Biology and Fertility of Soils (2021) 57:319–328
https://doi.org/10.1007/s00374-020-01535-z

ORIGINAL PAPER

Canola straw biochars produced under different pyrolysis temperatures and nitrapyrin independently affected cropland soil nitrous oxide emissions

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Received: 21 August 2020 / Revised: 16 December 2020 / Accepted: 23 December 2020 / Published online: 3 January 2021

Abstract

The effect of biochar and nitrapyrin (a nitrification inhibitor) applications on nitrous oxide (N_2O) emissions from a cropland soil was studied in a 35-day incubation experiment. The biochars were produced using canola (*Brassica napus* L.) straw under two pyrolysis temperatures: 300 (BC300) and 700 °C (BC700). Biochars (20 g kg^{-1} soil) and nitrapyrin (80 mg kg^{-1} soil) were applied alone or in combination. The cumulative N_2O emissions were affected by both biochar and nitrapyrin applications ($p < 0.05$, same below) but not by their interaction. Cumulative N_2O emissions were not affected by BC700, but were increased by BC300, as compared with the CK treatment (no biochar addition). Nitrapyrin significantly decreased cumulative N_2O emissions by inhibiting nitrification, whether biochar was applied or not. There were positive relationships ($p < 0.05$) between cumulative N_2O emissions and soil microbial biomass carbon to nitrogen ratio, nitrate and dissolved organic nitrogen concentrations, and net nitrification rates. Our results show that biochars need to be appropriately selected (such as the use of BC700) that do not increase N_2O emissions, while the effectiveness of nitrapyrin in reducing N_2O emissions was not affected by the co-application of biochars. We conclude that the co-application of biochar and nitrapyrin may be able to both increase soil C sequestration by the addition of stable C contained in the biochar and reduce N_2O emissions from agricultural production systems.

Keywords Greenhouse gas · Biochar · Microbial biomass · N_2O emission · Nitrification inhibitor

Introduction

Nitrous oxide (N_2O), one of the most critical greenhouse gases, was estimated to have 265 times the global warming potential to that of CO_2 on a 100-year time frame (Stocker et al. 2013) and has also been implicated in destroying the atmospheric ozone layer (Ravishankara et al. 2009). Land-use activities such as intensively managed agriculture and forestry are the primary cause of increased anthropogenic N_2O emissions (Jia et al. 2019). Emissions of N_2O from anthropogenic sources, dominantly caused by fertilizer nitrogen (N) application in agriculture, have recently been reported to exceed the highest emission scenarios previously projected (Tian et al. 2020). Thus, reducing N_2O emissions from agricultural production systems is an urgent matter (Baah-Acheamfour et al. 2016; Omonode et al. 2017; Smith 2017; Tian et al. 2020).

Biochar, an organic material derived from biomass through pyrolysis without or with limited oxygen, has been shown to potentially reduce greenhouse gas emissions when applied to the soil (Lehmann and Joseph 2015). The effect of biochar application on N_2O emissions is often variable. Some meta-analysis studies showed that biochar addition reduced N_2O emissions by 12 to 54% (Cayuela et al. 2014, 2015; Liu et al. 2018). However, some have shown that N_2O emissions

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were increased by biochar application. For instance, in a 12-day incubation study, the application of a biochar produced by the pyrolysis of green waste at 550 °C increased N_2O emissions by 54% in agricultural soils (Sánchez-García et al. 2014), as a result of enhanced nitrification. Similarly, a biochar made from spruce woodchips at ~ 450 °C increased N_2O emissions from an abandoned grassland soil indirectly through increasing soil water content and reducing plant N uptake (Saarnio et al. 2013).

Many abiotic and biotic mechanisms have been proposed to explain biochar effects on N_2O production (Kammann et al. 2017); most of these mechanisms are dependent on soil and biochar properties (Saarnio et al. 2013; Lehmann and Joseph 2015; Levesque et al. 2020). Pyrolysis temperature is one of the primary determinants of biochar characteristics (Clough et al. 2013; Lehmann and Joseph 2015). Biochars produced under high-pyrolysis temperatures usually have a greater surface area and higher organic C adsorption capacity that have a greater ability to adsorb nitrate (NO_3^-) and other nutrients as compared with biochars produced under low-pyrolysis temperatures (Mukherjee and Zimmerman 2013). Many have studied the effect of biochars made from different feedstocks or under different pyrolysis conditions on N_2O emissions; however, studies on the effect of canola (*Brassica napus* L.) straw biochar on N_2O emissions are rare, especially canola straw biochars pyrolyzed under different temperatures, while canola is a major agricultural crop and the proper disposal of its residue will benefit the agriculture sector. A better understanding of the effect of biochars made from canola straw under different pyrolysis temperatures on N_2O production from the soil is needed.

Another widely used method to mitigate soil N_2O emissions is the application of nitrification inhibitors; nitrification is one of the main processes responsible for N_2O emissions from agricultural soils. The product (NO_3^-) from nitrification is also subject to losses through denitrification (which is another process that produces N_2O) and leaching. Among the nitrification inhibitors, nitrapyrin is widely used and can effectively decrease N_2O emissions by inhibiting the activities of the bacteria *Nitrosomonas* (Dittmar et al. 2009).

