings arbitrarily based using an angle of 40° [i.e., R=0.77; 75]. Variable vectors pointing in the same direction represent direct relationships, opposing vectors indicate indirect relationships, and unrelated variables have vectors perpendicular to each other.

Results

Soil Physical, Chemical, and Biological Properties

Unless otherwise stated, the following comparisons are relative to the unamended control. Neither the manures nor the biochars alone influenced the Brown soil Db and there were minor differences among treatments (Fig. 1a). The SCM+Slow decreased the Brown soil Db 9.4%. Likewise, the SCM+Slow reduced the Db 10.3% (on average) compared with SCM alone and SCM+Fast. The SCM treatments had a 3.8% (on average) lower Db compared with LHM treatments (statistics not shown). None of the treatments affected the Black soil Db (Fig. 1a). The SCM+Slow decreased the Db 8.1% compared with SCM alone and SCM+Fast. The Brown soil Db was 34.6% (on average) greater than the Black soil (Fig. 1a; Table 4). The SCM+Slow increased the Brown soil WHC 14.7%, along with the Brown (11.3%) and Black (4.9%) soil WHC compared with SCM alone (Fig. 1b). The LHM alone and LHM+Slow increased the Black soil WHC 8.4% (on average). There were no significant interactions among the main effects on the WHC (Table 4), and therefore, the following summaries are valid: (i) the Black soil had a 39.2% (on average) greater WHC than the Brown soil; ii) adding manure to soil increased the WHC 4.0% (on average) compared with unmanured soil; and iii) the Slow biochar increased the WHC 3.4% (on average) compared with soil without Slow biochar. The SCM+Slow, SCM+Fast, and LHM+Slow reduced the Brown soil WFPS 4.8% (on average), while the Slow biochar alone had 4.7% greater WFPS than SCM+Slow (Fig. 1c). With the Black soil, the LHM+Fast and SCM+Slow increased the WFPS 3.4% (on average), while the LHM+Fast had 2.51% (on average) more WFPS compared with LHM+Slow and SCM+Fast. The Brown soil had 18.2% (on average) greater WFPS than the Black soil (statistics not shown).

Table 4

ANOVA comparing the effect of animal manures and biochars, alone or in combination, on selected soil properties, cumulative PRS™-probe ammonium (NH₄-N) and nitrate (NO₃-N) supply rates, along with cumulative N₂O, CO₂, and CH₄ fluxes, during a six-week incubation, associated with two contrasting Saskatchewan agricultural field soils.^a

Effect	Db ^b	WHC ^c	WFPS ^d	EC ^e	pH	CEC ^f	WEOC ^g	WEON ^h	WEOC:WEON
Soil	<.001	<.001	<.001	<.001	0.004	<.001	<.001	<.001	0.008
Manure	0.676	0.016	0.196	<.001	0.011	0.619	0.142	<.001	<.001
Biochar	0.002	0.009	0.196	0.184	0.049	0.119	0.048	0.714	0.311
Soil × Manure	0.059	0.061	<.001	0.003	0.838	0.501	0.889	0.818	0.029
Soil × Biochar	0.707	0.496	0.191	0.278	0.476	0.487	0.808	0.581	0.314
Manure × Biochar	0.019	0.215	0.513	<.001	0.063	0.686	0.144	0.481	0.269
Soil × Manure × Biochar	0.246	0.302	0.556	0.006	0.308	0.964	0.351	0.548	0.641
Effect	MB-C ⁱ	MB-N ^j	MB-C:MB-N	MeQ ^k	NH ₄ -N	NO ₃ -N	N ₂ O	CO ₂	CH ₄
Site	0.391	0.056	0.002	<.001	0.177	<.001	<.001	0.010	0.924
Manure	0.519	0.749	0.507	0.555	<.001	<.001	<.001	0.855	0.065
Biochar	0.399	0.093	0.918	0.881	<.001	0.656	<.001	0.627	0.474
Site × Manure	0.967	0.861	0.626	0.885	0.012	0.125	0.064	0.596	0.029
Site × Biochar	0.732	0.015	0.951	0.754	0.648	0.961	0.028	0.651	0.527
Manure × Biochar	0.315	0.830	0.431	0.246	<.001	<.001	0.006	0.208	0.863
Site × Manure × Biochar	0.047	0.198	0.352	0.661	0.275	0.123	0.188	0.008	0.138
^a Significant (<i>P</i> <0.05) effects are bolded. Note: if there are significant interaction effects, then main effect <i>P</i> values must be ignored.									
^b Bulk density.									
^c Water-holding capacity.									
^d Water-filled pore space.									
^e Cation-exchange capacity.									
^f Electrical conductivity.									
^g Water-extractable organic carbon.									
^h Water-extractable organic nitrogen.									
ⁱ Microbial biomass-carbon concentration.									
^j Microbial biomass-nitrogen concentration.									
^k Metabolic quotient (qCO ₂).									

The Slow biochar alone reduced the Brown soil EC 45.0%, while the SCM+Slow increased the EC 33.5% compared with SCM alone (Fig. 1d). For the Black soil, LHM+Fast increased the EC 24.7% compared with LHM alone. The LHM alone had a higher Brown (29.0%) and Black (31.6%) soil EC compared with SCM alone. The Slow biochar alone and SCM+Fast increased the Brown soil pH 5.0% (on average), with no treatments influencing the Black soil pH (Fig. 1e). The SCM+Fast increased the pH 4.1% (on average) compared with SCM alone in both soils. There were no significant interactions among the main effects on pH (Table 4), and therefore, the following summaries are valid: (i) the Brown soil had a higher pH (6.1%; on average) compared with the Black soil; ii) the SCM treatments had a higher pH (3.2%; on average) than the LHM treatments; and iii) the Slow biochar increased pH 1.8% (on average) compared with soil without biochar. The SCM alone was the only treatment to affect the Brown soil CEC (22.4% increase; 16.3 vs 13.3 cmol(+) kg⁻¹, respectively), but co-applying either biochar negated this effect (data not shown). No treatments affected the Black soil CEC (data not shown). The Black soil had 3.2× greater CEC compared with the Brown soil (43.5 vs 13.7 cmol(+) kg⁻¹, respectively; Table 4). Except for the Fast biochar alone, all treatments decreased the Brown soil WEOC 19.5% (on average), while no treatments influenced the Black soil WEOC (Fig. 1f). There were no significant interactions among the main effects on WEOC (Table 4), and therefore, the following summaries are

valid: (i) the Black soil had 2.6× (on average) more WEOC compared with the Brown soil; and ii) the Slow biochar reduced the WEOC 5.1% (on average) compared with soil without biochar. The LHM alone increased the Brown soil WEON 39.2%, with no treatments impacting the Black soil WEON (Fig. 1g). There were no significant interactions among the main effects on WEON (Table 4), and therefore, the following summaries are valid: (i) the Black soil had 3.3× (on average) more WEON compared with the Brown soil; and ii) soils with LHM had 40.6% (on average) greater WEON compared with soils without LHM. The LHM treatments decreased the Brown soil WEOC:WEON ratio 36.7% (on average), along with having a 29.4% (on average) smaller WEOC:WEON compared with SCM treatments for both soils (Fig. 1h). The SCM+Fast increased the Brown soil WEOC:WEON 20.5% compared with SCM alone.

