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Biochar

ORIGINAL RESEARCH

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Effects of biochar, hydrochar and nitrogen fertilization on greenhouse gas fluxes, soil organic carbon pools, and biomass yield of a boreal legume grassland

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Abstract

Char amendment is an option to lower climatic impact of agricultural soils. However, their effect can vary depending on char and soil properties, vegetation type and their interactions. Nutrient poor and acidic soils of boreal region could benefit from char amendment. We conducted a three-month long mesocosm study representing a typical boreal forage-legume grassland to understand the effects of char application on greenhouse gas (GHG) emissions, soil organic carbon (SOC) pools and biomass yield. We examined biochar and hydrochar for changes in soil properties, gross nitrogen transformation rates, SOC and its fractions, biomass yield and all three major GHG fluxes. We assessed our results from two different perspectives; one, when chars were added at a uniform rate with fertilizer nitrogen (N) following the farmer's practice and two, when chars were added based on the char C amount without fertilizer N. We show that only N_2O emissions (not CO_2 and CH_4) were affected when chars were added at a uniform rate with fertilizer N. Biochar increased N_2O emissions significantly compared to control whereas hydrochar restricted N_2O relative to control and lowered significantly compared to biochar treatments. Biochar with N amendment significantly increased gross NO_3^- production (gross nitrification) and N_2O emissions, indicating a linkage between increased nitrifier activity and N_2O emissions. Hydrochar with N amendment showed lower gross nitrification rates and N_2O emissions, indicating a reduced nitrifier activity and N_2O emissions compared to biochar. Interestingly, hydrochar without N amendment showed lowest N_2O emissions with few N_2O uptake events and similar gross NO_3^- consumption and production rates, hinting an enhanced soil N_2O reduction/sink mechanism, especially with actively photosynthesizing vegetation. Both chars increased soil particulate organic C (POC) significantly mainly owing to both chars themselves being carbon. The mineral associated organic C (MAOC) remained unaltered. Interestingly, we found significantly lower soil MAOC per unit of char C with biochar than with hydochar amendment, especially when endpoint soil MAOC was compared with initial soil MAOC. Our results suggest that destabilization of MAOC increased more with biochar than with hydrochar, especially with N fertilization and in the presence of actively photosynthesizing vegetation. This was further supported by a significantly greater rise in microbial biomass carbon with hydrochar than with biochar amendment. The total biomass yield remained unaffected. However, biochar with the applied N reduced the timothy grass yield compared to control, implying a reduced uptake of applied N by timothy. Our results shed light on the complex interactions among chars, soil, vegetation and N management. Therefore, future studies should focus on assessing the char amendment impacts including both plant and soil and at the whole agricultural

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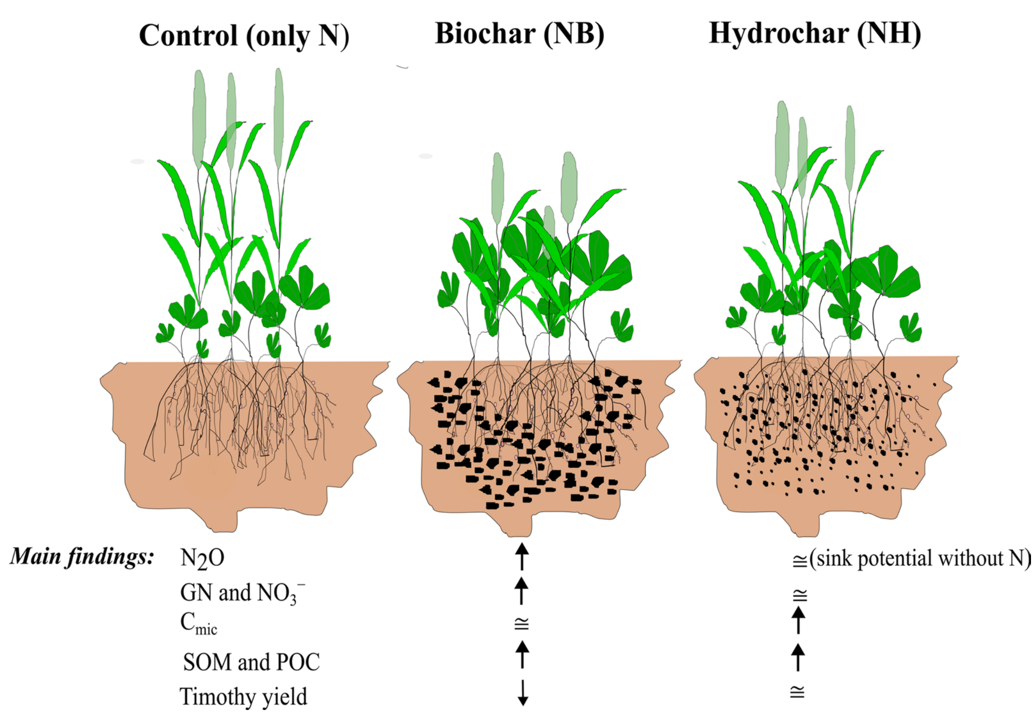
field scale. Chars manufactured from diverse feedstocks need to be investigated for their impacts in diverse agricultural ecosystems, paving the way for their large-scale use.

Keywords Biochar and hydrochar, Boreal legume-forage, Nitrogen fertilization, POC and MAOC, Greenhouse gases, Gross nitrogen transformation

Graphical Abstract



1 Introduction

Increased global food demand has raised the need for synthetic nitrogen (N) fertilizer applications in agriculture (Salo et al. 2013). However, due to inefficient use of N by plants, agricultural ecosystems are prone to lose considerable amount of fertilizer N into surrounding water bodies and ground waters, causing eutrophication and acidification (Lucander et al. 2021), and into the atmosphere as gaseous nitrous oxide (N_2O) causing climate warming and ozone depletion (Ravishankara et al. 2009). In fact, agriculture is the main anthropogenic source of N_2O accounting for ~ 70% of total N_2O emissions during 2000–2014 (Xu et al. 2020). Concurrently, N fertilization can also make agricultural soil as a carbon dioxide (CO_2) source by enhancing microbial respiration (Sun et al. 2022; Ryan and Law 2005). Therefore, measures to reduce the impact of agriculture on the water bodies and climate are urgently needed. One of such measures is to use char e.g., biochar in agricultural soils.

Char is a pyrolyzed biomass and preserved carbon as it contains condensed aromatic compounds that are stable against microbial decomposition (Lehmann et al. 2015). When used in soil as an amendment, char has been shown to produce positive effects and such effects are particularly emphasized for agricultural soils to play a greater role in feeding the rising global population. Several previous studies point towards more positive and less negative impact of char use in agricultural soil, and the reported positive impacts include, soil greenhouse gas (GHG) emissions mitigation (He et al. 2017; Joseph et al. 2021), improving soil nutrients (e.g., N and phosphorus (P)) transformation and crop yield (Gao and DeLuca 2020), and morphological traits of plant roots (Xiang et al. 2017). Nevertheless, some contradicting results exist, such as increase in all three major GHG gases (He et al. 2017; Jones et al. 2011; Kalu et al. 2021b; Smith et al. 2010; Zhang et al. 2012;), hinting that char amendments may not always result in a positive outcome. Such differences in effects of biochar amendments have been linked primarily to physiochemical properties of charsto a variety of feedstocks used in making them and their production methods and interactions with the inherent properties of soils (Aller 2016).

A variety of feedstocks and production methods lead to distinct physiochemical properties of chars, such as elemental compositions, porosity and surface area, which affect their quality and application benefits (Spokas and Reicosky 2009; Sun et al. 2014). Additionally, using the same feedstock, the conventional pyrolysis can produce biochar whereas hydrothermal carbonization (HTC) can produce hydrochar and these both (hydrochar and biochar) chars have distinct physiochemical characteristics, which also affect their behavior in the soil (Aller 2016; Masoumi et al. 2021). For instance, the surface area in biochar represents the amount of internal porosity whereas in hydrochar it represents the actual external surface area (Fuertes et al. 2010; Sevilla et al. 2011). In biochar, surface area representing the amount of internal porosity (e.g., macropores) is key to biochar functions related soil aeration, hydrology and microbial activities (Atkinson et al. 2010) whereas in hydrochar it would relate to function of sorption and can affect the availability nutrients in the soil. Therefore, different results could be expected when implying the same property (e.g., surface area) for biochar and hydrochar. Similarly, hydrochar is known to be more acidic than biochar (Fuertes et al. 2010) and is considered to be more effective in sorption of basic nutrients such as P (Libra et al. 2011). Moreover, use of the same biochar on different soils (e.g., sandy acidic vs calcareous loamy) can produce different results (Watzinger et al. 2014), suggesting that it is not only char but also its interaction with soil that plays an important role. Therefore, studies suggest that biochar should be tailored to specific soils (Aller 2016) and should be used carefully. For example, the use of high C/N biochar in nutrient rich soil could reduce plant N uptake because of immobilization and should be avoided (Atkinson et al. 2010). Such decision could help address and achieve agricultural sustainability issues such as C-sequestration, biomass yield improvement, reduce nutrient leaching and GHG emissions mitigation. In agreement with this, studies suggest that because of its liming effect, biochar could be a good option as an amendment for agricultural soils that are N poor and acidic in nature (Biederman and Harpole 2013; Jeffery et al. 2017)—the ones that are in boreal region e.g., Finland.

Most soils in Finland are acidic (pH < 7) and biochar amendment to them has been shown to produce beneficial results. Some of such results are negative priming (Kalu et al. 2024), reduced N_2O emissions (Peltokangas et al. 2023; Saarnio et al. 2013) and nitrate (NO_3^-) leaching (Karhu et al. 2021), and improved CH_4 uptake (Karhu et al. 2011), water retention (Rasa et al. 2018), nitrogen use efficiency (NUE) and biomass productivity (Kalu et al. 2021b, 2022). Nevertheless, some contradicting results have been also reported such as increased CO_2 and N_2O emissions (Kalu et al. 2022; Saarnio et al. 2013) and increased surface runoff of P (Saarnio et al. 2018). The aforementioned results are outcomes of studies conducted using other than typical boreal grassland soil (e.g., crop legumes, faba beans, Kalu et al. 2022) and mostly biochar (not hydrochar). Grasslands have been studied, however, only as a monoculture (Kalu et al. 2021a; Saarnio et al. 2013, 2018). Additionally, varying amendment effects of biochar and hydrochar are expected because of their varying physiochemical properties (Aller 2016). Also, in some cases, the same properties of biochar and hydrochar (see the text about surface area above) could result in different results. Therefore, studies researching the amendment effect of hydrochar in conjunction with biochar are needed to better understand the effects of char types on boreal agricultural soils. To the best of our knowledge, the impact of biochar and hydrochar amendments on a typical boreal grassland, e.g., legume-based forage systems is not yet explored and understood.