Applying biochar and nitrification inhibitors together has been proposed to be a viable approach to enhance biochar's benefit as a soil amendment (Chen et al. 2019). The combined application of biochar and 3,4-dimethylpyrazole phosphate has been shown to decrease N_2O emissions more than biochar application alone (Chen et al. 2019). However, Li et al. (2015) demonstrated that applying wheat straw biochar and nitrapyrin together increased N_2O emissions by 9% in a managed vegetable field compared with nitrapyrin application alone. In contrast, there were no significant differences between combined or individual application of nitrapyrin and maize straw biochar in N_2O emissions (Niu et al. 2018). Biochar may adsorb nitrification inhibitors when they are applied together and reduce the effectiveness of the nitrification inhibitor (Li et al. 2020). To the best of our knowledge, no one has tested the interaction of canola straw biochars that are produced at different pyrolysis temperatures and nitrapyrin on soil N_2O emissions. We were interested in understanding if the co-application of canola straw biochar would reduce the effectiveness of nitrification inhibitors and if canola straw biochars made under different pyrolysis temperatures behave differently when co-applied with nitrification inhibitors. In this study, we conducted a 35-day laboratory incubation experiment with two specific objectives: (1) to investigate the interactive effects of biochars produced under different pyrolysis temperatures and nitrapyrin on soil N_2O emissions, and (2) to examine the relationships between soil characters and cumulative N_2O emissions.

Materials and methods

Soil sampling and biochar production

Five canola fields near Edmonton (53° 32′ 0″ N, 113° 30′ 0″ W), Alberta, Canada, were selected as the soil sampling sites (each site represents a block in our experiment). The sampling sites were located more than 20 km from each other. The cropland was canola rotated with wheat or fallow. The soils at those sampling sites were classified as Black Chernozems based on the Soil Classification Working Group (1998). One composite surface mineral soil sample (0–10 cm) was collected from each of these five sampling sites. Each composite sample consists of ten core samples collected 5 m from each other using a 5-cm diameter auger to obtain a representative sample for each site. Each composite sample was treated as a replicate (block) in the lab incubation experiment. The soil samples were kept fresh and gently sieved through a 2-mm sieve after litter and plant roots were removed. The samples were stored in a refrigerator under − 20 °C for no more than 2 weeks until further analysis. The soil pH ranged from 4.3 to 6.5, total C from 20.6 to 74.7 g kg^{-1}, total N from 2.0 to 7.0 g kg^{-1}, exchangeable NH_4^+ from 0.36 to 6.27 mg kg^{-1}, and NO_3^- from 1.4 to 35.4 mg kg^{-1} (see below for the methods of analysis of those soil properties).

Biochars were produced in a limited oxygen environment using a slow-pyrolysis process (Kwak et al. 2019). Before pyrolysis, the canola straw was chopped to pieces less than 10 mm long and oven-dried at 60 °C for 24 h. The biochars were then produced by heating the canola straw at different temperatures, with a heating rate of 10 °C min^{-1}. For producing low- and high-pyrolysis temperature biochars, the final temperature was set at 300 (BC300) and 700 °C (BC700), respectively. The chemical and physical properties of the biochars can be found in Supplementary materials (Table S1).

Incubation procedure

In the laboratory incubation experiment, we used a completely randomized block design with biochar applied at three levels: no biochar addition (CK), the addition of biochar produced at 300 °C (BC300), and biochar produced at 700 °C (BC700) using canola straw as the feedstock, and nitrapyrin applied at two levels: with and without nitrapyrin addition, as the treatments. Biochar was applied at 2% (*w/w*; oven-dry weight basis, equivalent to 4.5 Mg ha^{-1}) (Pokharel et al. 2018), and nitrapyrin was applied at 80 mg kg^{-1} soil (*w/w*, equivalent to 180 kg ha^{-1}) (Islam et al. 2007). Two parallel incubation experiments were conducted. The first one was conducted in 500-mL mason jars using 100-g (oven-dry equivalent) fresh soil for determining N_2O emission rates. The second set was conducted in 1.5-L mason jars using 300-g (oven-dry equivalent) fresh soil for measuring the changes in soil chemical and microbial properties over time in the incubation experiment. The biochar and/or nitrapyrin was thoroughly mixed with the soil before placing into the mason jars. The soils in the mason jars were adjusted to 40% water holding capacity (WHC) using deionized water and were pre-incubated for 3 days at 25 °C in the dark to stabilize microbial activities. After the 3-day pre-incubation, the water content was adjusted to 60% WHC, which was maintained for the 35-day incubation period by adding water every 2 days based on weight loss.