The SCM alone decreased the Brown soil MB-C concentration 25.1%, but no treatments affected the Black soil MB-C concentration (Fig. 1i). The Slow biochar alone had contrasting effects on the MB-N concentration depending on the soil type: decreasing the Brown soil MB-N concentration 30.8% vs increasing the Black soil MB-N concentration 3.0× (Fig. 1j). Likewise, the Slow biochar alone had a 4.2× greater Black soil MB-N concentration than the Fast biochar alone. Adding biochars with the manures had no effect on the MB-C or MB-N concentrations within either soil compared with applying LHM or SCM alone. The only treatment influencing the MB-C:MB-N within either soil was the LHM+Fast (1.3× increase) within the Brown soil (Fig. 1k). The LHM alone had a 30.6% larger MB-C:MB-N compared with SCM alone. The SCM+Slow and SCM+Fast increased the MB-C:MB-N 30.2% (on average) compared with SCM alone. The SCM alone increased the MeQ 53.0% within the Brown soil, but the addition of either biochar negated this effect (Fig. 1l). No treatments impacted the MB-C:MB-N or MeQ of the Black soil (Figs. 1k,l). The Black soil had a 2.9× (on average) greater MB-C:MB-N and a 90.1% (on average) larger MeQ than the Brown soil (Figs. 1k,l; Table 4).

Soil Nitrogen Availability

All treatments decreased the $\text{NH}_4\text{-N}$ supply in both soils (8.8 to 44.2% reduction), except for the Fast biochar alone (Fig. 1m). The Slow biochar alone decreased the $\text{NH}_4\text{-N}$ availability 34.7% (on average) within both soils. The SCM alone reduced the $\text{NH}_4\text{-N}$ supply more in the Brown (39.5%) and Black (17.0%) soils compared with LHM alone. Although neither biochar added with the LHM affected the $\text{NH}_4\text{-N}$ supply of either soil compared with LHM alone, co-applying either biochar with SCM increased the $\text{NH}_4\text{-N}$ availability within both soils 34.9% (on average) compared with SCM alone. The LHM+Slow had a greater impact (4.6× increase) on the Brown soil $\text{NO}_3\text{-N}$ supply than the LHM alone or LHM+Fast (3.6× increase; on average). The SCM alone also increased the Brown soil $\text{NO}_3\text{-N}$ availability 74.4%, but this SCM effect was negated by co-applying either biochar (Fig. 1n). Neither biochar alone influenced the Brown soil $\text{NO}_3\text{-N}$ level. The LHM+Slow increased $\text{NO}_3\text{-N}$ availability 37.0% compared with LHM alone, while SCM+Slow decreased $\text{NO}_3\text{-N}$ availability 38.8% compared with SCM alone. Within the Black soil, the LHM alone did not influence the $\text{NO}_3\text{-N}$ level (Fig. 1n), however, both LHM+Slow (75.6%) and LHM+Fast (39.0%) increased $\text{NO}_3\text{-N}$ availability. The addition of Slow biochar alone decreased the $\text{NO}_3\text{-N}$ supply 37.8%. The Black soil had 2.1× (on average) greater $\text{NO}_3\text{-N}$ availability than the Brown soil (Fig. 1n; Table 4).

N_2O , CO_2 , and CH_4 Fluxes

The LHM treatments, with or without biochar, were the only treatments to impact the Brown soil N_2O emissions (6.3 to 10.5× greater; Fig. 2a). The LHM+Slow reduced the N_2O emissions 43.1% compared with LHM alone. Similarly with the Black soil, adding LHM, with or without biochar, increased the N_2O emissions (2.1 to 3.1×). Co-applying either biochar with LHM reduced the N_2O emissions 31.5% (on average) compared with LHM alone. The SCM+Fast reduced the Black soil N_2O emissions 49.8%, although neither biochar added with SCM influenced the N_2O emissions from either soil compared with SCM alone (Fig. 2a). The N_2O emissions from the unamended Black soil were 5.8× greater than the untreated Brown soil (statistics not shown). Neither biochar alone affected the N_2O emissions from either soil. The Fast biochar alone was the only treatment to influence the Brown soil CO_2 fluxes (34.0% increase) and supported 33.7% greater CO_2 fluxes than Slow biochar alone (Fig. 2b). For the Black soil, LHM+Fast (38.3%) and Slow biochar alone (27.3%) increased the CO_2 fluxes (Fig. 2b). The LHM+Fast had 37.3% greater CO_2 fluxes than LHM+Slow. Regardless of soil type, neither biochar co-applied with the manures affected the CO_2 fluxes compared with either manure alone. Both soils were CH_4 sinks during the incubation period (Fig. 2c). The SCM alone and SCM+Slow increased the Brown soil CH_4 consumption 59.4% (on average), while LHM alone and LHM+Fast decreased (i.e., a less negative value) CH_4 consumption 45.5% (on average). The SCM alone and SCM+Slow supported 2.9× (on average) greater methanotrophy than LHM alone and LHM+Fast, with SCM+Slow also being a 45.3% stronger CH_4 sink compared with SCM+Fast. The LHM+Slow increased CH_4 consumption 94.1% compared with LHM alone. No treatments affected the Black soil CH_4 flux and only minor differences were observed among treatments (Fig. 2c). The LHM alone was a 66.2% stronger CH_4 sink compared with SCM alone. Although neither biochar alone impacted the CH_4 fluxes associated with either soil, SCM+Fast increased the Black soil methanotrophy 2.1× compared with SCM alone (Fig. 2c).