It is therefore essential to assess whether chars could potentially mitigate GHG emissions and improve C sequestration and biomass yield of legume-based forage systems in the boreal region. We conducted a mesocosm study representing a typical boreal forage-legume (timothy and red clover). Our study has three objectives and hypothesis as follows. 1. To examine the effects of biochar and hydrochar amendments on emissions of all three GHG gases. We hypothesized that both char types would decrease all three GHG emissions (CO_2, N_2O and methane (CH_4)) due to their positive effects on soil basic edaphic properties such as microbial biomass, and two key mineral N (NO_3^- and ammonium (NH_4^+)) content and their transformation rates. 2. To examine the effects of biochar and hydrochar amendments on biomass yield and NUE. We hypothesized that both chars would have an improving effect on biomass yield due to improved NUE. 3. To examine the effects of biochar and hydrochar amendments on soil organic carbon (SOC) fractions; particulate and mineral associated organic carbon (POC and MAOC). We hypothesized that both chars would increase the soil POC and MAOC content due to improved microbial C assimilation.

2 Materials and methods

2.1 Soil sampling, char production and mesocosms preparation

The soil for the study was collected from one of the Luke's research platforms located in Anttila (63° 9′ 48.69′′

N, 27° 14′ 3.29′′ E), Maaninka in Eastern Finland. Soil samples were collected using a front-loader equipped tractor from ~ 0–20 cm depth and from 28 different locations to encompass the heterogeneity of the entire field (6.3 ha). The mineral soil in Anttila is classified as fine sand to coarse silt (Table 1) according to the World Reference Base for Soil Resources (WRB) system. The mesocosms were prepared by packing the sieved (2 mm) soil into a polyvinyl (PVC) ring (Ø 0.18 m, id, 0.12 m). The bottom part of the ring, however, had three ventilations holes (id, ~ 0.6 mm) at equal distance and was covered with circular cotton mesh before adding the soil. To each mesocosm, 3.7 kg of sieved soil was added maintaining the field bulk density (1.01 g cm^{-3}). Soil packing was followed by hydrochar and biochar (Tables 1 and S1, further detail in supplementary information, *SI*) addition. Top ~ 6 cm of packed soil in the mesocosm was scrubbed and mixed with the char and was repacked. Both chars used in the experiment were made from birch and were provided by the University of Eastern Finland. Hydrochar was produced by birch bark with HTC at 220 °C whereas biochar was produced by dry birch firewood using pyrolysis at 600 °C (char production detail in supplementary information, *SI)*. We seeded mesocosms with 40 mg (~ 15 kg ha^{-1}) of timothy (*Phleum pratense* L. cv Nuutti) and 10 mg (~ 4 kg ha^{-1}) of red clover (*Trifolium pratense* L. cv. Ilte) and fertilized with 754.8 mg of N (60% at the beginning of experiment and 40% after the first harvest on day 42) corresponding to 75 kg N ha^{-1} following regional recommendations for legume grasslands. Subsequently, soil moisture was set to 45% water filled pore space (WFPS) and was balanced every second day. The mesocosms were then transported to a greenhouse for GHG flux measurements.

Table 1 Characteristics of soil and chars (mean ± sem, n = 3 to 5) used in the experiment

	Soil	Biochar	Hydrochar
Sand (%)	22		
Silt (%)	55		
Clay (%)	27		
EC (µS cm^{-1})	49.90 ± 2.85		
NO_3^- µg N g_{dw}^{-1}	23.50 ± 0.53		
NH_4^+ µg N g_{dw}^{-1}	0.69 ± 0.55		
Organic matter (%)	9.23 ± 0.04		
Microbial C (ug g_{dw}^{-1})	33.98 ± 6.76		
Microbial N (ug g_{dw}^{-1})	3.67 ± 1.13		
Gross nitrification (µg N g_{dw}^{-1} d^{-1})	7.40 ± 0.16		
Gross NO_3^- consumption (µg N g_{dw}^{-1} d^{-1})	5.88 ± 0.48		
Gross mineralization (µg N g_{dw}^{-1} d^{-1})	3.20 ± 0.08		
Gross NH_4^+ consumption (µg N g_{dw}^{-1} d^{-1})	4.14 ± 0.41		
pH	5.84 ± 0.03	8.6	4.7
N (%)	0.27 ± 0.01	0.71	0.18
C (%)	3.48 ± 0.08	94.0	67.10
C:N ratio	13.0 ± 0.37	132.02	368.68
Surface area (m^{-2} g)		271.6	72.6
Water-soluble N (mg kg^{-1})		2.0	110
Water-soluble P (mg kg^{-1})		7.10	71

2.2 Experimental design, gas flux measurement and calculation

We conducted our study from two different perspectives; one, when chars were added at a constant application rate (referred as RAR hereafter) with fertilizer N following the farmer's practice and two, when chars were added based on the char C amount (referred as RCC hereafter) without fertilizer N (Table 2). We considered first perspective following many earlier studies, and so establish three treatments, N, NB and NH (Table 2). The same amount char in NB and NH in perspective one was based on mesocosm surface area (m^2) and corresponds to 20-ton char ha^{-1}. This rate is considered to be well within the beneficial range (10–20 ton ha^{-1}) for Finnish climatic conditions (Brandstaka et al. 2010). The consideration of latter perspective relies on the fact that char is primarily composed of C and that C amount is the one of the key char

Table 2 Experimental perspectives, treatments and number of replicates (n)

	Perspective one	Perspective two	
Treatments	Relative to char application rate (RAR)	Relative to char carbon (RCC)	n
Background (NBH0)			4
Nitrogen (N) only	754.80 mg N per mesocosm		4
NB (N + Biochar)	N + 54.34 g char per mesocosm		4
NH (N + Hydrochar)	N + 54.34 g char per mesocosm		4
Biochar (B) only		40.75 g char per mesocosm	4
Hydrochar (H) only		50.94 g char per mesocosm	4

properties that mediates soil nutrient availability and responses of GHG producing biogeochemical process towards char. With second perspective we aim to better understand, especially the role of char C on GHG emissions, SOC pools and biomass yields in both presence and absence of N fertilizer. Therefore, we established two more treatments, B and H without N addition and compared their results against fertilized counterparts (NB and NH) after data normalization, i.e., by dividing the result values from NB and NH with the char C amount (kg_{charC}) added to each mesocosm. The different amount of char in B and H was based on mesocosm surface area and C% and reflects relatively similar amount of char C. Additionally, we also established treatment without N and char amendment (NBH0, Table 2) for assessing background effect on all studied variables.

We measured fluxes of CH_4, CO_2 and N_2O in total 14 occasions lasting 99 days in a greenhouse using closed (opaque) static chamber technique and calculated flux rates using linear increment in gas concentrations and ideal gas equation according to Bhattarai et al. (2019). The gas concentrations were analyzed with gas chromatography (G3591-80004) coupled with autosampler (Gilson GX-271). We calculated the cumulative value of all three GHGs for all treatments and to do that, we first converted fluxes to g m^2 d^{-1} for CH_4 and CO_2 and mg m^2 d^{-1} for N_2O from their original units. Then, using a linear interpolation method for gap filling we obtained the flux values for all 99 days for each replicate in each treatment. Then for each replicate, the flux values from each day were added to get a cumulative flux value. Replicate sums were then used to calculate mean cumulative fluxes for each treatment. See *SI* for further details.

2.3 Measurements of soil physiochemical properties and SOC fractions

We assessed soil mineral N content (NH_4^+ and NO_3^-), pH, electrical conductivity (EC), soil organic matter content (SOM), microbial biomass (carbon C_{mic} and nitrogen N_{mic}), bulk C and N, MAOC, and POC from initial and end point soil samples. Briefly, mineral NH_4^+ and NO_3^- concentrations were analzyed from soil-KCl (1 M) extracts with spectrophotometer using colorimetric method as described in Fawcett and Scott (1960) and Miranda et al. (2001), respectively. Soil pH and EC were measured from soil–water slurry. The C_{mic} and N_{mic} were determined from potassium sulfate (K_2SO_4) extracts using direct chloroform extraction method (Setia et al. 2012; Bhattarai et al. 2021) and concentrations were analyzed with a total carbon analyzer (TOC-L CSH, Shimadzu) equipped with total nitrogen unit (TNM) and autosampler (ASI-L). The SOM content was analyzed by combusting the oven-dried soil at 550 °C for two hours. Size and density fractionation techniques were used to analyze soil mineral-associated organic carbon (MAOC) and particulate organic carbon (POC). Each sample was wet-sieved into two size classes: 0.063–2 mm and < 0.063 mm, with the latter assumed to represent MAOC. In the 0.063–2 mm fraction, MAOC and POC were separated using sodium polytungstate (SPT) solution adjusted to a density of 1.8 g cm^3 (Skipp and Brownfield 1993).

2.4 ^{15}N pool dilution and gross consumption and production of NO_3^- and NH_4^+

To estimate gross consumption (GC) and production (GP) rates of NO_3^- and NH_4^+, we performed ^{15}N pool dilution (IPD) using the end point soil samples according to Kirkham and Bartholomew (1954). Briefly, 4 g of soil was supplemented with 0.3 ml of ^{15}N tracer solutions; 1.29 mM $^{15}N-NO_3$ tracer solution ($Na^{15}NO_3$, ≥ 98 atom%, Sigma-Aldrich, MO, USA) for NO_3^- and 0.2 mM $^{15}N-NH_4$ tracer solution ($^{15}NH4Cl_4$ ≥ 98 atom%, Sigma-Aldrich, MO, USA) for NH_4^+. We added 10% of initial NO_3^- and NH_4^+ pools as a ^{15}N tracer solution. The reactions were stopped with 30 ml 1 M KCl at 4 and 24 h after tracer addition and subsequently, KCl extracts were collected. Micro-diffusion of extract to acid traps was performed and $^{15}N/^{14}N$ was quantified with elemental analyzer-isotope ratio mass spectrometry (EA-IRMS, Thermo Finnigan DELTA XP Plus, Bremen, Germany) in isotope laboratory at University of Eastern Finland which were further used to calculate the gross rates. See *SI* for further detail.