Gas sampling and measurement

N_2O flux measurements were conducted on days 0 (after pre-incubation), 1, 3, 5, 7, 10, 13, 18, 23, 28, and 35. On each of those sampling days, the 500-mL mason jars were sealed tightly with lids that come with silicone pads for N_2O flux measurement. Once the mason jars were sealed, they were pumped with 10-mL syringes three times to make the air in the headspace uniform, and a 5-mL gas sample was taken using a 10-mL syringe. The gas sampling was repeated three times every 8 h. On non-sampling days, the 500-mL mason jars were covered with aluminum foil punched with a few small holes to minimize water loss from the jars but allow air in the headspace to be exchanged with the atmosphere. At each gas sampling, the 5-mL gas sample was injected into a pre-evacuated 3-mL glass container to create a positive pressure for the N_2O measurement. A gas chromatograph (Varian CP-3800, Mississauga, Ontario, Canada) that had an electron capture detector (detection limit: 5–20 μg L^{-1}) was used to analyze the N_2O concentrations in the collected gas samples. Daily N_2O fluxes were then calculated. The N_2O emissions on days not measured were calculated by linear interpolation. The cumulative N_2O emissions were summed from daily N_2O emissions over the incubation period (Yu et al. 2019).

Soil analysis

The soil was destructively sampled using a spatula on days 1, 7, 14, 21, 28, and 35 from the 1.5-L mason jars. For measuring the dissolved organic C (DOC) and N (DON) concentrations, 10 g of moist soil samples was extracted using 50 mL K_2SO_4 solution (0.5 mol L^{-1}) by shaking at 250 rpm for 30 min and then filtered through Whatman No. 42 filter papers. The C and N concentrations in the extracts were determined using a TOC-TNM1 analyzer (Shimadzu Corporation, Kyoto, Japan). Soil exchangeable NH_4^+–N and NO_3^-–N in the extracts described above were analyzed colorimetrically. The NH_4^+–N in the extracts was determined using the indophenol blue method (Keeney and Nelson 1982). Briefly, a 0.5-mL aliquot of the filtered extract was incubated in a 40 °C water bath for 30 min, then reacted with 0.2 mL phenol-nitroprusside and 1.7 mL of diluted hypochlorite reagent to form indophenol-blue in a test tube. The absorbance was read at 636 nm on a spectrophotometer (Genesys 10-S, Thermo Scientific Inc., Rochester, NY); a 0.5 M K_2SO_4 was used as the blank (Keeney and Nelson 1982). The NO_3^-–N concentration in the extract was determined using the vanadium oxidation method (Miranda et al. 2001). First, a 20-μL aliquot of the filtered extract was transferred into a 2.0-mL microcentrifuge tube, then 1.0 mL vanadium regent was added into the microcentrifuge tube. The microcentrifuge tubes were placed in the dark at room temperature (25 °C) for 24 h, after which the absorbance was read at 540 nm on the spectrophotometer described earlier against the absorbance of a blank (0.5 mol L^{-1} K_2SO_4) (Miranda et al. 2001). The net nitrification rates (NNR) were calculated from the changes in the NO_3^-–N pool size between two incubation intervals (Han et al. 2012).

Soil pH was measured using a Thermo Scientific pH meter (710A, Beverly, MA) in a 1:5 soil:water (w:v) suspension. We used the chloroform fumigation-extraction method to determine soil microbial biomass C (MBC) and N (MBN) (Brookes et al. 1985). Briefly, both fumigated (fumigated in the dark, 24 h, at room temperature) and non-fumigated fresh samples were extracted using 1:5 soil: 0.5 mol $L^{-1}K_2SO_4$ solution (w:v). Carbon and N concentrations in the extracts were measured using the TOC-TNM1 analyzer described above to calculate MBC and MBN using an extraction coefficient of 0.45 (Jenkinson et al. 2004).

Data analysis

All data analyses were carried out using R (R Core Team 2018). Assumptions of normality of distribution were evaluated by the Shapiro-Wilk's test, and homogeneity of variance was tested using the Levene's test, before further data analysis. The effects of fixed factors (nitrapyrin and biochar) on cumulative N_2O emissions were tested using the linear mixed-effects model (LMM), with sampling site or replication as random factors. Natural logarithmic transformations were

applied to the N_2O data before data analysis (to make the distribution of the residuals normal), while the untransformed N_2O data are presented in this paper. Other data were not transformed as they met the normality of distribution and heterogeneity of variance assumptions. For the repeated measurements of soil pH, NO_3^-, exchangeable NH_4^+, DOC, DON, MBC, and MBN, the LMM was used with nitrapyrin and biochar as the fixed effects, and sampling site (as a random effect) was nested in sampling day. The calculated net nitrification rate was analyzed using the LMM with nitrapyrin and biochar as the fixed effects and sampling site as the random effect. When the analysis of variance (ANOVA) showed significant effects, the least square means were compared with the 'lsmeans' function in the *emmeans* package at $p \leq 0.05$. Relationships between soil properties and cumulative N_2O emissions were explored using Pearson correlation analysis. Soil chemical properties significantly correlated with cumulative N_2O emissions were analyzed with linear regression to quantify the response of cumulative N_2O emissions to each chemical variable. All statistical significance was set at α = 0.05 unless otherwise stated.