Principal Component Analysis

The PCA ordination accounted for 75.4% of the variability in the two principal axes and identified several distinct groupings among the measured soil variables associated with the N_2O , CO_2 , and CH_4 fluxes during this six-week study, with and without, the addition of manure and/or biochar (Fig. 3). Specifically, variable clustering clearly indicated the following relationships: N_2O fluxes were directly related to NO_3-N availability and indirectly related to pH and WEOC:WEON; CO_2 fluxes were related to several variables either directly (CEC, MB-C:MB-N, MeQ, WEOC, WEON, and WHC) or indirectly (Db, EC, MB-C, MB-N, and WFPS); and the CH_4 fluxes were directly related to NH_4-N availability (Fig. 3).

Discussion

Soil Physical, Chemical, and Biological Properties

The observed soil type differences are primarily due to their differing parent materials, with the Black soil of high clay content developed from a weakly calcareous glaciolacustrine deposit having lower pH and EC levels compared with the Brown loam textured soil developed on moderately calcareous shale-modified glacial till. The contrasting SOC levels of the two soils result from the historically drier Brown soil zone supporting lower plant production and associated above- and below-ground residue inputs to soil, compared with the wetter Black soil zone region of the province. The lower Black soil Db is related to its higher SOC content, while the Black soil greater WHC and CEC are a function of its higher clay and SOC amounts (Table 1). Accordingly, the higher WFPS in the Brown soil is associated with its higher Db, coupled with lower porosity and volumetric moisture content (data not shown). The relative lack of treatment effects on the Black soil WEOC and WEON, compared with the Brown soil, is consistent with the much higher SOC and total N levels within the Black soil (Table 1), which would tend to mask treatment effects more.

The tendency of SCM to reduce Db and increase CEC compared with LHM, especially within the Brown soil, is due to the greater organic matter content of SCM per unit weight. Likewise, the improved WHC of both soils following either manure addition is primarily related to the reduced Db (and associated increased porosity) from the added organic matter. The higher EC following LHM addition compared with SCM is caused by the high EC of the LHM (1,480 dS m^{-1}). The elevated pH following SCM addition compared with LHM is attributed to a liming effect, given the high concentration of base cations within SCM, such as calcium (33,800 mg kg^{-1}), magnesium (12,200 mg kg^{-1}), potassium (9,500 mg kg^{-1}), and sodium (1,400 mg kg^{-1}), along with carboxyl and phenolic hydroxyl functional groups neutralizing H^+ [76-78]. The increased Brown soil CEC following SCM addition is due to the humic acid substances within SCM [79]. The greater WEON (and corresponding decrease in WEOC:WEON) within the LHM treatments is attributed to its greater water soluble organic N content compared with SCM. The reduced Brown soil MB-C concentration with SCM alone is consistent with its enhanced MeQ compared with the unamended control, along with its smaller MB-C:MB-N than LHM alone (Figs. 1k,l). An increasing MeQ indicates decreasing heterotrophic respiration efficiency, where microbes tend to mineralize C instead of assimilating (i.e., immobilizing) it in their biomass, and is consistent with the observed inverse relationship between MeQ and both MB-C and MB-N (Fig. 3). The increased MeQ could be due to moisture stress associated with the surface hydrophobicity of composted SCM when initially applied to soil [80] or changing microbial community structure. The hydrophobicity of SCM was not an issue with the Black soil, given its greater WHC (Fig. 1b). Additionally, the wider MB-C:MB-N with LHM alone compared with SCM alone, likely reflects the proliferation of autotrophic nitrifiers, specifically ammonia-oxidizing Archea (AOA) and bacteria (AOB), following LHM addition containing predominantly NH_4-N .

The reduction in Brown soil EC with Slow biochar alone is presumably due to electrolyte sorption by the biochar, while the increased Brown soil pH following the addition of alkaline biochar is often reported [81]. The increased WHC of both soils with the Slow biochar only is consistent with Szmigielski et al. [51], who reported the Slow biochar increased WHC more than the Fast biochar regardless of application rate. Furthermore, the greater porosity and specific surface area of the Slow biochar, relative to the Fast biochar (Table 2), are recognized to collectively enhance soil aeration, WHC, sorption capacity, and microorganism refugia [82,19,20]. It is important to note the agronomic impact of this increased WHC, albeit minor, can have on crop productivity; particularly, within semi-arid Saskatchewan, where soil moisture deficits often limit annual and perennial plant species growth, including short-rotation coppice (SRC) willow production [83-85]. Specifically, in the companion four-year field study to this incubation work [36], isotopic fractionation $\delta^{13}C$ composition data served as a proxy for historic water-use efficiency and, therefore, an index of moisture stress. The biochar-amended Brown and Black soils reduced the moisture stress of annual crops over a four-year period, compared with the crops growing in plots without biochar. Although both biochars reduced crop moisture stress, the positive effect was more pronounced with the Slow biochar (R. Hangs, unpublished data). The reduction in soil WEOC with the Slow biochar alone is likely due to its large sorptive capacity (Table 2) and biochar's affinity to sorb low-molecular weight organic acids and organic pollutants [86-90]. Szmigielski et al. [51] found the Slow and Fast biochars reduced herbicide activity in soil through sorption. Additionally, in the third year of the companion field study [36], the added biochars mitigated wild oat (*Avena fatua*) antagonism against wheat (*Triticum aestivum* L.) growth; presumably by mollifying allelochemical activity via sorption (R. Hangs, unpublished data). The contrasting effects of the Slow biochar alone on the Brown soil MB-C and MB-N (decreased) compared with the Black soil (increased) could be related to a greater sensitivity of the heterotrophic community to reduced WEOC availability in the Brown soil containing less SOC (Table 1).

Compared with SCM alone, the decreased Db and increased WHC of SCM+Slow for both soils, along with a reduction in the Brown soil WFPS with SCM+Slow and SCM+Fast, may indicate a synergistic interaction between SCM and the biochars. Considering both SCM and biochar amendments are known to improve Db, WHC, and WFPS [77,50,91], co-applying these low-density materials may contribute to the improved soil structure, aeration, and hydraulic properties, especially within the higher Db Brown soil. The decreased Brown soil CEC and MeQ observed with SCM+biochars,

compared with SCM alone, may be due to biochar sorption of SCM-related humic substances and SCM-WEOC, which reduced the soil CEC and labile-C available for heterotrophic metabolism, considering the recalcitrant nature of WEOC associated with composted SCM [92].

The Brown loam soil properties generally were more responsive to the imposed treatments than the Black clay soil in this study, which is consistent with the companion four-year field study [36], along with most reports indicating poorer quality soils are more responsive to biochar additions than higher quality soils. Overall, the added manures and biochars, alone or in combination, had a minor impact on the measured soil physical, chemical, and biological properties of both soils and likely reflects the good quality of the young temperate prairie soils used. Moreover, the limited treatment effects may be explained by only a single manure application, along with the relatively low rate of biochar used in this study. Recent investigative and meta-analyses indicate >10 Mg biochar-C ha⁻¹ is generally required to influence young fertile temperate soil properties [93,35,94].

