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RESEARCH ARTICLE

Cattle manure biochar and earthworm interactively affected CO_2 and N_2O emissions in agricultural and forest soils: Observation of a distinct difference

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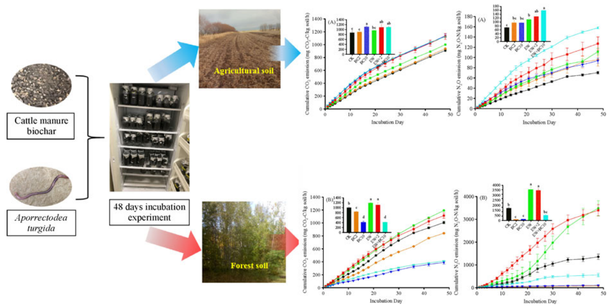
HIGHLIGHTS

- Earthworms increase CO_2 and N_2O emissions in agricultural and forest soil.
- 10% biochar suppresses CO_2 and N_2O emissions in forest soil.
- Biochar interacted with earthworm to significant affect CO_2 and N_2O emissions.

GRAPHIC ABSTRACT



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ABSTRACT

The application of manure-derived biochar offers an alternative to avoid the direct application of manure to soil causing greenhouse gas emission. Soil fauna, especially earthworms, can markedly stimulate carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions from soil. This study therefore investigated the effect of cattle manure biochar (added at rates of 0, 2%, or 10%, coded as BC0, BC2 and BC10, respectively) application, with or without earthworm *Aporrectodea turgida*, on emissions of CO_2 and N_2O and changes of physic-chemical properties of agricultural and forest soils in a laboratory incubation experiment. The BC10 treatment significantly enhanced cumulative CO_2 emissions by 27.9% relative to the untreated control in the agricultural soil. On the contrary, the BC2 and BC10 treatments significantly reduced cumulative CO_2 emissions by 16.3%–61.1% and N_2O emissions by 92.9%–95.1% compared to the untreated control in the forest soil. The addition of earthworm alone significantly enhanced the cumulative CO_2 and N_2O fluxes in agricultural and forest soils. Cumulative CO_2 and N_2O fluxes were significantly increased when BC2 and BC10 were applied with earthworm in the agricultural soil, but were significantly reduced when BC10 was applied with earthworm in the forest soil. Our study demonstrated that biochar application interacted with earthworm to affect CO_2 and N_2O emissions, which were also dependent on the soil type involved. Our study suggests that manure biochar application rate and use of earthworm need to be carefully studied for specific soil types to maximize the climate change mitigation potential of such management practices.

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1 Introduction

Soils act as either important sources or sinks for both carbon dioxide (CO_2) and nitrous oxide (N_2O), with approximately 20% and 62% of global CO_2 and N_2O emissions, respectively, originating from the soil (Wu et al., 2015). The CO_2 is released from soils through the respiratory metabolism of plant roots and bryophytes, as well as the decomposition of plant litter and soil organic matter (SOM) by both soil microorganisms and fauna (Zhu et al., 2018). The N_2O is produced through microbial nitrification under aerobic (oxidative) or denitrification under anaerobic (reductive) conditions (Fest et al., 2009). Agricultural soil is the most significant contributor to global N_2O emissions, and they account for 58% of anthropogenic N_2O emissions (Nelissen et al., 2014). Forest soils have large stocks of soil organic carbon (SOC), accounting for more than 70% of all SOC worldwide; even a small change in SOC storage could significantly affect atmospheric CO_2 concentrations (Li et al., 2013). Agricultural and forest soils are, therefore, significant sources of GHG emissions. Agricultural and forest lands comprise the largest land cover type in Canada. Thus, seeking a sustainable management strategy that can decrease the agricultural and forest soil CO_2 and N_2O emissions is crucial.