2.5 Shoot and root biomass, root/shoot ratio, yield, and nitrogen use efficiency

For biomass, we assessed root and shoot biomass and their ratios, biomass yield and NUE. Roots were separated from the soil by hand-picking and washed with water, then oven-dried at 60 °C for 48 h and reported as dry biomass. On both harvests, fresh biomass was quantified immediately after the harvest whereas the cut grass was oven-dried at 60 °C for 48 h, weighed and reported as dry biomass, which was further used to calculate the yield (g_{dw} m^{-2}) and NUE. As for NUE we calculated, partial factor productivity (NUE_{PFP}, g_{dw} $g^{-1}{}_N$) and agronomic efficiency (NUE_{Ag}, Δg_{dw} $g^{-1}{}_N$) of applied N according to Dobermann (2007). We choose to calculate NUE_{PFP} as it has high relevance to farmers as it integrates the efficiency of both indigenous and applied N whereas NUE_{Ag} excludes the inclusion of yield from indigenous N (e.g., NH_4^+ and NO_3^- via mineralization and nitrification, respectively) which would allow us to compare the yield as an effect of char amendment solely in the presence of exogenous fertilizer N (Dobermann 2007). See *SI* for further detail.

2.6 Statistical analyses

To compare the mean values, we used one-way and two-way analysis of variance (ANOVA). The two-way ANOVA was used to examine the difference between GC and GP of NO_3^- (for all treatments) and NH_4^+ (for only B and H), and between total yield of timothy and red clover. In both ANOVAs, we used Tukey HSD as a post-hoc test and considered statistical significance in means of variables when $p<0.05$. Normality was tested prior to analysis with Sapiro-Wilk normality test and Q-Q and histogram plots and when needed, $\log_{10}$ transformation was applied to obtain normal distribution. The data that were $\log_{10}$ transformed were: CO_2 and N_2O flux rates and EC for RAR and CO_2 flux rates and total CO_2, root and shoot biomass, GC and GP rates of NO_3^- and N_{mic} for RCC. For those data which did not exhibit normal distribution even after $\log_{10}$ transformations, we used non-parametric Kruskal–Wallis test and compared their means with Dunn's post-hoc test using Benjamini Hochberg adjustment. No statistical test was carried out for mean GP and GC rates of NH_4^+ for N, NH and NB treatments because of weak recovery of added $^{15}NH_4^+$ leading to smaller sample size (n = 1 per treatment) of GP and GC rates. We examined the correlations between GHG (fluxes and their total emissions) and plant and soil variables by using R package CORRPLOT (Wei and Simko 2017). All statistical tests were done separately for RAR and RCC. For all statistical tests we used original variable values; however for comparing results from B and H treatments in RCC we normalized the data (except for NUE_{PFP}, NUE_{Ag}, pH and EC) prior to statistical tests by dividing the values with the amount of C (in kg) added via char. This normalization was needed because treatment NB and NH had varying amount of char C unlike B and H. All statistical analyses were performed with R (version 4.4.0, R Core Team 2024). See *SI* for further detail.

3 Results

3.1 Fluxes and cumulative emissions of CH_4, CO_2 and N_2O

The mean fluxes of CH_4 were negative implying its uptake, CO_2 were positive implying its emissions and N_2O were both negative and positive implying both emissions and uptake to atmosphere throughout the experimental period (Fig. 1a-b).

3.1.1 CH_4

The CH_4 uptake did not change except an uptake on Day 62 in RCC, and it was significantly higher with only hydrochar (H, −0.023 mg C m^{-2} h^{-1} $kg^{-1}{}_{charC}$, p < 0.001) compared to only biochar (B,-0.010 mg C m^{-2} h^{-1} $kg^{-1}{}_{charC}$) and its fertilized counterparts, NH (Fig. 1b). The cumulative CH_4 uptake was statistically insignificant in all treatments in RAR and RCC, although there were some variations in magnitude e.g., it was lower in B than H in RCC (Fig. 1c).

3.1.2 CO_2

In general, the CO_2 emissions increased gradually with time, however remained insignificant between treatments in majority of the measurement days, except on Days 1, 2, 6, and 10 and after both harvests (Fig. 1a-b). During these occasions, CO_2 emissions were significantly ($p<0.05$) higher only on the hydrochar treatments; NH > N (control) in RAR and H > B in RCC (Fig. 1a-b). Both harvests (d42 vs. d43 and d98 vs. d99) decreased CO_2 emissions significantly ($p<0.05$) in all treatments, except in NH in RAR (Fig. 1a-b). The cumulative CO_2 emissions were statistically insignificant in all treatments in RAR and RCC, although there were some variations in magnitude e.g., it was higher in H than B in RCC (Fig. 1d).

3.1.3 N_2O

The emissions of N_2O decreased gradually with time. Although decreased, biochar amendment showed a greater number of significantly ($p<0.05$) higher emissions (Fig. 1a-b). Of all 14 occasions, N_2O emissions were higher on six occasions in NB compared to control (N) in RAR and on eight occasions in B compared to hydrochar (H) in RCC (Fig. 1a-b). The N_2O emissions in NB and B were up to 42- and 11.84- fold higher, respectively. The N_2O emissions were higher ($p<0.05$, up to 33-fold) only two of 14 occasions in NH compared to control in RAR. In RAR, harvesting did not change the N_2O emissions, except in NBH0 where soil turned from a sink (−0.15 µg N m^{-2} h^{-1}) to a source (5.58 µg N m^{-2} h^{-1}) in the first harvest (d42 vs. d43, $p<0.05$) (Fig. 1a). In RCC, treatment H was N_2O sink on day43 (the day following the first harvest) and d72 with uptake rates of −2.92 and −4.02 µg N m^{-2} h^{-1} $kg^{-1}{}_{charC}$, respectively, while NH, B, and NB acted as a N_2O source (Fig. 1b). The cumulative N_2O emission was significantly ($p<0.05$) higher in both NB (1.56-fold) and NH (1.4-fold) compared to control (N, 5.60 mg N m^{-2}) in RAR and significantly ($p<0.05$) lower in H (0.83 mg N m^{-2} $kg^{-1}{}_{charC}$) compared to B, NH and NB in RCC (Fig. 1e). Further, cumulative N_2O was significantly higher in biochar (NB, $p=0.003$) than hydrochar (NH) in RAR (Fig. 1e).

3.2 Effects on root and shoot biomass, root/shoot ratio, yield, and NUE

The biomass of root (R), shoot (S) and their ratios (R/S) and total yield remained unchanged except in NH in RCC which has higher total yield than B, H and NB