Soil Nitrogen Availability

The cumulative six-week NH₄-N (13.5-24.2 mg 10 cm² six weeks⁻¹) and NO₃-N (337.4-3456.9 mg 10 cm² six weeks⁻¹) supply rates are consistent with values reported elsewhere for Canadian prairie soils amended with LHM, SCM, or biochar [95,96,50,42]. The NO₃-N supply represented 97.9% (averaged across all treatments and soils) of the total inorganic N available and was two orders of magnitude larger than the NH₄-N supply; reflecting the rapid nitrification typically occurring within these temperate prairie agricultural soils [97]. For example, although 93% of the total LHM-N added was in the form of NH₄-N (7% organic N; data not shown), the LHM, with or without biochar, did not increase the NH₄-N supply within either soil (Fig., 1m). The decreased NH₄-N availability within both soils following manure addition, with and without biochar, may be attributable to a combination of enhanced NH₄-N oxidation to NO₃-N by AOA and AOB, along with net NH₄-N immobilization from autotrophic metabolism, along with heterotrophic mineralization of the added labile-C [98,99]. The greater NH₄-N levels observed with the Fast biochar alone compared with Slow biochar alone, within both soils, could be the result of its greater CEC protecting sorbed NH₄-N from autotrophic and heterotrophic activity [100,101,32]. The enhanced net mineralization (i.e., increased NH₄-N supply rate over and above immobilization) with the SCM+Slow and SCM+Fast compared with SCM alone, within both soils, is likely a combination of the biochars augmenting heterotrophic mineralization of the added SCM-organic N, while protecting a portion of the mineralized NH₄-N from microbial metabolism through sorption mechanisms. Additionally, the increased WHC of SCM+Slow (Fig. 1b) would also increase the supply of diffusion-limited nutrients like NH₄-N.

The greater Black soil NO₃-N availability compared with the Brown soil is indicative of the larger mineralizable N content within the Black soil (Table 1). The higher NO₃-N supply associated with the LHM compared with SCM, with or without biochar, is consistent with previous work comparing these manures [1] and represents their contrasting predominant N forms and associated temporal availability: LHM (narrow C:N, inorganic, and immediate) vs SCM (wide C:N, organic, and slow-release). Additionally, the organic N within the composted SCM used is more recalcitrant and mineralizes slower than fresh SCM [102-105]. The SCM alone increased the Brown soil NO₃-N only; presumably due to the inherently higher Black soil NO₃-N level masking the relatively small amounts of mineralized SCM-organic N. The lack of fertilizer N effect from the added biochar-N (105 kg N ha⁻¹; on average) on the NO₃-N availability within either soil, is not surprising given the inherently good quality of these young temperate soils (particularly, the fine-textured Black soil), the low N content of willow stem feedstock, and the recalcitrant nature of lignocellulosic biochar-N [58,106,19]. However, Hangs et al. [50] reported that 20 Mg C ha⁻¹ of the Slow biochar immobilized soil N within the Brown soil and reduced NO₃-N levels (\approx 50%), with and without added urea (100 kg N ha⁻¹). In the same study, the Slow biochar did not affect the NO₃-N level within a loam Orthic Black Chernozem (Typic Haplocryoll), with or without urea. In this study, only 8 Mg C ha⁻¹ of the Slow biochar decreased the Black clay soil NO₃-N supply (Fig. 1n); primarily caused by biotic fixation (i.e., autotrophic and heterotrophic immobilization; Fig. 1j), considering its wider C:N ratio than the Fast biochar (Table 2). Such seemingly contradictory results reaffirm the importance of assessing the many soil×biochar interactions that may exist before generalizing biochar amendment effects (and application rates) across broad soil types.

The higher NO₃-N supply with the LHM+Slow than LHM alone and LHM+Fast within both soils (Fig. 1n), may be due to three biochar-related factors [107,108,93]: i) the Slow biochar provided a more favourable ecological habitat; resulting in enhanced autotrophic nitrification by AOA and AOB. ii) the Slow biochar contributes to faster movement of NO₃-N to the PRS™-probe ion-exchange resin membrane surface, given its greater porosity (Table 2); and iii) the Fast biochar presumably has a stronger anion-exchange capacity to fix adsorbed NO₃-N, which is common among inactivated lignocellulosic biochars produced at lower temperatures. Considering the majority of cereal crop plant N uptake from soil occurs within several weeks after plant emergence [109], the observed difference in plant available NO₃-N between the Slow and Fast biochars co-applied with LHM observed during this six-week study is consistent with the following first-year results from the companion field study [36]: i) applying the Fast biochar with LHM reduced the production of barley (*Hordeum vulgare* L.) grain (25%) and total biomass (29%) at both field sites, along with straw (29%) at the Brown site compared with LHM alone; ii) both LHM alone (72%) and LHM+Slow (57%) increased barley N uptake, whereas LHM+Fast had no effect; and iii) less fertilizer ¹⁵N was recovered by barley growing within the Fast biochar plots compared with Slow biochar plots. The lower Brown soil NO₃-N level within the SCM+Slow compared with SCM alone, may be caused by reduced NH₄-N availability for nitrification, via biochar sorption, which evidently rendered a portion of the NH₄-N available for PRS™-probe measurement, but less accessible for microbial metabolism (Figs. 1k,m,n). Within the Black soil, the muted effects of either manure, alone or in combination with biochar, reflect the Black soil having more soil

organic matter and inherently stronger buffer capacity to maintain soil solution N concentration (Table 1). It is important to note, however, after the first year of the companion field study, the SCM+Slow increased the residual $\text{NO}_3\text{-N}$ content within both the Brown (55%) and Black (49%) soils compared with SCM alone [36]. Additionally, compared with either the SCM or Slow biochar applied alone, the SCM+Slow increased canola (*Brassica napus* L.) production (i.e., a high N-demanding crop) at the Brown (year 2) and Black (year four) sites [36]. Consequently, the co-application of the Slow biochar with SCM appears to reduce SCM-N availability within these two soils in the short-term (i.e., weeks; this study), but enhance SCM-N availability for plant uptake over the long-term (i.e., years; companion field study). The precise mechanism(s) is unclear, but the Slow biochar could be inhibiting SCM-N mineralization and/or immobilizing SCM-N in the short-term, and subsequently augmenting SCM-N mineralization and/or preserving liberated SCM-N ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and dissolved organic N) over the long-term via sorption [110,108,93,29].