Biochar is a recalcitrant carbon-rich material producing from the slow pyrolysis of organic wastes (such as crop residues, poultry manure, and waste wood chips, etc.) under limited oxygen conditions (Gong et al., 2018; Su et al., 2021). Biochar can be used to remove various pollutants and improve soil fertility (Xue et al., 2021). Moreover, biochar addition has been regarded as an efficient method for mitigating climate change due to its advantages, such as slow degradation rate and long residence time (Zhang et al., 2012). However, considerable variability in CO_2 and N_2O emissions from biochar-amended soils has been reported for different types of biochars in both laboratory and field studies. For example, cumulative CO_2 emissions were 2%–56% lower in bamboo plantation soils amended with bamboo biochar than the control, and cumulative CO_2 emissions were sharply decreased with increasing addition rate and biochar particle size (Chen et al., 2017), while cumulative CO_2 emissions were not affected by wheat straw biochar addition in agricultural soils (Hu et al., 2014). Generally, biochar may enhance CO_2 emissions from soil by increasing labile SOC pools, and stimulating the microbial mineralization of native SOC (Troy et al., 2013). Conversely, biochar may decrease soil CO_2 emissions by biochar absorbing soil derived-DOC (dissolved organic carbon), cation or mineral nitrogen and extracellular enzymes, thereby suppressing microbial activity and SOC mineralization (Zimmerman et al., 2011; Senbayram et al., 2019). Similarly, soil N_2O emissions may be enhanced or suppressed by the addition of different types of biochars, because of the large variability in physicochemical properties of biochars, which are highly dependent on the type of source materials, production method and the pyrolysis conditions (Cely et al., 2014; Brassard et al., 2018) and the rate of biochar application (Taghizadeh-Toosi et al., 2011).

Beef cattle breeding is raised in all Canada provinces in recent years, and inevitably generates a larger amount of cow manure. Cattle manure can cause many environmental issues without being properly handled. For example, it can be an important source of potentially dangerous pathogens and nitrates transported to the surface water and groundwater (Cao et al., 2016). And cattle manure could similarly produce vast GHG emissions during storage and processing (Ersoy and Ugurlu, 2020). On the other hand, cattle manure acts as a crucial a biomass resource due to its high content of organic matter and nutrients. Therefore, it is essential to explore effective strategies that can reduce GHG emissions from the disposal of Canadian beef cattle manure, prevent environmental pollution and properly reuse of biomass resources. Converting cattle manure to biochar via pyrolysis would be an alternative method of disposing this wastes. Using cattle manure biochar in soil amendment may significantly reduce GHG release from soil and improve the soil quality.

Soil fauna, especially earthworms, are likely to markedly impact soil biogeochemical processes and GHG emissions. Earthworms are well-known ecosystem engineers, can make either a positive or a negative impact on soil CO_2 and N_2O emissions through directly and indirectly affect the processes of organic matter decomposition, nitrification, denitrification, carbon and nutrient cycling, and the maintenance of soil structure through feeding, burrowing, and cast-forming activities (Paul et al., 2012; Wu et al., 2015; Sánchez-de León et al., 2018; Zhu et al., 2018). The earthworm gut provides an ideal microenvironment for denitrifying bacteria due to the moist, high-osmolarity, anoxic, the higher nitrate/nitrite content, and the increased availability of carbon, thereby enhancing soil N_2O emissions (Horn et al., 2003). On the contrary, some species of earthworms may reduce N_2O emissions by altering the soil structure and gas diffusion in the upper organic layer. Earthworms can comminute litter and process soil-litter mixtures in the gut, selectively feed on organisms, and excrete casts that have high mineral nutrient and labile carbon contents (Wachendorf et al., 2014). Earthworms casts can serve as a habitat for microorganisms due to their physical stability, higher pH, and higher availability of moisture, labile C (such as polysaccharides) and nutrients such as N, Ca^{2+}, Mg^{2+} and K^{+} (Briones et al., 2011). The high availability of labile C and microbial activities in earthworms casts can result in high C mineralization and thus high rates of CO_2 emissions. In addition, the mixing activities such as burrowing by earthworms may increase C mineralization due to increased contact between microorganisms and the SOC substrate. On the other hand, biogenic soil aggregates created by earthworms may negatively affect rates of decomposition and denitrification by decreasing the accessibility of labile C to microbes (Giannopoulos et al., 2010).

Earthworms activities can be influenced by biochar addition and the addition rate, which may subsequently affect soil CO_2 and N_2O emissions. Earthworms may avoid biochar due to poisonous substances in biochars, the dryness of biochar causing physical injury, or dramatic changes in soil pH. Conversely, earthworms may be attracted to biochar due to increased availability of minerals, or raising soil pH to a desired level (Namoi et al., 2019). *Aporrectodea turgida* is an endogenic species that is one of the most common earthworm species in North America. However, information concerning the contribution of this earthworms to and its interaction with biochar on CO_2 and N_2O flux in agricultural and forest soils is scarce. Thus, the aims of this study were to investigate 1) the effects of cattle manure biochar and earthworm *A. turgida* application on CO_2 and N_2O emissions in agricultural and forest soils, 2) the effects of cattle manure biochar and earthworm *A. turgida* application on soil chemical properties in both agricultural and forest soils.