Results

Effects of biochar and nitrapyrin on N_2O emissions

The highest daily N_2O emission rates were observed on either day 0 (BC300 addition, regardless of the nitrapyrin treatment) or day 1 (CK and BC700, regardless of the nitrapyrin treatment); the rate then sharply decreased until day 10, and then remained stable until day 35 (Fig. 1(a)). At the later stage of the incubation, soil N_2O emission rates were low and not different between treatments regardless of treatment (nitrapyrin and/or biochar addition; Fig. 1(a)). No interaction between the biochar and nitrapyrin treatments was observed on cumulative N_2O emissions (Fig. 1(b) and Table S2, $F = 0.23$, $p = 0.80$), which was affected by biochar ($F = 37.46$, $p < 0.001$) and nitrapyrin ($F = 10.91$, $p = 0.004$) application independently. The BC300 treatment increased cumulative N_2O emissions by 5.4 and 8.4 times compared to the CK and BC700 treatments, respectively (Fig. 1(c), in the original scale). Nitrapyrin addition reduced cumulative N_2O emissions by 42% compared to no nitrapyrin addition (Fig. 1(d)).

Effects of biochar and nitrapyrin on soil chemical and microbial properties

Repeated measures ANOVA showed that incubation time, biochar, nitrapyrin, and/or their interaction influenced soil pH, exchangeable NH_4^+, DOC, DON, MBC, MBN, and MBC:MBN ratio (Table S3). More specifically, on day 1, soil pH was higher in biochar addition (BC300 and BC700) than in the CK. The highest soil DOC was found in BC700, which was higher than that in CK and BC300. The highest soil MBC and MBC:MBN ratio were found in BC300, and were greater in BC300 than in CK and BC700. Biochar and nitrapyrin interacted to affect soil exchangeable NH_4^+, DON, and MBN. Soil NO_3^- concentration was not different across all treatments on day 1 (Table 1).

Soil pH and NO_3^- were affected by biochar and nitrapyrin when measured on day 35 (Table 2). Among the treatments, soil pH was the highest, while NO_3^- concentration was the lowest in the BC700 treatment. Similarly, soil pH was higher, and NO_3^- was lower with nitrapyrin than without nitrapyrin addition. Nitrapyrin application increased soil exchangeable NH_4^+ but decreased MBC:MBN ratio. The biochar affected DOC, MBC, and MBN. The highest soil DOC concentration was found in BC300, which was higher than that in CK and BC700. Both BC300 and BC700 increased soil MBC, while BC700 also increased soil MBN. Biochar and nitrapyrin interacted to affect soil DON: without nitrapyrin addition, soil DON was lowest in BC700 as compared with CK and BC300, while with nitrapyrin addition, soil DON was not affected by biochar addition (Table 2). In addition, without nitrapyrin addition, NO_3^- concentration increased over time (Fig. 2(a)), while with nitrapyrin addition, exchangeable NH_4^+ concentration increased over time (Fig. 2(b)).

Effects of biochar and nitrapyrin on soil net nitrification rates

Soil net nitrification rate (NNR) was affected by biochar ($F = 20.27$, $p < 0.01$) and nitrapyrin additions ($F = 63.86$, $p < 0.01$), but not by their interaction ($F = 1.96$, $p = 0.17$) (Fig. 3). The addition of BC700 significantly reduced soil NNR (− 0.23 mg N kg^{-1} day^{-1}), as compared with CK and BC300 addition (Fig. 3).

Relationships between soil properties and cumulative N_2O emissions

Cumulative N_2O emissions were positively related to soil NO_3^- concentration, DON, MBC:MBN ratio, and NNR; however, no relationships were found between N_2O emissions and soil pH, MBC, and MBN (Fig. 4). In addition, soil NNR was negatively related with soil exchangeable NH_4^+ while positively with NO_3^- (Fig. 4). The MBC:MBN ratio, NO_3^-, DON, and NNR explained 26.5, 19.8, 11.4, and 11.3%, respectively, of the variation in cumulative N_2O emissions (Table 3).

Discussion

Our results show that the cumulative N_2O emissions were not interactively affected by biochar and nitrapyrin applications,