N₂O Fluxes

The estimated six-week cumulative N_2O emissions ranged from 37.9-1956.8 mg m⁻² (Fig. 2a) and are consistent with Canadian temperate agricultural soils, with and without, LHM, SCM, or biochar addition [111,10,53,29,50]. The principal edaphic properties governing soil N_2O emissions are abundant inorganic N, labile SOC for denitrifying heterotrophs, and anoxic conditions [97]. These conditions were most prevalent within the Black soil (Tables 1 and 4; Figs. 1b,f,n), which helps to explain the larger N_2O losses from the unamended Black soil compared with the untreated Brown soil. Despite less WFPS within the Black soil (61.3%) than the Brown soil (72.4%), anaerobic microsites occur more frequently within clay soil [112,113]. Moreover, both soils surpassed the critical threshold of 60% WFPS for denitrification [114,111,115].

The relationship between LHM addition, with or without biochar, and elevated N_2O emissions from both soils may be the result of the following [116-119]: i) N_2O losses associated with autotrophic nitrification (Fig. 1n); ii) denitrifying community responding to increased $\text{NO}_3\text{-N}$ availability (Fig. 3); and iii) prolific heterotrophic activity in response to the added LHM dissolved organic-C and -N creating anaerobiosis within soil aggregate microsites. Accordingly, the greater N_2O emissions attendant with the LHM explain the lack of LHM alone effect on the Black soil $\text{NO}_3\text{-N}$ supply (Figs. 1n and 2a). Likewise, the relatively larger amounts of dissolved organic-C and -N within LHM supported higher rates of nitrification and denitrification compared with SCM-amended soils [Figs. 1n and 2a; 10].

Reduced N_2O emissions following biochar addition are generally considered to be associated with the following [108]: i) improved soil aeration/gas exchange due to reduced Db (and correspondingly greater porosity); ii) reduced WFPS; iii) biotic N immobilization and/or biochar sorption lowering soil $\text{NO}_3\text{-N}$ supply; and iv) elevated pH. All these factors likely contributed to lower N_2O emissions in this study, but the influence of biochar on soil pH (indirect) and $\text{NO}_3\text{-N}$ availability (direct) appeared to be the most relevant factors (Fig. 3). Interestingly, however, the reduced N_2O emissions from both soils with the LHM+Slow compared with LHM alone corresponded with enhanced $\text{NO}_3\text{-N}$ supply (Figs. 1n and 2a); thus, indicating enhanced LHM-N conservation by the Slow biochar. Likewise, the greater Brown soil N_2O emissions from the LHM+Fast than LHM+Slow, help account for the lower $\text{NO}_3\text{-N}$ level within the LHM+Fast and could be due to the more labile-C within the Fast biochar supporting greater N_2O emissions. Additionally, recent work involving western Canadian Chernozemic soils of similar textures, with and without the addition of a lignocellulosic slow pyrolysis biochar, suggests a narrowing WEOC:WEON is indicative of greater microbial metabolism [120], which agrees with the observed inverse relationship between WEOC:WEON and the microbially mediated pathways (i.e., autotrophic and heterotrophic) controlling the $\text{NO}_3\text{-N}$ supply and N_2O fluxes in our study (Fig. 3).

The greater Brown soil N_2O emissions with the LHM+Fast than LHM+Slow, may be due to reduced gas permeability and diffusivity, resulting from soil pore clogging associated with the much smaller Fast biochar particles (Table 2), causing less aeration and increased denitrification [19]. The Brown soil was more susceptible to reduced gas exchange, given its greater Db (i.e., lower pore space volume), less WHC, and WFPS compared with the Black soil (Figs. 1a-c). The reduced $\text{NO}_3\text{-N}$ availability following Slow+SCM (Brown soil) and Slow biochar alone (Black soil), without attendant increased N_2O emissions, could be due to the following [110,108,98,121,93]: formation of organo-biochar-mineral complexes that stabilized organic N and, therefore, slowed its mineralization, especially within clay soils; ii) preservation of SCM-N ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and dissolved organic N) via Slow biochar sorption; and iii) biotic N immobilization. Presumably, this N will become available eventually and these slow-release N mechanisms would explain the apparent synergism between SCM+Slow, to increase canola growth at both the Brown (year 2) and Black (year 4) field sites following a single application of SCM and Slow biochar [36]. As such, the results from this six-week incubation study are consistent with the meta-analysis of Gao et al. [93], who reported co-applications of biochar with organic fertilizers can improve inorganic N availability for crop uptake.

Although the capacity of lignocellulosic biochar to reduce soil N_2O emissions is well known [e.g., 122,123,124], the inability of either biochar alone to influence the N_2O emissions in our study is attributed to the relatively low rate of biochar applied and the inherently good N retention capacity of these two soils. Cayuela et al. [125] suggest lignocellulosic high-temperature slow pyrolysis biochars facilitate electron transport during denitrification; thus, coupled with its higher pH and greater surface area for N_2O sorption [33,126] compared with Fast biochar (Table 2), may have promoted more complete denitrification (i.e., N_2O reduction to N_2), thereby decreasing the $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ ratio. Increased pH under aerobic conditions favours $\text{NH}_4\text{-N}$ sorption by biochar and reduced nitrification, while under anerobic conditions supports complete reduction to N_2 [Fig. 3;

127]. In saturated alkaline soils, N_2 is the primary source of denitrification loss [97], which although not a greenhouse gas, results in decreased manure-N use efficiency.

CO₂ Fluxes

The estimated six-week cumulative CO₂ fluxes ranged from 665.2-1233.2 g m⁻² (Fig. 2b), and are consistent with Canadian temperate agricultural soils, with and without, LHM, SCM, or biochar addition [128,129,10,53,50,29]. The observed CO₂ fluxes were considered to be primarily governed by heterotrophic respiration, given the direct relationship between the CO₂ flux and WEON, WEOC, MB-C:MB-N, MeQ, and WHC, along with its indirect relationship with Db (i.e., porosity) and WFPS (Fig. 3). For example, the Slow biochar addition to the Black soil enhanced heterotrophic mineralization-immobilization as evidenced by the increased CO₂ flux and MB-N, with a corresponding reduction in NH₄-N and NO₃-N supply (Figs. 1j,n and 2b). Likewise, the enhanced Brown soil CO₂ flux with the Fast biochar alone, particularly compared with Slow biochar alone, may be caused by the greater amount of labile-C added with the Fast biochar stimulating heterotrophic activity. Larger CO₂ fluxes associated with lower temperature biochars, compared with higher temperature biochars, are often reported [e.g., 130,124,131] and help to explain the greater Black soil CO₂ flux (mineralization) and lower NO₃-N supply (immobilization) in the LHM+Fast than LHM+Slow (Figs. 1n and 2b). As such, the C:N values of our Slow (116) and Fast (51) biochars were not a reliable indicator of biochar-C mineralization potential compared with their volatile matter and fixed C contents (Table 2). Another useful metric for assessing the biochar-C mineralization potential is its hydrogen (H):C, which is indirectly related to its recalcitrance [132-134] and was relevant in this study.