2 Materials and methods

2.1 Soil, biochar, and earthworm

The agricultural and forest soils used in the present experiment were both classified as Othic Black Chernozem based on the Canadian system of soil classification (Soil Classification Working Group 1998). Soils (0–10 cm depth) were collected from a farm (52°19′ 13.05″ N, 113° 39′ 8.65″ W; elevation 992 m) near Red Deer, Alberta, Canada. An agricultural site and the adjacent forested site were established, and soil samples were taken at ten randomly selected locations within a 50 × 50 m area and then mixed homogeneously; six composite samples were created for each of the two sites. Fertilizer is typically applied to the agricultural soil for the wheat (*Triticum aestivum* L.) and canola (*Brassica* spp.) plantation in September (the first year) and April (the sencond year), consisting of urea and super phosphate with the application doses of approximately 80 kg N/ha and 25 kg P/ha, respectively. All collected soil samples were first air-dried, then passed through a 2 mm mesh sieve with roots, gravel and plant residues removed. The samples were kept at 4°C before using for the incubation experiment described below. The cattle manure biochar used in this study was produced by British Columbia Biocarbon Ltd. (Prince George, BC, Canada). Biochar was prepared using a pyrolysis unit with a continuous flow reactor at 750°C–850°C under oxygen-limited conditions. The selected properties of biochar were: pH 9.65±0.05, total carbon 83.05±1.16 g/kg, total nitrogen 4.78±0.15 g/kg, C/N 17.40±0.36, NH_4^+-N 3.45±0.2 mg/kg, NO_3^--N 101.24±4.17 mg/kg, DOC 2188.9±95.5 mg/kg, DON 139.3±15.9 mg/kg. Healthy adult or large juvenile earthworms (Endogeic species: *A. turgida*) of similar size were collected from the same agricultural field where the soil samples were collected by shovel and hand sorting, the earthworms (Endogeic species: *A. turgida*) were observed in both the agricultural soil and forest soil at the study site.

The earthworms were reared in the agricultural soil collected from the sampling site under a dark condition at room temperature (approximately 15°C). Earthworms were picked out from the soil, and their guts were cleared by placing them on moist filter papers for 48 h (15°C, under a dark condition) before the start of the incubation experiment. Then the earthworms were cleaned with distilled water, dried with paper towels, and the weight was recorded. After that, the earthworms in the incubated jars were released and mixed with pre-incubation soils, and then incubated for 48 days.

2.2 The microcosm experiment

Six treatments were set up in a completely randomized design as follows: soil only (CK, as control), soil + one earthworm (EW), soil + 2% w/w biochar (BC2), soil + one earthworm + 2% w/w biochar (EW + BC2), soil + 10% w/w biochar (BC10), soil + one earthworm + 10% w/w biochar (EW + B10). The treatment combinations were replicated four times for both the forest and agricultural soils. The rates of 2% and 10% were chosen because soil physicochemical properties and GHG emissions have been proven to be significantly changed in this range in previous researches (Jones et al., 2011; Bamminger et al., 2014). For each treatment, 100 g of air-dried soil was loaded into a 250-mL glass bottle, air-dried soil was rewetted with distilled water to 40% water holding capacity (WHC), and all bottles were pre-incubated at 20°C for 5 days. Thereafter, 2 g or 10 g oven-dry biochar was added to soil in each bottle to provide a biochar application rate equivalent to 20 Mg/ha or 100 Mg/ha to the 0–10 cm layer of soil. Then, the soil and biochar were mixed thoroughly. Earthworm treatments received one individual of *Aporrectodea turgida* (≈505 mg). All soil mixtures were initially adjusted to 60% of WHC, which was maintained throughout the incubation period and incubated in the dark in a climate-controlled growth chamber at 25°C for 48 days. All bottles were covered with a perforated aluminum foil and were tightly held with a rubber band. Water was sprayed into the soil every two days as needed to make up for water lost through evaporation.