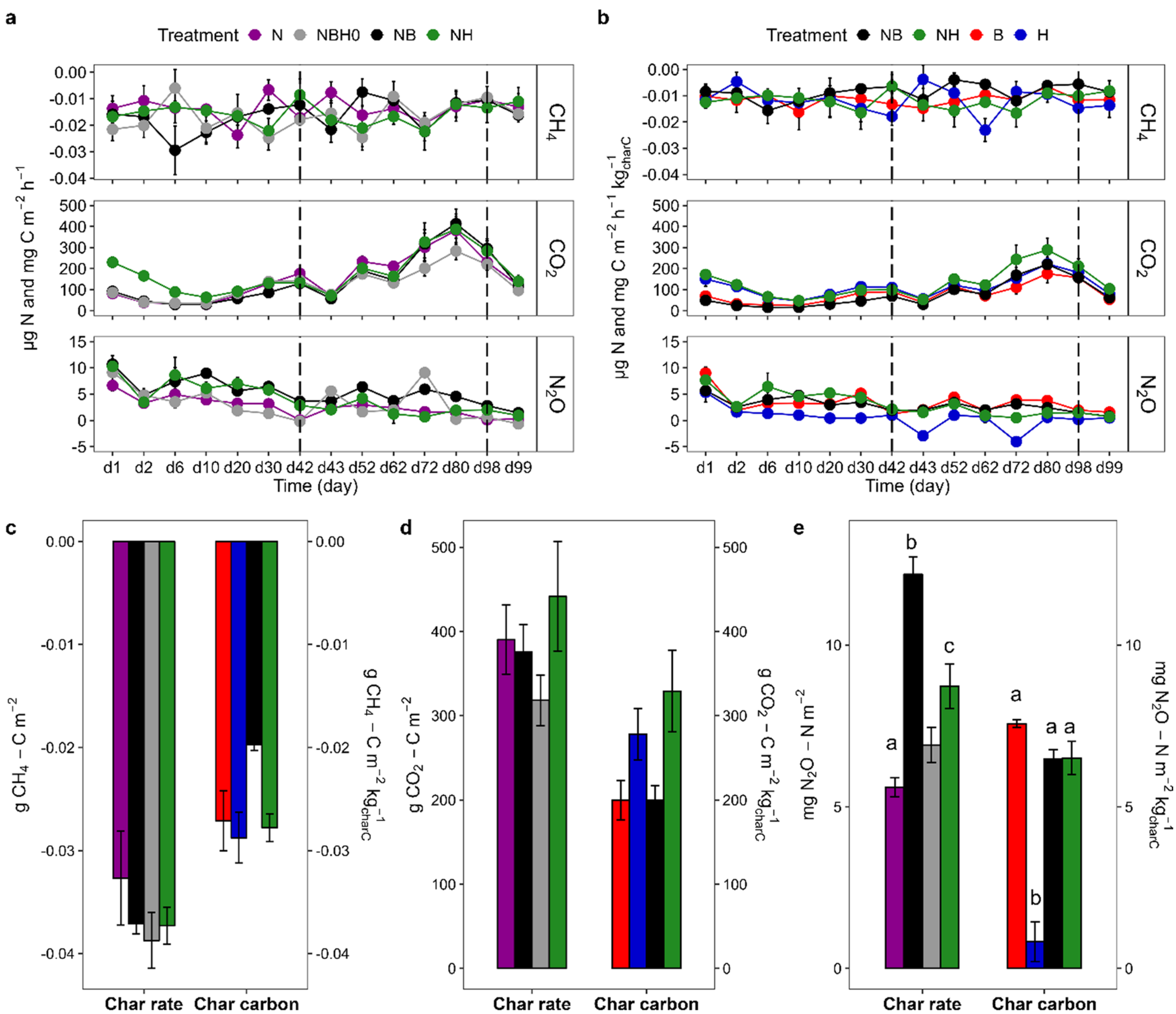


Fig. 1 Flux rates and cumulative emissions of methane (CH_4), carbondioxide (CO_2), and nitrous oxide (N_2O). Plot **a** represents the GHG flux rates (y-axis, µg N and mg C m^{-2} h^{-1} kg^{-1}) relative to char application rate (RAR) and **b** represents the flux rates (y-axis, µg N and mg C m^{-2} h^{-1} kg$^{-1}_{charC}$) relative to char carbon content (RCC) over 99 days (d1-d99, x-axis) of experiment. Plots **c**, **d**, and **e** represent the cumulative emissions of 99 days for CH_4, CO_2, and N_2O, respectively. The unit of cumulative emissions in plots **c**, **d**, and **e** are g C m^{-2} for CH_4 and CO_2 and mg N m^{-2} for N_2O for RAR (primary y-axis) and g C m^{-2} kg$^{-1}_{charC}$ for CH_4 and CO_2 and mg N m^{-2} kg$^{-1}_{charC}$ for N_2O for RCC (secondary y-axis). The colors in plots **a**-**e** indicates experimental treatments: "purple", nitrogen addition (N), "black" nitrogen with biochar addition (NB), "green" nitrogen with hydrochar addition (NH), "grey" no nitrogen nor char addition (NBH0, background), "red" biochar without nitrogen addition (B), and "blue" hydrochar without nitrogen addition (H). Each flux value and cumulative emission bar represents the mean and error bars denote standard error of mean (n = 4). Each two vertical dashed lines in plots a and b denote first (day 42) and second (day 98) harvest, respectively. The error bars, in some cases in plot a are smaller than the size of the symbols. Letters above each bar in plots c—e represent the statistical significance ($p < 0.05$) in the cumulative emissions between the treatments in RAR and RCC separately and their absences denote no statistical difference. Statistically significant results for the daily flux means in plots **a** and **b** are reported in the text.

(Table 3, Fig. 2a-b). When individual plant species were compared between treatments, only timothy yield differed and was significantly ($p < 0.05$) lower only in NB than N in RAR and was significantly higher only in NH than in H in RCC (Fig. 2a-b). When both plant species in each treatment were compared, timothy yield was significantly ($p < 0.05$) higher than red clover only in N (RAR) and NH (in both RAR and RCC) and red clover yield was significantly ($p < 0.05$) higher than timothy yield in only H (Fig. 2b). As a general observation, mean yield of timothy was higher than that of red

Table 3 Soil pH, electrical conductivity (EC), organic matter (OM) content, dry shoot and root biomass and their ratios and water filled pore space (WFPS%) in RAR and RCC

	Relative to char application Rate (RAR)					**Relative to char carbon content (RCC)**		
	NBH0	**N**	**NB**	**NH**	**B**	**H**	**NB**	**NH**
pH	6.03 ± 0.02[a]	5.95 ± 0.01[ab]	5.90 ± 0.01[b]	5.90 ± 0.02[b]	6.10 ± 0.23	6.01 ± 0.01		
EC	14.50 ± 0.62[a]	20.50 ± 1.3[ab]	23.80 ± 1.50[b]	24.10 ± 4.0[b]	15.30 ± 0.60	15.70 ± 0.69		
Organic matter (%)	9.13 ± 0.03[a]	9.09 ± 0.02[a]	10.12 ± 0.07[b]	10.31 ± 0.13[b]	7.04 ± 0.05[ab]	8.11 ± 0.01[c]	5.39 ± 0.04[a]	7.70 ± 0.10[bc]
Dry shoot biomass (g and g $Kg^{-1}{}_{charC}$)	6.98 ± 0.54[a]	11.10 ± 0.67[b]	10.96 ± 0.74[b]	10.32 ± 1.24[b]	5.03 ± 0.60	5.68 ± 0.43	5.83 ± 0.39	8.41 ± 0.82
Dry root biomass (g and g $Kg^{-1}{}_{charC}$)	3.54 ± 0.54	5.24 ± 0.57	4.36 ± 0.47	4.76 ± 1.10	1.87 ± 0.27	2.84 ± 0.39	2.32 ± 0.25	4.12 ± 0.78
Root to shoot ratio	0.51 ± 0.08	0.48 ± 0.05	0.40 ± 0.02	0.48 ± 0.04	0.37 ± 0.02	0.52 ± 0.11	0.40 ± 0.02	0.48 ± 0.04
WFPS%	39.89 ± 0.42	38.67 ± 0.25	38.44 ± 0.29	38.86 ± 0.62	39.79 ± 0.13[a]	39.76 ± 0.34[a]	28.96 ± 0.46[b]	20.45 ± 0.15

Here, values are mean ± sem of four replicates except for treatment N and NH for root to shoot ratio, which were n = 3. Different alphabets as superscripts denote statistically significant ($p < 0.05$) difference in the mean values whereas their absences indicate statistical insignificance. Exact *p*-values are reported in the text

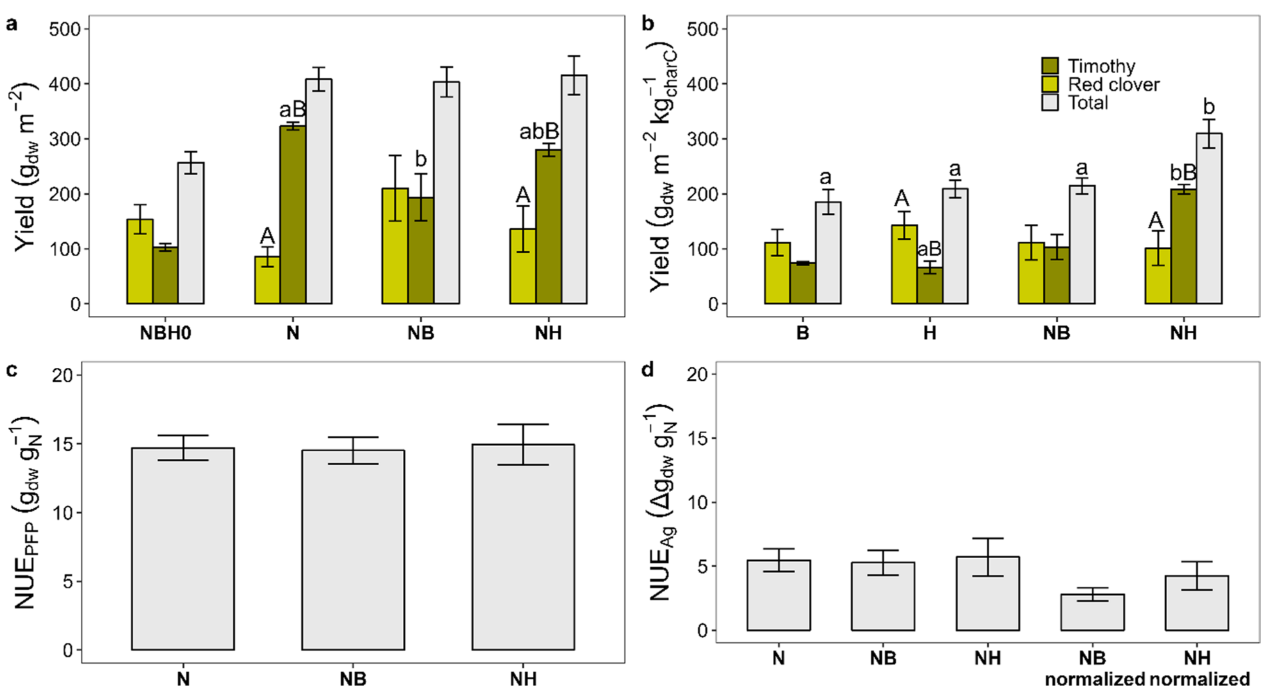


Fig. 2 Biomass yields and nitrogen use efficiency. Plots **a** and **b** represent the yields in RAR (g_{dw} m^{-2}) and RCC(g_{dw} m^{-2} $kg^{-1}{}_{charC}$) respectively. Plots **c** and **d** represent the partial factor productivity of applied N (NUE_{PFP}, g_{dw} $g^{-1}{}_N$) and agronomic efficiency of applied N (NUE_{Ag}, Δg_{dw} $g^{-1}{}_N$), respectively. Colors in plots **a** and **b** represent the different yields; grey, total yield, light green, red clover yield and dark green, timothy yield. Each bar represents the mean and error bars denote standard error of mean (n = 4), except for treatment N and NH for NUE_{PFP} and NUE_{Ag}, which are n = 3. Letters above each bar represent the statistical significance ($p < 0.05$). The small letters are used to denote the significant differences in red clover, timothy and total yield between the treatments whereas capital letters denote the significant differences between timothy and red clover yield in each treatment. Absence of either of letters denotes no statistical difference

clover in fertilized (N, NB, and NH) soil, whereas this relationship was reversed in unfertilized (NBH0, B, and H) soil (Fig. 2a-b). Both NUE_{PFP} and NUE_{Ag} remained insignificant in NB and NH compared to N, although normalized mean NUE_{Ag} was higher by 34% in NH than in NB (Fig. 2c-d).

3.3 Effects on edaphic variables, microbial biomass and gross N transformation rates

Soil pH and EC decreased significantly in NB and NH compared to NBH0 in RAR (Table 3). Soil OM% increased significantly the NB and NH treatments compared to the N treatment in the RAR system. In the RCC system, OM% was higher in the H treatment than in the B treatment, and also higher in NH than in NB (Table 3). Soil mean NO_3^- concentrations was significantly higher only in NB compared to N in RAR and in NB and NH compared to B in RCC (Fig. 3a-b). Interestingly, only NB had significantly higher mean GN than N in RAR, supporting the increased NO_3^- concentrations (Fig. 3a). The mean GM rates were significantly higher in B than in H while mean GC rates of NH_4^+ were significantly higher than mean GM rates in both B and H in RCC (Fig. 3b). Soil mean C_{mic} was significantly higher in NH compared to NBH0, N and NB in RAR and in both hydrochar (H and NH, $p < 0.05$) than B and NB in RCC (Fig. 3e-f). The variables that did not vary statistically at all between treatments were GC of NO_3^-, NH_4^+ content, and N_{mic} in both RAR and RCC, GC vs. GN within each treatment in RAR and GN in RCC (Fig. 3).