Considering manure and biochar amendments are well known to promote microbial abundance, activity, and diversity by improving soil physical and chemical properties [e.g., 135,136,137], the inverse relationship between CO₂ flux and both MB-C and MB-N were surprising (Fig. 3). For example, the C and N substrates added with the LHM and SCM treatments would be expected to increase heterotrophic respiration, especially within the lower SOC-containing Brown soil. Biochar is capable of immobilizing CO₂ through surface sorption and precipitation reactions, to form CaCO₃ (due to increased pH and calcium availability associated with the biochar), but these are relatively minor CO₂ sinks [136]. Likewise, CO₂ sorption reactions with soil minerals and organic matter, along with pedogenic (i.e., secondary) CaCO₃ recrystallization do occur in calcareous soils, but likewise, are minor CO₂ sinks [138,139]. Instead, however, these apparently contradictory results likely reflect the concurrent opposing influence of autotrophic (i.e., CO₂ sink) and heterotrophic (i.e., CO₂ source) communities on the measured CO₂ fluxes [i.e., net CO₂ emissions; 50]. Specifically, treatments adding labile-C and -N were simultaneously supporting both autotrophic respiration (i.e., CO₂ consumption) and heterotrophic respiration (i.e., CO₂ production). The inherent complexity of the treatments varying influence on autotroph and heterotroph activity collectively impacted the measured CO₂ fluxes and were manifest by the significant ($P < 0.01$) interaction among all main treatment effects (Table 4) and minor treatment effects on CO₂ flux (Fig. 2b). These concurrent CO₂ sink/source pools also help to explain the highly variable MeQ data in this study (Fig. 1l), along with numerous inconsistent reports of MeQ for more than a decade following biochar additions that varied markedly depending on soil N availability [86,140]. Heterotrophic respiration has long been considered the largest regulator of soil CO₂ flux and sole consideration when calculating MeQ [141]. However, unless the proportional influence of autotrophic and heterotrophic respiration on measured CO₂ flux can be quantified, then caution needs to be exercised when basing inferences regarding soil ecosystem health on MeQ data, which does not account for autotrophs (i.e., their biomass or CO₂ consumption) during the measurement period.

CH₄ Fluxes

Well-drained temperate agricultural soils typically are sinks for atmospheric CH₄ and our study was no exception; with average CH₄ consumption after six weeks among treatments (28.3-90.0 mg m⁻²; Fig 2c) consistent with Canadian temperate agricultural soils, with and without, LHM, SCM, or biochar addition [129,10,50,29]. Like the CO₂ fluxes, the measured CH₄ fluxes are a net balance of CH₄ methanogenesis and methanotrophy. Several edaphic factors favour methanotrophy [142-144]: (i) enhanced aggregation, structure, and porosity causing more microaerophilic niches and, therefore, greater CH₄ diffusivity to aerobic microsites; (ii) alkaline pH; (iii) low EC; (iv) moisture content at/or below FC (and/or less than 60% WFPS); and (v) large C:N accentuated by wide C:N amendments. The imposed treatments positively affected these physical and chemical properties to varying degrees, albeit slightly, for both soils (Figs. 1a-e). Consequently, the observed methanotrophy in this study was primarily influenced by NH₄-N availability (Fig. 3). For example, the much greater CH₄ consumption with the SCM alone than LHM alone was principally due to the comparatively large amount of NH₄-N added with the LHM manure causing a shift in autotroph (primarily AOA) methanotrophic activity, which preferentially oxidize NH₄-N instead of CH₄, following the addition of NH₄-N or NH₄-N forming inorganic or organic fertilizers [145-147]. In the absence of plants, this shift in autotrophic methanotrophy to nitrification is evidenced by increased NO₃-N availability concurrent with decreased CH₄ consumption [50] and was most apparent when comparing the greater Brown soil NO₃-N supply, coupled with less CH₄ oxidation, associated with LHM alone than SCM alone (Figs. 1n and 2c). Furthermore, rapid nitrification often favours N₂O production, along with reduced CH₄ consumption (and vice-versa), which agrees with the enhanced Brown soil N₂O emissions and reduced CH₄ sink strength associated with the LHM alone and LHM+Fast compared with the unamended control and SCM alone (Figs. 2a,c). It is desirable to observe the Slow biochar co-applied with

LHM mitigated the elevated N_2O emissions (both soils) and decreased CH_4 consumption (Brown soil) typically associated with LHM amendments (Figs. 2a,c). Presumably, this biochar-induced effect involved enhanced abiotic and biotic N immobilization, in addition to promoting greater methanotrophic activity. Interestingly, despite greater Black soil N_2O emissions with the LHM alone than SCM alone, the LHM alone promoted more CH_4 consumption than SCM alone (Figs. 2a,c) and could be related to the more favourable soil aeration and porosity following LHM addition (i.e., Db and WHC; Figs. 1a,b). The increased Black soil methanotrophy with the SCM+Fast than SCM alone is probably due to the preservation of mineralized SCM-N from nitrification through biochar sorption; indicated by its greater $\text{NH}_4\text{-N}$ supply (Fig. 1m). Enhanced CH_4 consumption following biochar amendment is often reported [e.g., 143,50,148] and is attributed to not only its improvement of the edaphic factors favouring CH_4 oxidation, but also, CH_4 retention via biochar sorption further facilitating CH_4 oxidation [149-151]. However, the inability of either biochar alone to affect the CH_4 consumption of either soil, along with no treatment effects on the Black soil methanotrophy is not uncommon [151,31,34] and likely reflects the relatively low rate of biochar added and inherent good quality of these young temperate prairie soils.