2.3 Analyses

pH (1:5 soil:water ratio, w/v) was detected using a digital pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA). A portion of the air-dried soil samples was mechanically ground with a ball mill (Mixer Mill MM200, Thomas Scientific, Swedesboro, NJ, USA) to a fine powder for total carbon and nitrogen analyses. Total carbon and nitrogen concentrations of soil samples were analyzed with an elemental analyzer (Vario MICRO cube, Elementar Analysensysteme GmbH, Germany). To determine NH_4^+ and NO_3^- concentrations, soil samples were extracted with a 2 mol/L KCl solution at the ratio of 1:5 (w:v) and shaken on a horizontal shaker (30 min at 250 r/min), then soil extraction solution was filtered through Whatman No. 42 filter papers. NH_4^+ and NO_3^- concentrations in the filtrate were analyzed colorimetrically using the indophenol blue and the vanadium oxidation methods, respectively (Pokharel and Chang, 2019). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted from fresh samples with deionized water (sample to water ratio of 1:10, w/v), and determined by a TOC analyzer (TOC-5000A, Shimadzu, Kyoto, Japan) after filtering through a 0.45-μm membrane filter.

Gas samples were taken on days 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 21, 24, 27, 30, 36, 42 and 48. For gas sampling, the flasks were tightly closed with a lid with a septum, and 20 mL of gas was collected at 0 and 24 h. The CO_2 and N_2O concentrations (ppm) were measured using a gas chromatograph (Varian CP-3800, Mississauga, Canada), equipped with an electron capture detector to quantify N_2O, and a thermal conductivity detector to quantify CO_2 concentration, respectively. The cumulative CO_2 and N_2O emissions, as well as average CO_2 (mg/kg/h) and N_2O emissions rates (μg/kg/h) were calculated following the procedures and equations introduced by Wang et al. (2018a) and Wang et al. (2018b).

2.4 Statistical analyses

The statistical analysis was conducted in SPSS, version 20.0 (SPSS Inc., Chicago, IL, USA), and all figures were generated using Sigmaplot version 12.5 for Windows (Systat Software, Inc., San Jose, CA, USA). Two-way ANOVA with posthoc Tukey's HSD test was conducted to tested for the effects of different factors on cumulative CO_2 and N_2O emissions from the agricultural and forest soils as well as the final soil chemical properties after 48 days of incubation, with one-factor being the earthworm and the other factor being the biochar application. The significance of the differences in these measured variables between different treatments was also tested by one-way ANOVA and Tukey's HSD test with a significant level of $P < 0.05$. The redundancy analysis (RDA) was used to assess the multivariate relationships between soil physicochemical parameters and cumulative CO_2 and N_2O emissions on day 48 using Canoco for Windows version 5.0.

3 Results and discussion

3.1 Soil CO_2 emissions

In the agricultural soil, the CO_2 emission rates in BC10 and EW + BC10 were highest on day 1, decreasing remarkably through day 3, thereafter, maintaining a high CO_2 emission rate from day 4 to day 14, then gradually decreased until day 36, after which the rates kept stable until the experiment ended (Fig. 1(A)). The other treatments maintained a high CO_2 emission rate from day 1 to day 14, then declined rapidly until day 36 and then remained stable.

Two-way ANOVA showed that cumulative CO_2 emissions were significantly affected by the biochar amendments ($P < 0.001$), earthworm addition ($P = 0.002$) and their interaction ($P = 0.016$) after 48 days of incubation (Table 1). The BC10 treatment caused a 27.9% increase in cumulative soil CO_2 emissions ($P < 0.05$) compared with the CK (Fig. 2(A)). While, the cumulative soil CO_2 emissions showed no significant differences between EW, BC2 and CK. The combined addition of earthworm and biochar (at 2% and 10%) increased cumulative soil CO_2 emissions by 26.3% (EW + BC2) and 26.9% (EW + BC10), respectively, compared with the control ($P < 0.05$). This could be due to the average CO_2 emission rates were generally elevated in treatments containing biochar and earthworm amendments compared to CK throughout the 48 day experiment period (Fig. 3(A)).