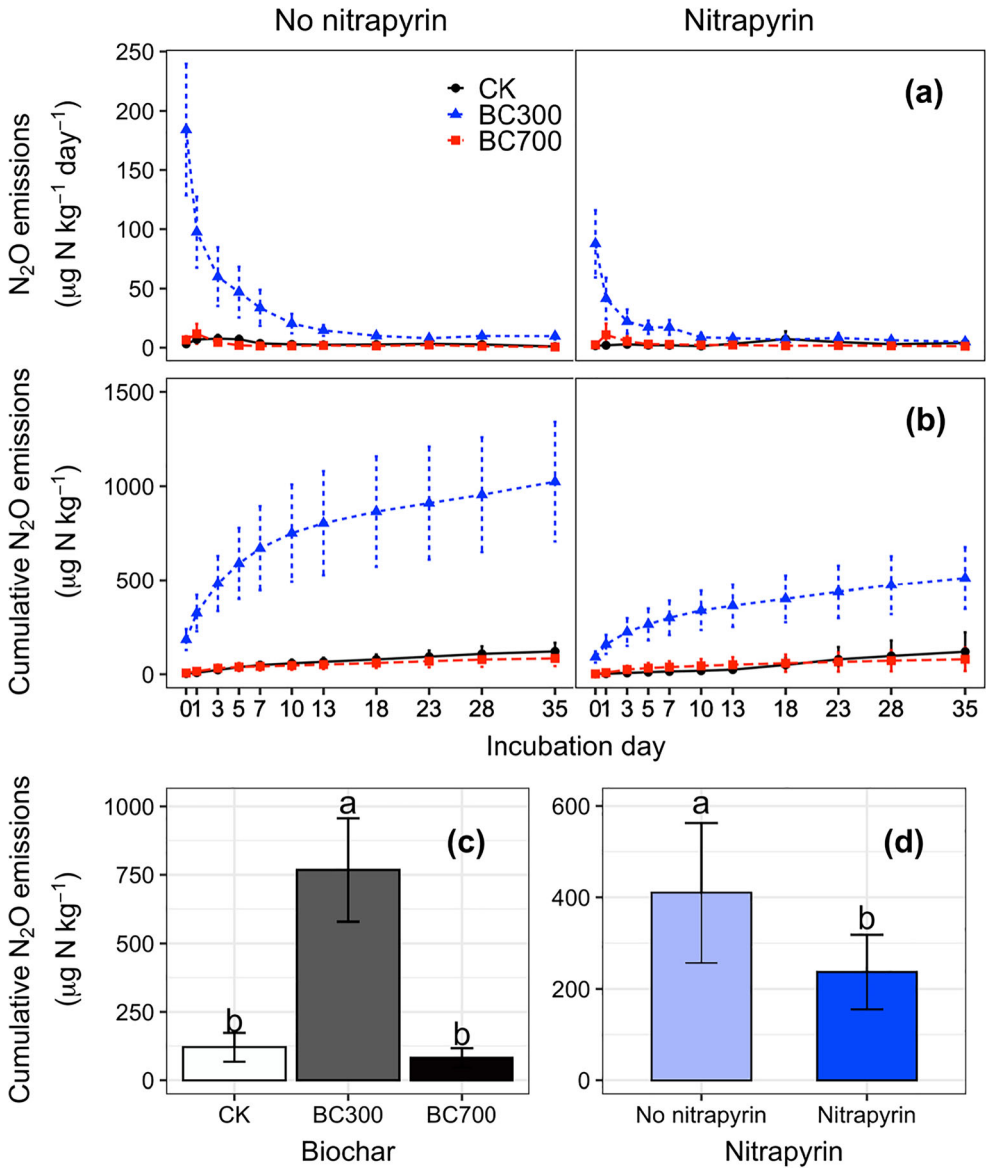

Fig. 1 (a) Daily N_2O emissions (mean ± SE; same below), (b) cumulative N_2O emissions, (c) the main effect of biochar on cumulative N_2O emissions, (d) the main effect of nitrapyrin on cumulative N_2O emissions from a cropland soil. Different lowercase letters in (c) and (d) indicate significant differences between biochar/nitrapyrin treatments (P = 0.05; Tukey's test). CK, no biochar addition; BC300, the addition of biochar produced under low-pyrolysis temperature (300 °C); BC700, the addition of biochar produced under high-pyrolysis temperature (700 °C)

indicating that the co-application of biochars produced at different pyrolysis temperatures and nitrapyrin will not reduce the effectiveness of nitrapyrin in reducing nitrification rates and N_2O emissions. Many researchers found that amending soil with biochar can reduce soil N_2O emissions (Borchard et al. 2019; Duan et al. 2020; Wu et al. 2013). However, our results show that the pyrolysis temperature used for biochar production significantly influenced cumulative N_2O emissions; the addition of BC300, but not BC700, increased cumulative N_2O emissions compared to the CK.

The main difference in N_2O emissions between BC300 and BC700 occurred in the first few days of the incubation. Similar to that reported in Ameloot et al. (2013), the increased N_2O emission rate in the early few days in the BC300 treatment might have been caused by the higher volatile matter content in the BC300 biochar. Biochars produced at a low pyrolysis temperature usually have a higher volatile matter content than those produced at a high pyrolysis temperature (Lehmann et al. 2011; Mukherjee and Zimmerman 2013). Biochar addition can affect the function and abundance of N_2O-producing nitrifiers and denitrifies by altering soil chemical properties (Braker and Conrad 2011). The BC300 treatment markedly increased soil MBC and the MBC:MBN ratio on day 1 compared with the CK and BC700 treatments (Table 1), indicating that the soluble C introduced by BC300 addition might have caused a short-term positive priming effect, resulting in the enhancement of microbial growth (Tan and Chang 2007) and the concurrent immobilization of soil mineral N (Deenik et al. 2010), and eventually increasing N_2O fluxes in the early stage of the incubation experiment. This finding is consistent with Nelissen et al. (2012) where they found that NH_4^+ consumption by microbial populations was

Table 1 The effects of biochar (BC) and nitrapyrin (NI) on soil properties on day one of a laboratory incubation experiment