3.4 Changes in soil MAOC and POC content

Of the total soil organic carbon, MAOC was higher than POC and further MAOC was considerably (up to 90%) higher in finer aggregates (< 0.63 mm) of soil (Fig. 4a and Fig S1). In RAR, only POC (not MAOC) varied significantly (Fig. 4a). The amount of POC was significantly ($p < 0.05$) higher in hydrochar (NH) and biochar (NB) amendments compare to N, NBH0 and initial POC amount in the soil (Fig. 4a). Although the differences were not statistically significant, the mean MAOC content was slightly higher in biochar (NB) and hydrocar (NH) than initial soil MAOC content (Fig. 4a).In RCC, only MAOC (not POC) varied, and it was significantly lower in biochar with fertilization (NB) than in only hydrochar (H) (Fig. 4b). However, there was a greater number of significant differences in POC and MAOC when compared with initial (dashed line vs bars) POC and MAOC in the soil (Fig. 4b). The hydrochar amendment with and without fertilization (NH, H,) showed significantly higher POC content than the initial soil (Fig. 4b). In contrast, biochar with and without fertilization (NB, B) amendment showed significantly lower soil MAOC than the initial soil (Fig. 4b).

3.5 Relationships between GHG fluxes and soil and plant attributes

Correlations between GHG fluxes and total emissions and plant and soil variables varied between RAR and RCC (Fig. 5). The total CO_2 emissions showed a positive correlation with shoot and total biomass yield, C_{mic} and char's CN ratio in both RAR and RCC. Additionally, total CO_2 emissions showed a positive correlation with root biomass and gross NO_3^- consumption in RCC. While total CH_4 uptake did not exhibit any relationship with no variables in RAR, it showed an only negative correlation with total CO_2 emissions, C_{mic}, OM, MAOC and WFPS% in RCC. Interestingly, the total N_2O emissions correlated positively with GN, OM and both forms of SOC (MAOC and POC), char's surface area and NO_3^- concentrations in RAR whereas only with char's surface area in RCC. Additionally, the total N_2O emissions correlated negatively with WFPS% in RAR and char's CN ratio and OM in RCC.

(See figure on next page.)

Fig. 3 Soil mineral nitrogen concentrations, gross production and consumption rates of mineral nitrogen and microbial carbon and nitrogen. The left panel (plots **a**, **c** and **e**) represents the variables relative to char application rate (RAR) and the right panel (plots **b**, **d** and **f**) represents the variables relative to char carbon content (RCC). Plots **a** and **b** shows mineral NO_3^- concentrations (grey bars) and its gross consumption (light green bars) and production (i.e., nitrification (GN), orange bars) rates. Similarly, **c** and **d** show mineral NH_4^+ concentrations (grey bars, secondary y-axis) and its gross consumption (light green bars) and production (i.e., mineralization (GM), orange bars) rates. Plots **e** and **f** show microbial carbon (C_{mic}, light blue bars) and nitrogen (N_{mic}, dark blue bars). X-axis in all plots represents the treatments and y-axis represents the units of respective variables; in left panel, the unit of NO_3^- and NH_4^+ concentrations is µg N $g^{-1}{}_{dw}$ (secondary y-axis), both gross rates are µg N $g^{-1}{}_{dw}$ d^{-1} (primary y-axis) and C_{mic} is µg C $g^{-1}{}_{dw}$ and N_{mic} is µg N g^{-1}dw. In right panel, all the units are same, however presented in relative to kg of char C. Each bar represents the mean and error bars denote standard error of mean (n = 4), except for treatment N and NH n = 3 in both gross rates of NO_3^- transformation, and for treatment N, NH, NB n = 1 and for treatment B n = 3 for both gross rates of NH_4^+ transformation. The small letters in plots **a** and **b** denote statistical differences in NO_3^- contents between the treatments, in plot **d** denote statistical differences in gross mineralization (NH_4^+ production) rates between treatments B and H and in plots **e** and **f** denote statistical differences in C_{mic} between the treatments. The capital letters in plot **a** denote the significant differences in gross nitrification (NO_3^- production) rates between the treatments and in plot **d** denote significant differences between gross mineralization and gross consumption rates of NH_4^+ in B and H treatment. Absence of letters denotes no statistical difference

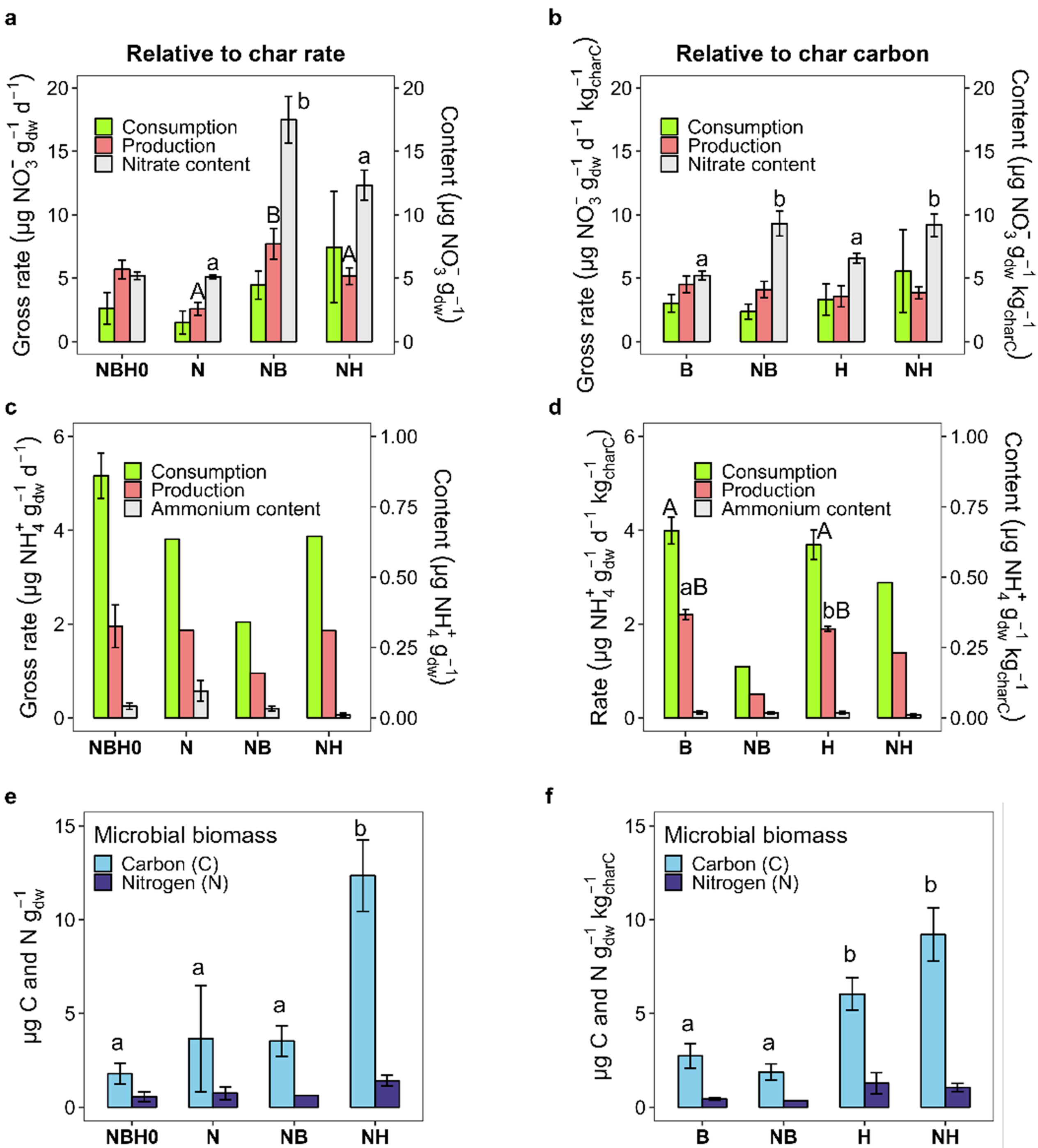


Fig. 3 (See legend on previous page.)

4 Discussion

4.1 No considerable effects on CH_4 uptake

We found that the soil CH_4 uptake remained rather unaffected by amendments of both chars as the cumulative uptake rates were similar across all treatments (Fig. 1c, in RAR). An increment in soil anoxia with char amendments can be expected as char particles tend to occupy the soil voids replacing oxygen. This effect can inhibit the activity of methanotrophs—microbes consuming CH_4, and thereby char amendments can result in suppressed soil CH_4 oxidation as seen in previous studies with biochar (Priemé and Christensen 1999).

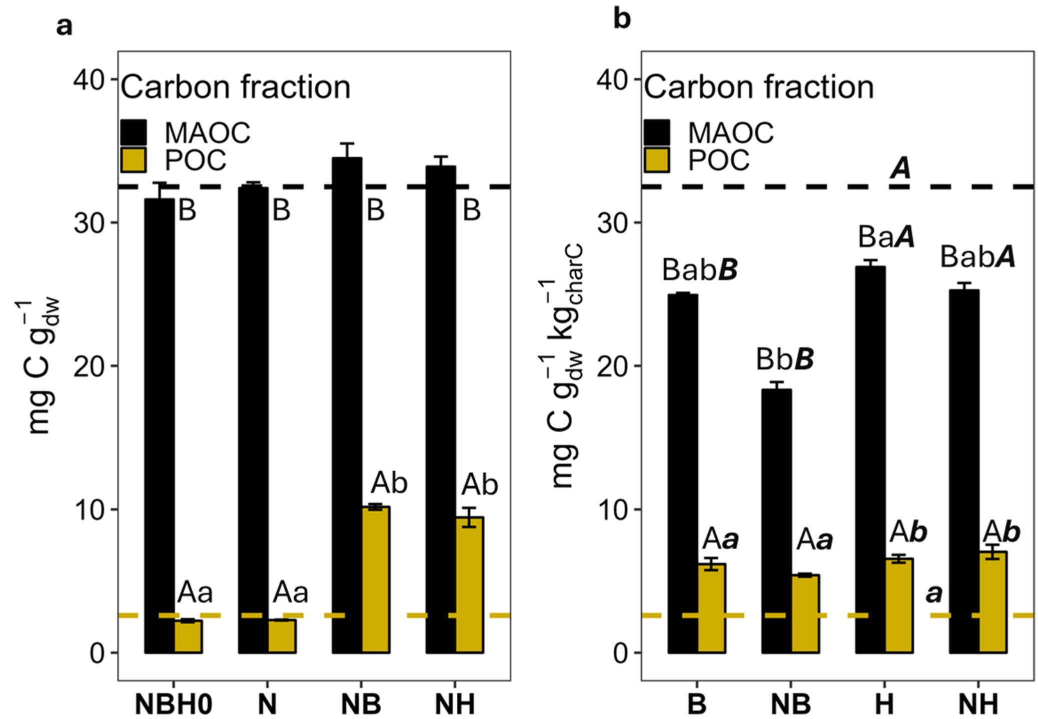


Fig. 4 Amount of mineral associated (MAOC) and particulate (POC) soil organic carbon. Plots **a** and **b** represent MAOC (black bars) and POC (light brown bars) concentrations in relative to char application rate (mg C g$^{-1}{}_{dw}$) and char carbon content (mg C g$^{-1}{}_{dw}$ kg$^{-1}{}_{charC}$), respectively in different experimental treatments (x-axis). The respectively colored horizontal dash lines in both plots represents the mean initial MAOC (32.05 ± 2.87 mg C g$^{-1}{}_{dw}$, mean ± sem) and POC (2.61 ± 0.07 mg C g$^{-1}{}_{dw}$, mean ± sem) amount in the soil. Each bar represents the mean and error bars denote standard error of mean (n = 4). Letters above each bar in both plots indicates the statistical significance ($p < 0.05$). In both plots, the small letters denote the significant differences in POC and MAOC between the treatments whereas capital letter denote the significant differences between POC and MAOC in each treatment. In plot b, cursive small bold letters denote statistical significance between initial POC (dashed line) and end point POC (bars) in each treatment whereas capital bold letters denote statistical significance between initial MAOC (dashed line) and end point MAOC (bars) in each treatment. In both plots, any absence of letters denotes no statistical difference