Implications for Lifecycle Analyses of SRC Willow Bioenergy Production

Widespread biofuel adoption will be aided by these fuels being accepted as CO_2e -negative in their influence on atmospheric GHG concentrations [152]. Over the past two decades, the life cycle analysis (LCA) of net GHG emissions associated with SRC willow bioenergy production has progressed from initially being considered a weak C-source to a currently being deemed a large sink for atmospheric C, as increasing above- and below-ground C-sink crediting data have become available [50]. Likewise, if accounted for, the reduced GHG emissions associated with the co-firing of willow biomass with coal for electricity generation within large jurisdictions [e.g., Saskatchewan; 153,154] would further support the CO_2e -negative narrative of SRC willow bioenergy. This evolving perspective is an excellent example of how expanding LCA boundary conditions can profoundly influence subsequent conclusions. Consequently, the GHG mitigation benefits of bioenergy fuel chain co-products, such as biochar, warrant consideration within LCAs [155,30,156]. Currently, there is a general lack of interest in commercial scale slow pyrolysis biochar production, due to the perceived absence of any market value [157]. However, if the agroecosystem benefits of slow pyrolysis biochar can be valued, then a further economic justification of SRC willow exists for growers [158]. In our study, the Slow biochar effectively reduced the LHM-related N_2O emissions, while increasing the soil CH_4 sink strength, compared with LHM alone. Moreover, neither biochar co-applied with manure affected the CO_2 fluxes in either soil compared with the manures alone.

As such, co-applying the willow biochar with manure appears to not only support present agronomic initiatives seeking novel approaches to maximize manure-N use efficiency within temperate regions [159], but also, represents an innovative practice for mitigating GHG fluxes associated with land-applied manure, which would help demonstrate Canada's compliance to its Paris Agreement commitment [14,15]. Considering our study examined short-term GHG fluxes after a single application of manure and biochar under controlled conditions, we recommend assessing the long-term GHG fluxes *in situ* over several field seasons, after a single biochar application (at a variety of rates) and following repeated annual manure additions (representative of conventional practices), to validate our results and/or augment our understanding of the mechanisms governing our observed GHG fluxes. If consistent results are found under field conditions, then new LCAs encompassing an even broader perspective (i.e., "womb to tomb") of SRC willow bioenergy production could incorporate the GHG mitigating benefits of co-applying biochar with manure, to further improve the estimated net GHG emissions associated with this renewable energy alternative.

Conclusion

Few studies have investigated biochar's potential to mitigate GHG fluxes associated with manure applied to Canadian temperate soils, and to our knowledge, this is the first to examine the combination of biochar with LHM. The estimated six-week cumulative GHG fluxes in this study were consistent with Canadian temperate agricultural soils, with and without, LHM, SCM, or biochar addition. The N_2O emissions were primarily affected by soil $\text{NO}_3\text{-N}$ supply and pH, while the relationship between WEOC:WEON (as an index of microbially mediated N dynamics) and both $\text{NO}_3\text{-N}$ supply and N_2O emissions was also identified. Although the observed CO_2 fluxes were principally governed by heterotrophic respiration, the lack of treatment effects on CO_2 fluxes, despite augmenting the labile-C and -N levels (particularly within the poorer quality Brown soil), indicates concurrent stimulation of autotroph and heterotroph activity. Consequently, these co-existing opposing CO_2 sink/source pools, confounded the CO_2 fluxes measured in this study and we suspect many more. Therefore, caution is required when interpreting MeQ values that neither account for autotroph biomass nor autotrophic CO_2 consumption. Both soils were CH_4 sinks during the incubation period and CH_4 consumption was chiefly affected by $\text{NH}_4\text{-N}$ availability and its impact on methanotrophic autotrophs that preferentially oxidize $\text{NH}_4\text{-N}$ instead of CH_4 . The impact of manure amendment on these GHGs, alone or in combination with biochar, was more apparent with LHM than SCM and reflects their contrasting predominant N forms and associated temporal availability. Despite using the same willow feedstock, several physical and chemical Slow biochar properties favourably impacted the GHG fluxes: large fixed C content, specific surface area, and particle size; low bulk density and volatile matter content; wide C:N; narrow H:C; and high pH.

Co-applying biochar with animal manure, particularly LHM, appears to be an effective management approach for improving N_2O and CH_4 fluxes in these young temperate prairie soils. However, given the short-term nature of our study, long-term field studies are needed across the broad

geoclimatic gradient within Saskatchewan, to validate our results and assess the permanence of the beneficial biochar influence observed. If favourable biochar legacy effects on manure-GHG fluxes exist under field conditions, then we suggest future willow bioenergy LCAs expand their boundaries by including the manure-related GHG mitigating influence of co-applying willow biochar with animal manure, to improve the net GHG balance of SRC willow bioenergy and, therefore, more attractive to renewable energy policy makers. We recommend assessing the long-term GHG fluxes *in situ* over several field seasons, after a single biochar application at a variety of rates and following repeated annual manure additions representative of conventional practices.

To further elucidate the role of co-applied biochar on manure-N dynamics for developing management strategies aimed at increasing manure-N use efficiency and, therefore, benefit both environmental and agronomic interests, we suggest the following work: i) determine if willow biochar affects manure-related NH_3 and N_2 fluxes when co-applied with LHM and SCM; ii) investigate the relative contributions of autotrophic (i.e., CO_2 consumption) and heterotrophic (i.e., CO_2 production) respiration on measured CO_2 fluxes by incorporating $^{13}\text{CO}_2$ into GHG experimental designs; and iii) the anion-exchange capacity of biochar is a metric rarely reported and is unknown for our biochars. Considering $\text{NO}_3\text{-N}$ is both environmentally and agronomically important within neutral to alkaline prairie soils, exploring the relationship between biochar- $\text{NO}_3\text{-N}$ sorption and associated long-term $\text{NO}_3\text{-N}$ availability following fertilizer N amendments (inorganic or organic) is warranted.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Both authors contributed to the study conception and design, material preparation, data collection, along with manuscript writing and editing. Both authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

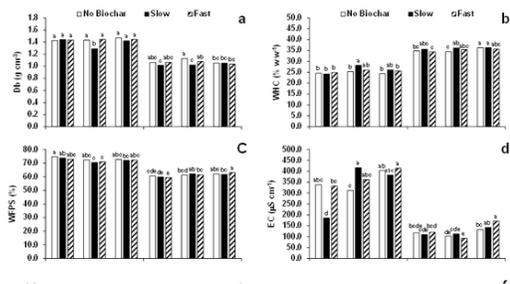


Figure 1

Mean (n=4) soil **a** bulk density (Db), **b** water-holding capacity (WHC), **c** water-filled pore space (WFPS), **d** electrical conductivity (EC), **e** pH, **f** water-extractable organic carbon (WEOC), **g** water-extractable organic nitrogen (WEON), **h** WEOC:WEON ratio, **i** microbial biomass carbon (MB-C), **j** microbial biomass nitrogen (MB-N), **k** MB-C:MB-N; **l** microbial metabolic quotient (MeQ; qCO_2), **m** PRS™-probe ammonium (NH_4 -N) supply rate, and **n** PRS™-probe nitrate (NO_3 -N) supply rate associated with two contrasting Saskatchewan agricultural field soils during a six-week incubation study, with and without, animal manures (100 kg N ha^{-1} ; liquid hog manure or solid cattle manure) or biochar (8 Mg C ha^{-1} ; Slow or Fast pyrolysis), alone or in combination. Note: for each variable and soil type, means with the same letter are not significantly different ($P > 0.05$) using LSD.