Soil property	No nitrapyrin			Nitrapyrin				*P* values‡		
	CK§	BC300	BC700	CK	BC300	BC700	SEM†	NI	BC	NI × BC
pH	4.94^{b}	5.00^{a}	5.02^{a}	4.96^{b}	5.01^{a}	5.04^{a}	0.41	0.195	*< 0.001*	0.561
NO_3^-–N (mg kg^{-1})	27.74^{a}	16.12^{a}	19.36^{a}	24.38^{a}	12.80^{a}	16.01^{a}	6.31	0.604	0.251	0.139
NH_4^+–N (mg kg^{-1})	1.92^{z}	1.73^{z}	2.57^{yz}	7.48^{xy}	2.15^{yz}	11.05^{x}	1.72	*< 0.001*	*0.002*	*0.003*
DOC (mg kg^{-1})	354.65^{c}	428.97^{b}	572.43^{a}	366.01^{b}	418.40^{b}	493.40^{a}	58.22	0.116	*< 0.001*	0.076
DON (mg kg^{-1})	71.22^{xy}	85.90^{x}	43.26^{y}	69.72^{xy}	60.67^{xy}	64.67^{xy}	18.39	0.795	0.062	*0.034*
MBC (mg kg^{-1})	342.61^{b}	463.12^{a}	371.34^{b}	293.54^{b}	414.05^{a}	322.27^{b}	44.36	*0.004*	*< 0.001*	0.862
MBN (mg kg^{-1})	52.43^{xy}	54.28^{xy}	62.82^{xy}	49.66^{y}	64.01^{x}	54.31^{xy}	5.06	0.838	*0.027*	*0.022*
MBC: MBN ratio	6.62^{b}	7.86^{a}	6.35^{b}	5.76^{b}	7.00^{a}	5.49^{b}	0.46	*0.009*	*0.001*	0.058

[a–c] Within a row, different superscripts indicate significant differences of the least square means due to biochar addition

[x–z] Within a row, different superscripts indicate significant differences of the least square means due to nitrapyrin × biochar interaction

† Pooled (largest) SE of least square means

§ CK, no biochar addition; BC300, the addition of biochar produced under low-pyrolysis temperature (300 °C); BC700, the addition of biochar produced under high-pyrolysis temperature (700 °C)

‡ Observed significance levels for main effects of nitrapyrin, biochar, and the nitrapyrin × biochar interaction. *P* values less than 0.05 are highlighted in italics

higher in the soil applied with biochar produced under a low pyrolysis temperature (350 °C) as compared to that applied with biochar produced under a high pyrolysis temperature (550 °C) or no biochar addition. On the other hand, the BC700 in this study had a large surface area (Table S1) and aromatic-C that would result in a high adsorption capacity, which might decrease the availability of substrates for microbial populations (Clough et al. 2013; Lehmann et al. 2011). Furthermore, the addition of BC700 might have increased soil aeration, which would reduce denitrification rates, as increased oxygen concentration in the soil inhibits denitrifying microbial activities (Van Zwieten et al. 2010).

Soil pH was less than 7 throughout the incubation in all treatments (Table 2). However, with a higher pH of the BC700 biochar than the BC300 biochar (Table S1), soil amended with BC700 had the highest pH (Table 2). An increase in soil pH might increase the N_2O-reductase (denitrifies) activities, and eventually, increase N_2 formation from N_2O and decrease the N_2O to N_2 ratio (Singh et al. 2010).

Table 2 The effects of biochar (BC) and nitrapyrin (NI) on soil properties on day 35 of a laboratory incubation experiment

Soil property	No nitrapyrin			Nitrapyrin				*P* values‡		
	CK§	BC300	BC700	CK	BC300	BC700	SEM†	NI	BC	NI × BC
pH	4.88^{c}	5.32^{b}	5.57^{a}	5.15^{c}	5.58^{b}	5.82^{a}	0.48	*< 0.001*	*< 0.001*	0.052
NO_3^-–N (mg kg^{-1})	95.59^{a}	77.84^{a}	37.62^{b}	33.49^{a}	33.30^{a}	1.35^{b}	11.41	*< 0.001*	*< 0.001*	0.191
NH_4^+–N (mg kg^{-1})	1.55^{a}	1.08^{a}	0.94^{a}	52.00^{a}	45.94^{a}	50.84^{a}	8.28	*< 0.001*	0.894	0.912
DOC (mg kg^{-1})	315.96^{b}	404.15^{a}	352.58^{b}	311.00^{b}	399.19^{a}	347.62^{b}	46.36	0.773	*0.002*	0.870
DON (mg kg^{-1})	130.09^{x}	138.74^{x}	67.31^{y}	142.19^{x}	130.62^{x}	146.46^{x}	22.57	*< 0.001*	*0.013*	*0.001*
MBC (mg kg^{-1})	282.79^{b}	370.18^{a}	328.57^{a}	274.91^{b}	362.31^{a}	320.69^{a}	38.23	0.558	*< 0.001*	0.331
MBN (mg kg^{-1})	30.21^{b}	43.46^{ab}	61.75^{a}	47.60^{b}	60.85^{ab}	79.15^{a}	9.41	0.066	*0.031*	0.367
MBC: MBN ratio	12.69^{a}	14.29^{a}	5.20^{a}	7.21^{a}	5.72^{a}	4.80^{a}	3.15	*0.044*	0.112	0.340

[a–c] Within a row, different superscripts indicate significant differences of the least square means due to biochar addition