However, our study showed similar CH_4 uptake rates to control after char, which aligns with some previous studies (Karhu et al. 2021; Kulmala et al. 2022; Van Zwieten et al. 2009). This suggests that the applied chars had mere effect on the activities of both methanogens (CH_4 producers) and methanotrophs at least during the period of our study. Nevertheless, when assessed in relative to char carbon content, biochar especially with N amendments (NB) seemed to show reduced soil CH_4 uptake compared to hydrochar regardless of N (H and NH) amendments (Fig. 1c). Although statistically insignificant, the lower soil CH_4 uptake per kilogram of biochar C indicates that char with higher C content (Table 1) may support creating soil conditions that are optimal for soil microbes producing methane. This is also supported by a strong negative correlation between total CH_4 uptake and total CO_2 emissions with latter being positively correlated further with total biomass yield, C_{mic} and the C/N ratio of

(See figure on next page.)

Fig. 5 Spearman correlation between greenhouse gases and soil and plant variables with relative to char application rate **a** and char carbon content **b**. The strength of purple colour in the legend indicates the significant positive correlation ($p < 0.05$), whereas the strength of red orange in the legend indicates the significant negative correlation ($p < 0.05$).The empty boxes in both correlation plots represents insignificant association between the variables. Here, variables are pH, EC = electrical conductivity, OM = soil organic matter, POC = particulate organic carbon, MAOC = mineral associated organic carbon, NH_4^+ = ammonium concentrations, NO_3^- = nitrate concentrations, WFPS = water filled pore space, C_{mic} = microbial biomass carbon, Root (R) = root biomass, Shoot (S) = shoot biomass, RS_ratio = root to shoot ratio, T_Yield = total aboveground biomass yield, Ti_Yield = total aboveground timothy yield, Rc_Yield = total aboveground red clover yield, GC_ NO_3^- = gross consumption of NO_3^-, GN = gross nitrification, CH_4 = CH_4 flux, CO_2 = carbondioxide flux, N_2O = N_2O flux, T_CH_4 = cumulative CH_4 uptake, T_CO_2 = cumulative carbondioxide emissions, T_N_2O = cumulative N_2O flux, Char_SA = surface area of char, Char_CN = char CN ratio and Fertilier_N = added total amount fertilizer N

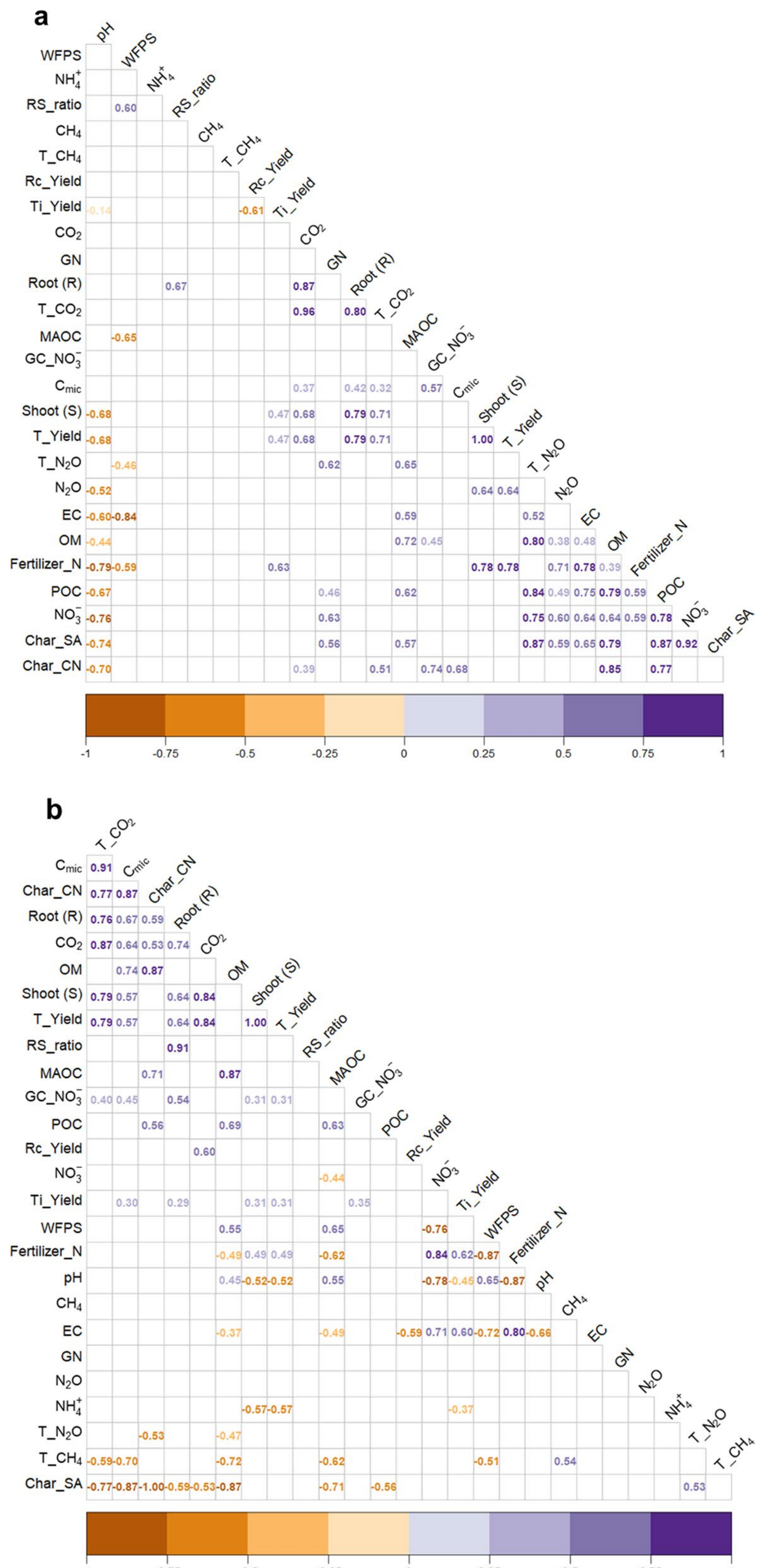


Fig. 5 (See legend on previous page.)

chars (Fig. 5b). This highlights the importance of char C in addition to char amount for a legume-based grasslands and suggests that char C should be considered as an important aspect while planning char amendments to legume-based forage cropping system as shown by recent meta-analysis (Farhangi-Abriz et al. 2024) and also interpreting results about char performance as an amendment (e.g., applied amount vs. applied C via char).

4.2 Biochar restricts CO_2 emissions while hydrochar shows a potential to increase it

Overall, amendment of both chars did not change soil respiration as cumulative CO_2 emissions did not vary in both RAR and RCC (Fig. 1d). However, hydrochar seemed to possess stronger potential to promote soil respiration as we found significantly greater number of occasions, especially during early phase of experiments where higher CO_2 emissions occurred with hydrochar amendments than with biochar (Figs. 1a, b and d). This result agrees with some previous studies with biochar and could likely be associated with the abiotic and/or biotic loss of char carbon itself and/or positive priming (Kuzyakov et al. 2009; Smith et al. 2010; Zimmerman et al. 2011). Moreover, a meta-analysis (Maestrini et al. 2015) concludes that char with low C content (or higher CN ratio), here in our case, hydrochar (than biochar, Table 1), has a greater potential to induce positive priming effect on native SOC. This is also supported by a strong positive correlation between total CO_2 emissions and total biomass yield and root biomass and the C/N ratio of chars (Fig. 5a-b).

The soil CO_2 emissions because of progressively growing vegetation and after its removal (the two harvests) seemed unaffected by both chars as CO_2 emissions between treatments showed a similar trend. The CO_2 emissions between all treatments were statistically insignificant and showed a typical increasing emission pattern in all treatments with vegetation growth, especially after day 10 when vegetation started to grow rapidly until the harvests (Fig. 1a, b). The effect of harvest was evident, as the CO_2 emissions decreased significantly in all treatments following the both harvests (Fig. 1a-b). This suggests that vegetation affects CO_2 emissions positively regardless of char characteristics (e.g., char C content and surface area) as supported by a strong positive correlation between CO_2 emissions and total biomass yield (Fig. 5a-b).

Interestingly, when we the proportion of CO_2 emission reduction after harvests, i.e., after the removal of fresh/labile C source, it was hydrochar (both, NH, and H) that showed greater reduction, up to 9–10% higher than biochar (both, NB, and B) amendments during both harvests. This suggests that, despite having similar biomass yield and NUE compared to biochar (Fig. 2a, c, d), the CO_2 emission from hydrochar amendment is more strongly linked with soil-root mediated microbial activities, which is also supported by a positive correlation between root biomass and CO_2 (total emissions and CO_2 flux) (Fig. 5a-b). A significant increase in C_{mic} (Fig. 3e-f) with hydrochar amendments indicates that fresh labile C via roots could have been easily accessible by soil microbes increasing their activities and efficiency to metabolize accessed labile C into microbial biomass and CO_2 (Fig. 5). On the contrary, C_{mic} with biochar amendments (Fig. 3e-f) was smaller than that with hydrochar and similar to that of control (N addition only) which aligns with previous studies (Ameloot et al. 2013; Karhu et al. 2011; Knoblauch et al. 2021). This indicates that the labile C via roots could have been made unavailable, probably occluded into biochar pores (Kalu et al. 2024) for microbial decomposition and CO_2 production. The support of char for microbial growth depends on their ability to absorb/adsorb soil nutrients and retain their microbial enzyme catalyzing capacity, usually with poor microbial growth with greater surface area of the char (Ameloot et al. 2013; Bailey et al. 2011; Lammirato et al. 2011). Indeed, our result of significant increase in C_{mic} with hydrochar amendment and a negative correlation between the surface area of char and CO_2 emissions (Fig. 5b) suggest that char with smaller surface area such as hydrochar in our case could result in higher soil CO_2 emissions. These results of CO_2 emissions and reductions before and after the harvests especially after amendments of chars with smaller surface area (e.g., hydrochar) indicate that interactions between soil microbes-vegetation-char are crucial for soil C dynamics and require further investigation.