In the forest soil, all treatments showed a similar temporal pattern; the CO_2 emission rates increased during the initial stage of the incubation experiment, then gradually decreased until the end of the incubation (Fig. 1(B)). The average CO_2 emission rates was higher in EW and EW + BC2 than in the CK, but lower in other treatments than in the CK over the entire incubation (Fig. 3(B)). The total cumulative CO_2 emissions, after 48 days of incubation, was significantly affected by the biochar ($P < 0.001$), earthworm ($P < 0.001$) as well as their interactions ($P < 0.001$) (Table 1). The BC2, BC10 and EW + BC10 treatments decreased cumulative soil CO_2 emissions by 16.3%, 61.1% and 59.2%, respectively, as compared with the control ($P < 0.05$) (Fig. 2(B)). In contrast, the EW and EW + BC2 treatments increased cumulative soil CO_2 emissions by 18.8% and 11.0%, respectively, as compared with the control ($P < 0.05$).

The above results indicated that BC2, BC10 decreased the CO_2 emission rate compared to CK in the forest soil; however, biochar addition at 10% increased the CO_2 emission rate as well as the cumulative CO_2 emissions in the agricultural soil. The different treatment effects on CO_2

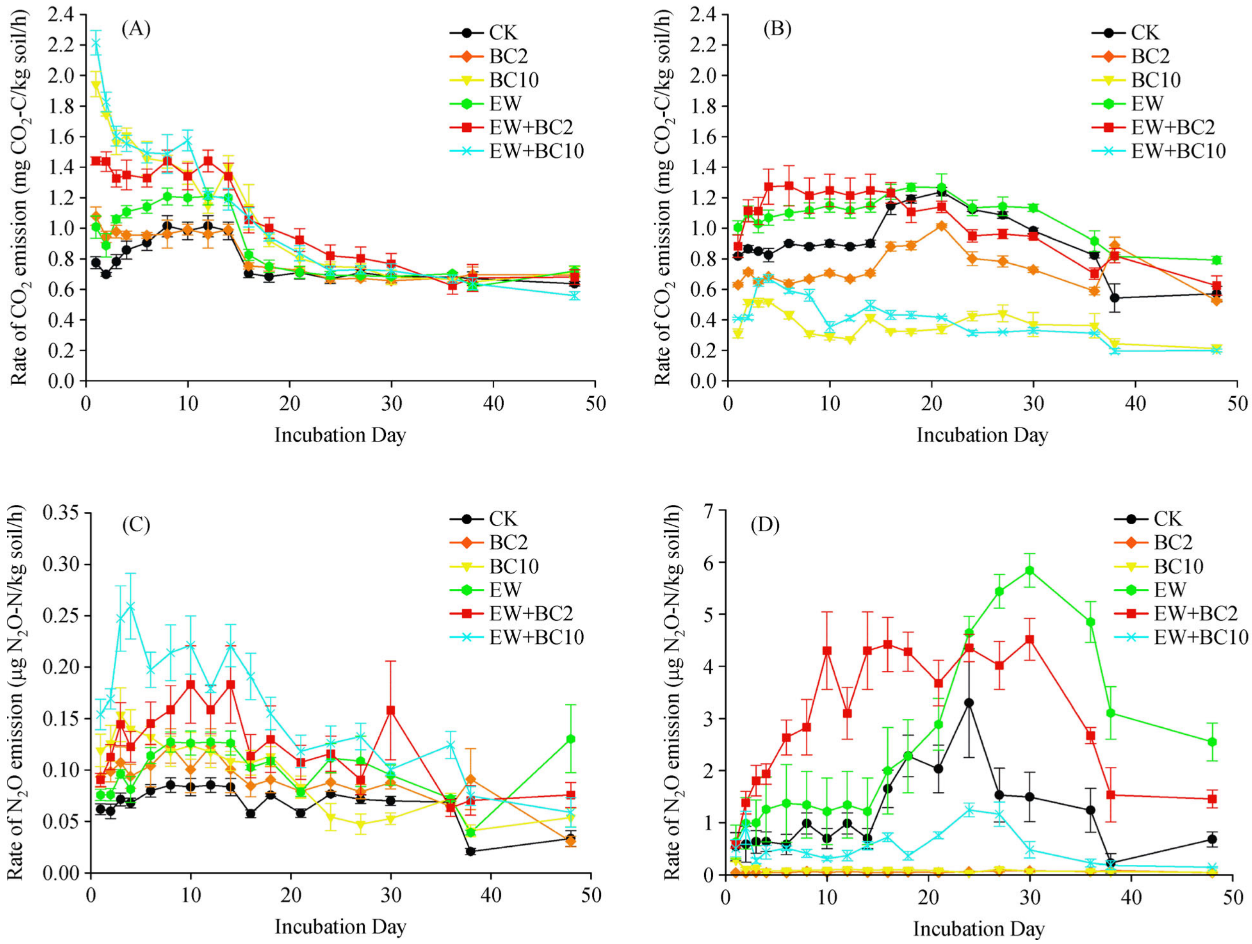

Fig. 1 Changes of CO_2 emission rate and N_2O emission rate during a 48-day incubation: (A) the dynamics of CO_2 emissions from the agricultural soil, (B) the dynamics of CO_2 emissions from the forest soil, (C) the dynamics of N_2O emissions from the agricultural soil and (D) the dynamics of N_2O emissions