[x–y] Within a row, different superscripts indicate significant differences of the least square means due to nitrapyrin × biochar interaction

† Pooled (largest) SE of least square means

§ CK, no biochar addition; BC300, the addition of biochar produced under low-pyrolysis temperature (300 °C); BC700, the addition of biochar produced under high-pyrolysis temperature (700 °C)

‡ Observed significance levels for main effects of nitrapyrin, biochar, and the nitrapyrin × biochar interaction. *P* values less than 0.05 are highlighted in italics

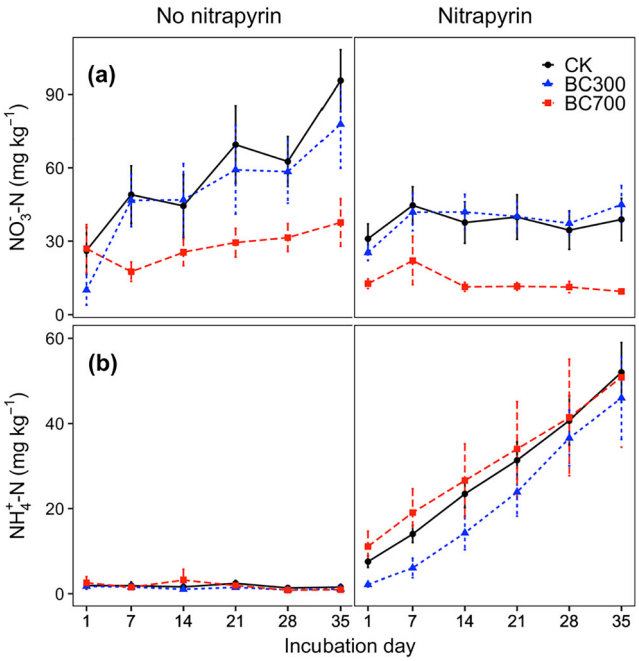

Fig. 2 Effects of biochar and nitrapyrin on (a) nitrate (mean ± SE; same below) and (b) ammonium concentrations. CK, no biochar addition; BC300, the addition of biochar produced under low-pyrolysis temperature (300 °C); BC700, the addition of biochar produced under high-pyrolysis temperature (700 °C)

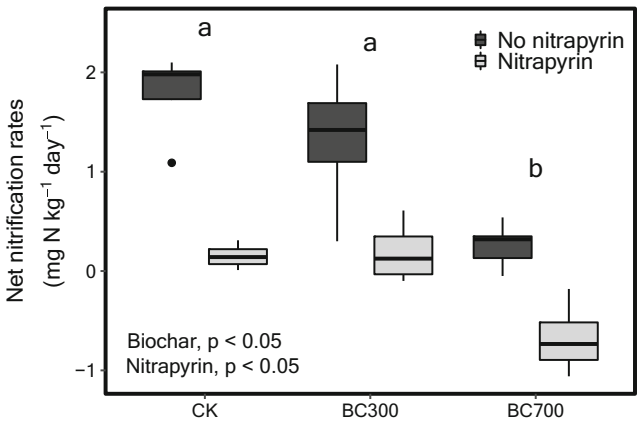

Fig. 3 Effects of biochar and nitrapyrin on net nitrification rates. The black dot represents an outlier. Different lowercase letters indicate significant differences between biochar addition treatments regardless of the nitrapyrin treatment (P = 0.05; Tukey's test). CK, no biochar addition; BC300, the addition of biochar produced under low-pyrolysis temperature (300 °C); BC700, the addition of biochar produced under high-pyrolysis temperature (700 °C)

The lower net nitrification rates in the BC700 treatment compared to the CK and BC300 treatments are consistent with the lack of BC700 treatment effect on N_2O emissions (Figs. 2 and 3 and Table 2); the lower net nitrification rates means that BC700 suppressed nitrification rates and reduced N_2O emissions, which counteracts the potential contribution of the volatile matter contained in the biochar that may enhance N_2O emissions. Furthermore, under 60% WHC, nitrification would be the dominant process contributing to N_2O production (Inubushi et al. 1996). The strong relationship between net nitrification rates and cumulative N_2O emissions (Fig. 4 and Table 3) illustrates the contribution of nitrification to N_2O emissions in the 35-day incubation. However, larger N_2O emissions occurred after the application of BC300 in the earlier stage of the incubation, and there were no significant differences in net nitrification rates between the BC300 and CK treatments, suggesting that other processes such as denitrification were enhanced by the priming effect and contributed to N_2O emissions. Biochar application can alter the activities of a range of enzymes related to C and N cycling (Pakheral et al. 2020) and genes that regulate N transformations in the soil (Ji et al. 2020), thereby influencing N_2O emissions. The closure of the mason jars for measuring N_2O emissions and the high greenhouse gas (such as CO_2) concentrations in the BC300 applied soils during the earlier incubation stage might have created a temporary anaerobic condition that enhanced the activities of denitrifiers (Sherlock and Goh 1983).