4.3 Biochar increases while hydrochar restricts N_2O emissions

We found that biochar possesses a greater potential than hydrochar to increase soil N_2O emissions as indicated by significantly higher cumulative N_2O emissions in biochar (NB) than in hydrochar compared to control (Fig. 1e, in RAR and RCC). Our finding of increased N_2O emissions after biochar (NB) addition aligns with previous only few studies (Gao and DeLuca 2020; Nelissen et al. 2012; Prommer et al. 2014) and is in contrast with previous majority of studies (Cayuela et al. 2014; Joseph et al. 2021 and many references therein). This result could likely be associated with increased nitrification process (Nelissen et al. 2012; Prommer et al. 2014) influenced by char surface area. We found a strong positive correlation between char surface area, N_2O flux, NO_3^- concentrations and GN (Fig. 5a-b) suggesting that surface area of char was vital in creating soil conditions for N_2O production. An increased aerobic condition could be expected when char with greater surface area is introduced in the soil which could eventually support nitrification process—an oxic N_2O production process. This is further supported by our result of an increased mineral NO_3^- content and GN rates with biochar than with hydrochar amendments (Fig. 3a-b), indicating increased aerobic conditions and nitrifiers activities and possibly increased N_2O production via nitrifiers (Bremner 1997) making biochar amended soil as a stronger N_2O source. Based on NO_3^- content and GN compared to control (N), the hydrochar amendment also seemed to support nitrification similar to biochar (Fig. 3a-b). However, based on cumulative N_2O emissions and greater number of significantly lower weekly N_2O fluxes in hydrochar (NH) than in biochar (NB) amendments, it could be anticipated the some of the produced N_2O in hydrochar-amended soil could have been reduced (to N_2) before its release as surface emissions. This N_2O reduction could be mediated via either abiotic surface sorption (Cornelissen et al. 2013) and/or microbial N_2O reduction supported by either char being as a source of electron donor (Pascual et al. 2020) and/or by increasing abundance and activity of N_2O reductase gene (*nosZ*) containing microbes (Clough et al. 2013; Van Zwieten et al. 2014).

With available labile C (via similar yielding biomass, Table 3; Fig. 2) for denitrifiers in the soil and higher NO_3^- than control (N), it could be expected that denitrification may have contributed to emitted N_2O from both biochar (NB)- and hydrochar (NH)-amended mesocosms. However, based on the gross transformation rates of mineral NO_3^-, C_{mic}, and N_2O emissions, it could be anticipated that the role of denitrification in N_2O dynamics may have remained contrastingly different between biochar (NB)- and hydrochar (NH)- amended soil. Comparatively, reduced cumulative N_2O emissions (Fig. 1e), lower amount of NO_3^- content with its increased consumption rates (Fig. 3a-b), and a significantly increased C_{mic} and N_{mic} (Figs. 3e-f) in hydrochar (NH)- than in biochar (BH)- amended soils indicate that hydrochar amendment may have supported more N_2O reduction than N_2O production. In soils, the reduction of N_2O is carried out by two distinct groups of microbes that possess the *nosZ* gene: clade I and clade II (Hallin et al. 2018; Shan et al. 2021). The former is also known as traditional denitrifers and can not only reduce but also produces N_2O whereas the latter one is known to only reduce N_2O as it lacks N_2O producing enzymes (e.g., nirK/nirS) (Hallin et al. 2018). So, in NH treatment either complete dentification (reduction of NO_3^- to N_2) by clade I and/or direct N_2O reduction by clade II could be expected. The total CO_2 emissions showed a negative correlation with char surface area and a positive correlation with GC-NO_3^- (Fig. 5b), indicating a greater potential for complete dentification for hydrochar which had smaller surface than biochar (Table 1). Moreover, an increased reduction in soil N_2O emissions has been found with higher soil NO_3^- consumption, labile C and P concentrations (Gebremichael et al. 2022; O'Neill et al. 2020; Shen et al. 2021)—conditions resembling hydrochar (soluble P, Table 1) and its amendment effects in our study. Further, higher CO_2 emission (Fig. 1) and C_{mic} (Fig. 3e-f) with hydrochar ammendents indicate increased soil respiration, which could lead to decreasing O_2 concentration and increasing denitrification potential (Kammann et al. 2012; McCarty and Bremner 1992). On the contrary, same results with opposite trends in biochar (NB)-amended soils indicate that denitrifers may contribute to N_2O emissions in tandem with autotrophic nitrifers, although to a considerably lesser extent.

We found a negative correlation between soil pH and N_2O emission in RAR (Fig. 5a) indicating that N_2O emissions would increase with decreasing soil pH. This result is in agreement with a recent metanalysis (He et al. 2017). However, both (NB and NH) showed similar pH to control (N) (Table 3), similar to previous studies from boreal region (Soinne et al. 2020; Tammeorg et al. 2014). This suggests that their expected pH effect (liming for biochar and acidifying for hydrochar, Table 1) was masked by the acidifying effect of N of fertilization (Knoblauch et al. 2021). This is also further supported by a negative correlation between fertilization N and soil pH in char-freed conditions (Fig S2). These results suggest that N_2O flux dynamics in the studied soil are affected by other variables (as discussed above) n addition to char-induced pH changes in the soil. The mean WFPS% remained unaffected between the treatments in RAR (Table 3), suggesting that char had mere effect in altering soil moisture status. Nevertheless, we found a moderately negative correlation between total N_2O emissions and WFPS% in RAR (Fig. 5a). Char amendment in boreal region is shown to improve soil water retention, especially in clay soil (Rasa et al. 2018); however, we studied sandy loam soil. Earlier results on boreal clay soil and our contradicting resulting from sandy loam soil suggest that the effect of char amendment on soil moisture may depends on soil types and may have weaker impact on soil N_2O emissions dynamics and requires further investigation.

4.4 Soil N_2O sink with hydrochar and no N fertilization

Hydrochar seemed to perform better in the absence of fertilizer N, as it showed the potential to turn the soil into a N_2O sink. The lowest cumulative N_2O emissions (Fig. 1e) and more instances of N_2O uptake, reaching up to − 4.02 μg N m^{-2} h^{-1} $kg^{-1}{}_{charC}$ indicate that hydrochar without N amendment (treatment H) promoted the reduction of N_2O even more than hydrochar with N amendment (treatment NH). To our knowledge this effect of hydrochar on soil N_2O uptake from a legume-based forage system is the first one in the boreal region. Many of the previous studies have shown decreased N_2O emissions after biochar amendments; however, the uptake of N_2O as an amendments effect has not been reported before. The N_2O uptake in hydrochar without N amendment could likely be associated with combined effect of labile C, and the activity of *nosZ* I and II, albeit more intensely than that in hydrocahr with N amendment. First, the H treatment lacks an exogenous N_2O source, i.e., N fertilizer, making it more likely to emit less N_2O than hydrochar with fertilizer amendments. This is also further supported by the negative correlation between N fertilization and GN (Fig S2) in the absence of both chars indicating that nitrification was inhibited, thus suppressing N2O emissions — assuming nitrification serves as the main N_2O source process in the studied soil. Second, increased microbial immobilization of C and N (C_{mic} and N_{mic}) and almost equal gross rates of NO_3^- production and consumption could create nutrient deficiency, particularly NO_3^- for N_2O producers. The observed negative correlation between total N_2O emissions and the CN ratio of the applied char—where the latter is positively correlated with total CO_2 emissions, Cmic, and MAOC in the RCC system (Fig. 5b), suggests that hydrochar amendment helps reduce soil N_2O emissions primarily by influencing soil C pool dynamics. It has been shown that soils with NO_3^- deficiency when amended with labile C tend to increase the activity of *nosZ* I and II and thus reduce N_2O more efficiently than in soil with high NO_3^- content (Senbayram et al. 2012).

4.5 Biochar affects aboveground timothy and red clover biomass in different ways

The root biomass, shoot biomass, total yield and NUE (both, NUE_{PFP} and NUE_{Ag}) did not differ at all in both RAR and RCC (Table 3; Fig. 2); however, individual yield of timothy and red clover varied between and within treatments (Fig. 2a-b). This effect could likely be associated with differences in nutrient utilization efficiency between timothy and red clover under different soil conditions generated by biochar and hydrochar amendments. A similar result of timothy and red clover yield between and within hydrochar (NH) and control (N) treatment suggests that hydrochar amendment does not alter the nutrient utilization efficiency of individual crop species compared to control. This is also supported by similar NUE between hydrochar (NH) and control (N) as it represents the production of biomass per unit of N utilized by plants (Fig. 2c). In contrast, biochar (NB) amendment reduced the timothy yield significantly and increased (although insignificant) the yield of red clover compared to control in RAR (Fig. 2a), although it showed a similar NUE compared to control. This is also supported by the negative correlation between yields of timothy and red clover in RAR (Fig. 5a) indicating that biochar amendment affects the nutrient utilization efficiencies of timothy and red clover differently.