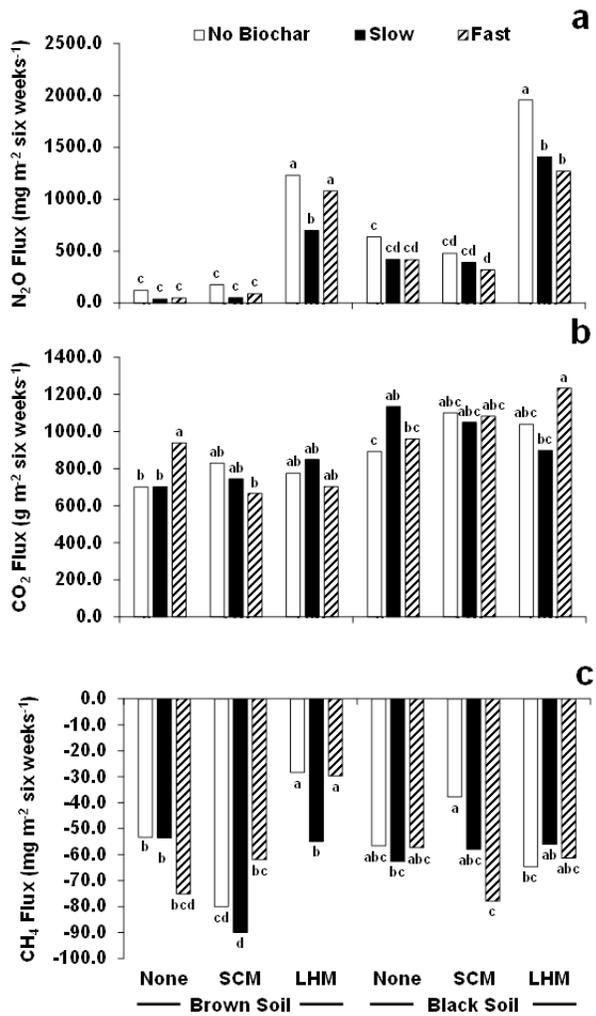


Figure 2

Mean (n=4) greenhouse gas fluxes associated with two contrasting Saskatchewan agricultural field soils during a six-week incubation study, with and without, animal manures (100 kg N ha⁻¹; liquid hog manure or solid cattle manure) or biochar (8 Mg C ha⁻¹; Slow or Fast pyrolysis), alone or in combination. Note: for each gas and soil type, means with the same letter are not significantly different ($P > 0.05$) using LSD.

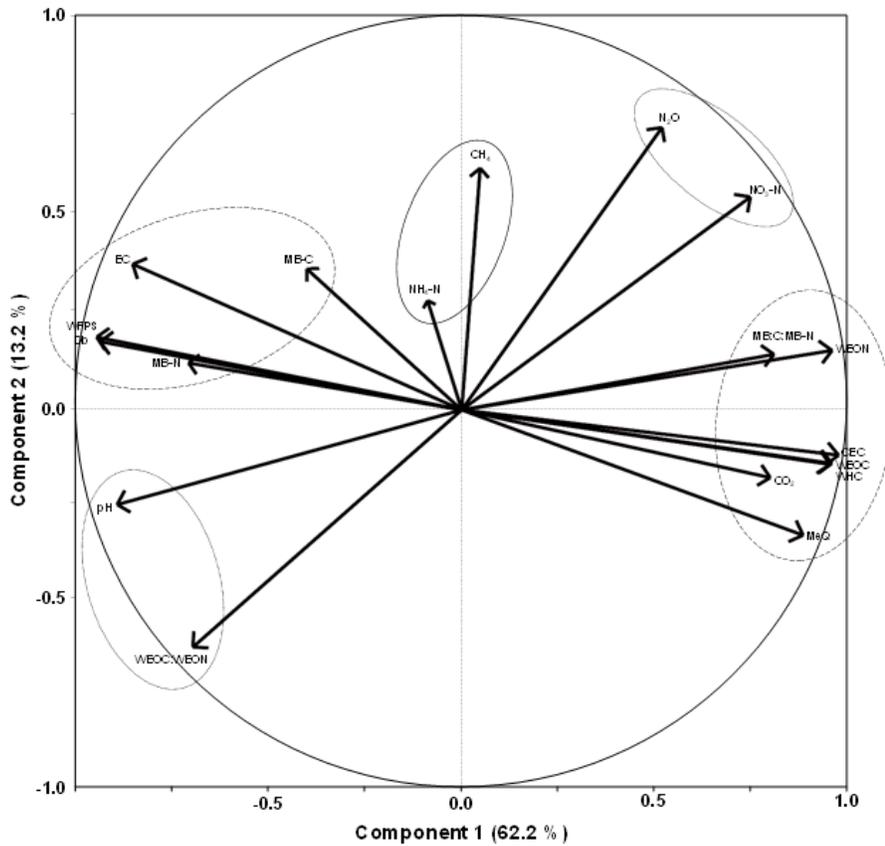


Figure 3

Principal component analysis of measured soil physical, chemical, and biological variables related to the greenhouse gas fluxes associated with two contrasting Saskatchewan agricultural field soils during a six-week incubation study, with and without, animal manures (100 kg N ha⁻¹; liquid hog manure or solid cattle manure) or biochar (8 Mg C ha⁻¹; Slow or Fast pyrolysis), alone or in combination. Eighteen variables were assessed: cumulative N₂O, CO₂, and CH₄ fluxes; bulk density (Db); cation-exchange capacity (CEC); electrical conductivity (EC); microbial biomass carbon (MB-C) and nitrogen (MB-N); MB-C:MB-N; microbial metabolic quotient (MeQ; qCO₂); pH; PRS™-probe ammonium (NH₄-N) and nitrate (NO₃-N) supply rates; water-extractable organic carbon (WEOC) and nitrogen (WEON); WEOC:WEON; water-filled pore space (WFPS); and water-holding capacity (WHC).

Supplementary Files

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