Another strong predictor for N_2O emission rates could be the H:C molar ratio of biochar. According to Cayuela et al. (2015), biochars with a H:C ratio < 0.3 have the ability to suppress N_2O emissions, and those with a H:C ratio > 0.5 are not effective in suppressing N_2O emissions. In our study, the H:C values of BC700 and BC300 were 0.26 and 0.95, respectively (Table S1), and the application of BC300 (biochar with a high H:C ratio) increased N_2O emissions. The lower H:C ratios imply higher redox activities and sorption abilities, such biochars can directly mediate the process of denitrification to produce more N_2 rather than N_2O (Klüpfel et al. 2014; Qiu et al. 2014; Zumft 1997).

Unlike many other studies that have shown that biochars are potentially beneficial in mitigating N_2O emissions (Cayuela et al. 2014), our research shows that canola straw biochar either increased (BC300) or had no effect (BC700) on cumulative N_2O emissions compared with no biochar addition. This result may be partly related to the canola straw being an N-rich feedstock that the produced biochar contained relatively high N concentration, especially when pyrolyzed at a low temperature, as compared to other feedstocks such as woodchips (Kwak et al. 2019). The N introduced by biochar application may cause a positive priming effect on soil N and increase the availability of substrates for microbes in a short period of time (Fiorentino et al. 2019). Therefore, we need to be cautious when selecting a feedstock and the pyrolysis condition for producing biochar to use as a soil amendment in order not to increase N_2O emissions.

Conclusions

Cumulative N_2O emissions from cropland soils were affected by biochar and nitrapyrin applications, but not their interaction. The cumulative N_2O emissions were increased by the BC300 treatment but were not affected by the BC700

Table 3 Simple linear regression models for relationships between cumulative N_2O emissions (μg N kg^{-1} soil) and soil properties

Model	*P* value	Variation explained (%)
N_2O = 6.15 + 37.69 MBC:MBN ratio	0.002	26.5
N_2O = 39.20 + 6.09 NO_3^-	0.010	19.8
N_2O = − 101.69 + 3.38 DON	0.039	11.4
N_2O = 230.20 + 205.90 NNR	0.045	11.3

MBC and N, microbial biomass C and N (mg kg^{-1}), respectively; DOC and N, dissolved organic C and N (mg kg^{-1}), respectively; NNR net nitrification rates (mg N kg^{-1} day^{-1})

treatment, as compared with the CK treatment. Significantly higher NH_4^+ consumption rates, MBC, and MBC:MBN ratio in soils with BC300 addition in the early stage of the incubation contributed to the higher cumulative N_2O emissions. The greater surface area, lower volatile matter content, and lower H:C value of the BC700 biochar suppressed net nitrification and NH_4^+ consumption rates, counteracting the stimulation on nitrification rates by the added dissolvable C, and eventually did not affect soil N_2O emissions relative to BC300. Nitrapyrin addition was effective in reducing net nitrification rates and cumulative N_2O emissions. Biochar addition did not affect the effectiveness of nitrapyrin in reducing N_2O emissions in the studied soil. Biochars need to be appropriately selected (such as BC700) in order not to increase N_2O emissions when applied to the soil. Since the effectiveness of nitrapyrin in reducing N_2O emissions was not affected by the co-application of biochar, we suggest that the co-application of biochar and nitrapyrin will be beneficial to both increase soil C sequestration through the addition of the stable C contained in biochar, and reduce N_2O emissions. Future research needs to investigate the effectiveness of biochars produced from different crop residues (feedstock type) and with different pyrolysis conditions, and their interactions with nitrification inhibitors, on reducing N_2O emissions under different soil conditions in long-term field studies.

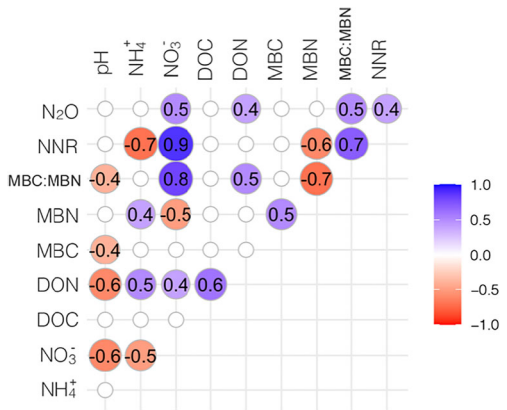

Fig. 4 Pearson correlation of soil properties and cumulative N_2O emissions in a 35-day laboratory incubation experiment. White circle indicates the correlations is not significant ($P > 0.05$). Color scheme with values indicates the Pearson correlation coefficient. DOC and N, dissolved organic C and N, respectively; MBC and N, microbial biomass C and N, respectively; NNR, net nitrification rates

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-020-01535-z.

Acknowledgments We thank Cole Gross, Christopher Nzediegwu, Dauren Kaliaskar, Na Chen, Prem Pokharel, Qian Wu, Yanchen Zhang, and Zilong Ma for their assistance in the laboratory. We also thank the Editor in Chief, the Regional Editor, and two anonymous reviewers for their constructive comments that improved earlier versions of this manuscript.

Funding We thank the China Scholarship Council (CSC) and the Natural Science and Engineering Research Council of Canada (NSERC) for funding. The first author was also supported by a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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