An improved yield of red clover in biochar treatment compared to control in our study (Fig. 2a) agrees with previous studies (Gao and DeLuca 2020; Tan et al. 2018). This could likely be associated with improved root morphological traits e.g., nodule mass (Quilliam et al. 2013; Xiang et al. 2017) of red clover and its nitrogenases activities (Farhangi-Abriz et al. 2024). Moreover, the presence of boron in biochar, similar to the one that we used in our study (Table S1), was linked to increased nitrogenase activity (Rondon et al. 2007), which could increase the yield of red clover more in biochar than in hydrochar compared to control. Timothy responds better than red clover to N fertilization (Termonen et al. 2020) as we have found in the control treatment (Fig. 2a) and in correlation analyses (a positive correlation between N fertilization and timothy yield Fig S2). However, the decreased timothy yield in biochar amendments (NB) compared to control even after receiving the same amount of mineral N fertilizer suggests that the efficiency of nutrient utilization, specially NO_3^- (primary N for plants), was affected negatively due to biochar. This effect could likely be associated with the greater surface area of biochar (Table 1), as it can immobilize greater amount of added fertilizer N onto their surfaces and pores via adsorption (Aller 2016), leading to its unavailability for plant growth. This is also hinted by reduced NUE_{Ag} in biochar (NB) amendments (Fig. 2d). Although not significant, a decreased normalized NUE_{Ag} in biochar (NB) compared to hydrochar (NH) amendments (Fig. 2d) suggests that biomass growing on biochar (NB)-amended soil was also using indigenous N (e.g., NO_3^- via nitrification) when added fertilizer becomes unavailable. Additionally, it could also suggests that biochar with high carbon content could alter the nitrogen acquisition strategies of roots after amendments likely by increasing root length and root tips (Xiang et al. 2017).

Interestingly, we found the opposite yield response, higher red clover than timothy yield in RCC and in the absence of fertilizer N (Fig. 2b). This suggests that biochar (B) and hydrocar (H) amendments act similarly and do not affect the nutrient use efficiency of vegetation unless synthetic fertilizer is supplied. In agreement with this, the total yield was significantly higher in hydrochar with fertilization (NH) than that without fertilization (H) (Fig. 2b), indicating the fertilization seems to benefit more when used with hydrochar. Similar effects were not evident when comparing total yields from biochar fertilization (NB) and without fertilization (B)(Fig. 2b), suggesting that fertilization had minimal effect when comparing yields per unit of char carbon content.

4.6 Soil MAOC stablizes better with hydrochar than biochar amendments

We found that soil MAOC remained unaffected by biochar (NB) and hydrochar (NH) amendments, although the mean MAOC of NB and NH seemed to increase slightly compared to control and to initial MAOC content when assessed in RCC (Fig. 4a). In contrast, we found a significant rise in soil POC content in biochar (NB) and hydrochar (NH) compared to control (N), suggesting that char itself was the main source of soil POC. This was also supported by normalized soil POC content which was similar and insignificant in both chars with and without fertilization amendments (Fig. 4b), a result corroborated by another short-term study (Garbuz et al. 2020). Due to the aromatic composition of the char, it is resistant to microbial degradation and therefore, it is retained long-term in soils (Lehmann et al. 2015).

Interestingly, we found a significant reduction in soil MAOC in biochar amendments (NB and B) when assessed for RCC (Fig. 4b), although both chars (NB and NH) showed equal rise in MAOC for RAR (Fig. 4a). Compared to initial soil MAOC, we found a significant reduction of soil MAOC in both biochar (NB and B) amendments (Fig. 4b).This suggests that the formation and stabilization of MAOC was better with hydrochar compared to biochar regardless of fertilizer N and could be related to char surface area and char C amount leading to microbial assimilation/necromass formation. In soil, MAOC is primarily formed by in-vivo assimilation of C-rich root exudates by soil microbes jointly with soil mineral particles (Lavallee et al. 2020). With similar above-ground biomass yields (Table 3) in both hydrochar (NH) and biochar (NB) amendments, a similar intensity in root exudation could be expected. However, due to larger surface area and higher C content of biochar (Table 1), a greater inhibition to microbial growth (see discussion about C_{mic} and Fig. 3e-f) could be expected leading to reduced microbial assimilation of C-rich root exudates and lesser formation of MAOC. In contrast, significantly higher C_{mic} and N_{mic} (Table 1) This suggests that hydrochar amendments promoted more effective formation and stabilization of mineral-associated organic carbon (MAOC), resulting in higher soil MAOC content compared to other amendments (Fig. 4b). These results are further supported by the strong negative correlation between surface area of char and C_{mic}, root biomass, and MAOC in RCC (Fig. 5b).

5 Conclusions

The aim of this study was to evaluate the effects of biochar and hydrochar amendments on GHG emissions and associated edaphic, soil microbial and plant attributes and C-sequestration potential of a typical boreal legume-forage grassland.

Our GHG results contradict to our first hypothesis as we found no change in cumulative CH_4 and CO_2 emissions while N_2O emissions were increased because of char amendments to legume-based forage system. We show that out of all three GHG, only N_2O emissions were affected considerably by both chars with N amendment. While CO_2 emissions and CH_4 uptake remained unaffected, biochar increased N_2O emissions substantially whereas hydrochar limited N_2O emissions compared to control. We found an increase in gross nitrification indicting an increase in activity of autotrophic nitrifiers, which explains higher N_2O emissions in biochar with N amendment. Hyrochar, exhibiting N_2O emissions similar to control, supported gross NO_3^- production and NO_3^- consumption equally indicating NO_3^- limitation in soil, which explains restricted N_2O emissions. Hydrochar showed greater number of N_2O utpake events than biochar (which had none) without N amendment, indicating the potential to make soil as a N_2O sink. Our second hypothesis remained rather neutral as we did not see significant increase in total (red clover + timothy) biomass yield and both NUE_{PFP} and NUE_{Ag}. While total biomass yield remained unchanged, biochar with N amendment showed reduced yield of timothy compared to yield of timothy in control, indicating that the response of timothy to fertilizer N acquisition was affected negatively. Our third hypothesis also remained rather neutral as we did not see significant increase in soil organic pools, MAOC and POC. Soil POC increased significantly in proportion to char addition; however, it was mainly because of char amendment itself not as result of photosynthetically fixed CO_2. Soil MAOC was slightly higher in both biochar and hydrochar with N amendment; however, differences were insignificant compared to control. Interestingly, MAOC per unit of char C was significantly lower in biochar with and without N amendment when compared to initial soil MAOC, indicating that biochar deaccelerates the formation of more recalcitrant soil carbon, which was also supported by lower microbial biomass in biochar with and without N amendment. In contrast, hydrochar with and without N amendment showed higher microbial C and no significant differences in MAOC per unit of char C with initial MAOC, indicating that hydrochar possesses the potential to stabilize the soil recalcitrant carbon better. We conclude that these effects were mainly because of the physiochemical (e.g., surface area) properties of the char (Fig. 6) and their interactions with soil inherent

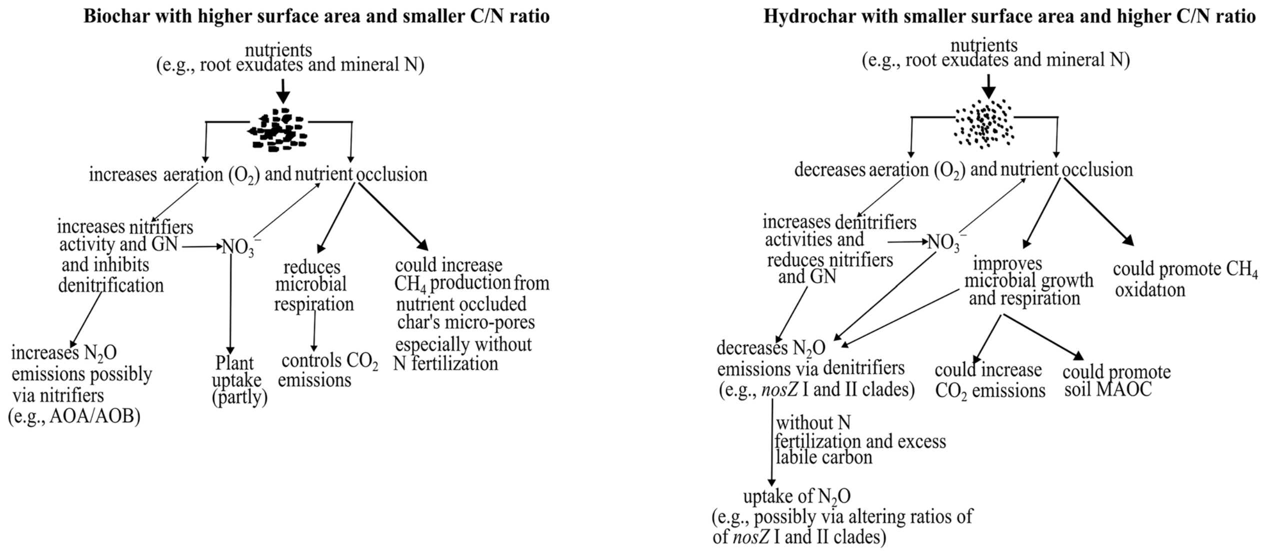


Fig. 6 A conceptual model showing interactions of biochar and hydrochar with legume-forage soil with vegetation. The proposed interaction mechanisms are based mainly on results from biochar (NB and B) and hydrochar (NH and H) amendment from our study and on earlier relevant studies that are referred in the discussion

architecture and plant-mediated changes in soil microbial communities.

In our study, we found several important aspects that hinted at clear effects of amendments, but they remained insignificant when compared to control. Some examples are, increased CH_4 uptake and CO_2 emission in hydrochar without N amendment, increased red clover yield in both chars with N amendment, and increased gross consumption of NH_4^+ in both char without N. These results showing no significant differences with control could likely because of short study period in laboratory conditions. Therefore, long-term field studies targeting ecosystems that are tightly coupled with milk and beef industries e.g., legume-forage ecosystem are needed. Moreover, for recommendation to large-scale use of char, more studies examining impacts of chars manufactured from diverse feedstocks in diverse agricultural ecosystems are needed, as the feedstock material influences the physiochemical composition of char, subsequently influencing its behavior in soil. Then only a soil and ecosystem specific char amendment policy can be developed and implemented, paving a path towards agricultural sustainability.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-025-00496-6.

Supplementary file 1.

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Authors contributions
The experimental design was conceived by HRB and supported by EH and NJS. HRB organized the soil sampling and collection of other necessary materials. HRB and EH initiated the experiments, which was later carried out independently by EH. Necessary guidance and supervision during the entire experimental work was provided mainly by HRB. EH did all the measurements and laboratory analyses (except gross N transformation rates, which was carried by HRB), processed the data, and performed the statistical test under the supervision of HRB. EH wrote the first draft, after which HRB finalized the manuscript with contributions from all the other authors. NJS was responsible for acquiring the research funds for the study.

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Data availability
Data related to this study is available at the Zenodo data repository via https://doi.org/10.5281/zenodo.15850279.

Declarations

Competing interests
All authors have no competing interests to disclose.

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