from the forest soil. Values are the mean (n = 4 replicates)±standard errors (bars). CK, soil only (control); BC2, 2% biochar; BC10, 10% biochar; EW, one earthworm; EW + BC2, one earthworm + 2% biochar; EW + BC10, one earthworm + 10% biochar.

Table 1 The results (F value and P value) of two-way ANOVAs on the effects of biochar and earthworm treatments on cumulative CO_2 and N_2O emissions from agricultural and forest soils after 48 days of incubation (n = 4)

Soil type	Treatments	Cumulative CO_2 emissions (mg CO_2-C/kg soil)			Cumulative N_2O emissions (μg N_2O-N/kg soil)		
		d.f.	F	P	d.f.	F	P
Agricultural soil	Biochar (B)	1	18.1	< 0.001	1	9.1	0.002
	Earthworm (E)	2	13.4	0.002	2	46.1	< 0.001
	B × E	2	5.3	0.016	2	2.1	0.154
Forest soil	Biochar (B)	1	564.7	< 0.001	1	71.9	< 0.001
	Earthworm (E)	2	77.7	< 0.001	2	184.1	< 0.001
	B × E	2	16.6	< 0.001	2	32.9	< 0.001

Notes: d.f.: degree of freedom.

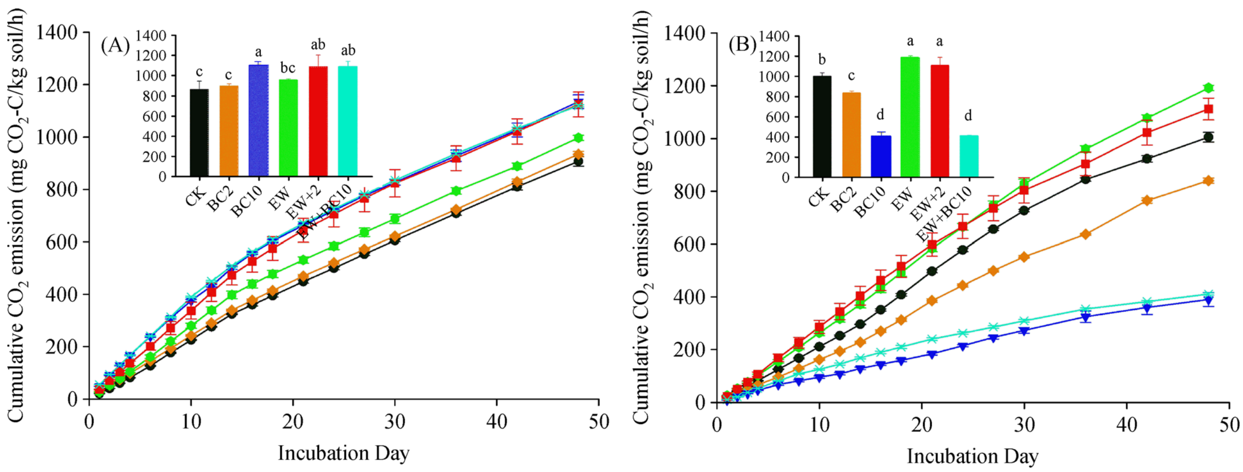

Fig. 2 Changes of cumulative CO_2 emissions during a 48-day incubation: (A) the cumulative CO_2 release from the agricultural soil and (B) the cumulative CO_2 emission from the forest soil. The inserts are the cumulative CO_2 emissions at the end of the 48-day incubation. Values are the mean (n = 4 replicates)±standard errors (bars). Different letters indicate significant differences between treatments at $P < 0.05$ according to the Tukey's HSD test. CK, soil only (control); BC2, 2% biochar; BC10, 10% biochar; EW, one earthworm; EW + BC2, one earthworm + 2% biochar; EW + BC10, one earthworm + 10% biochar.

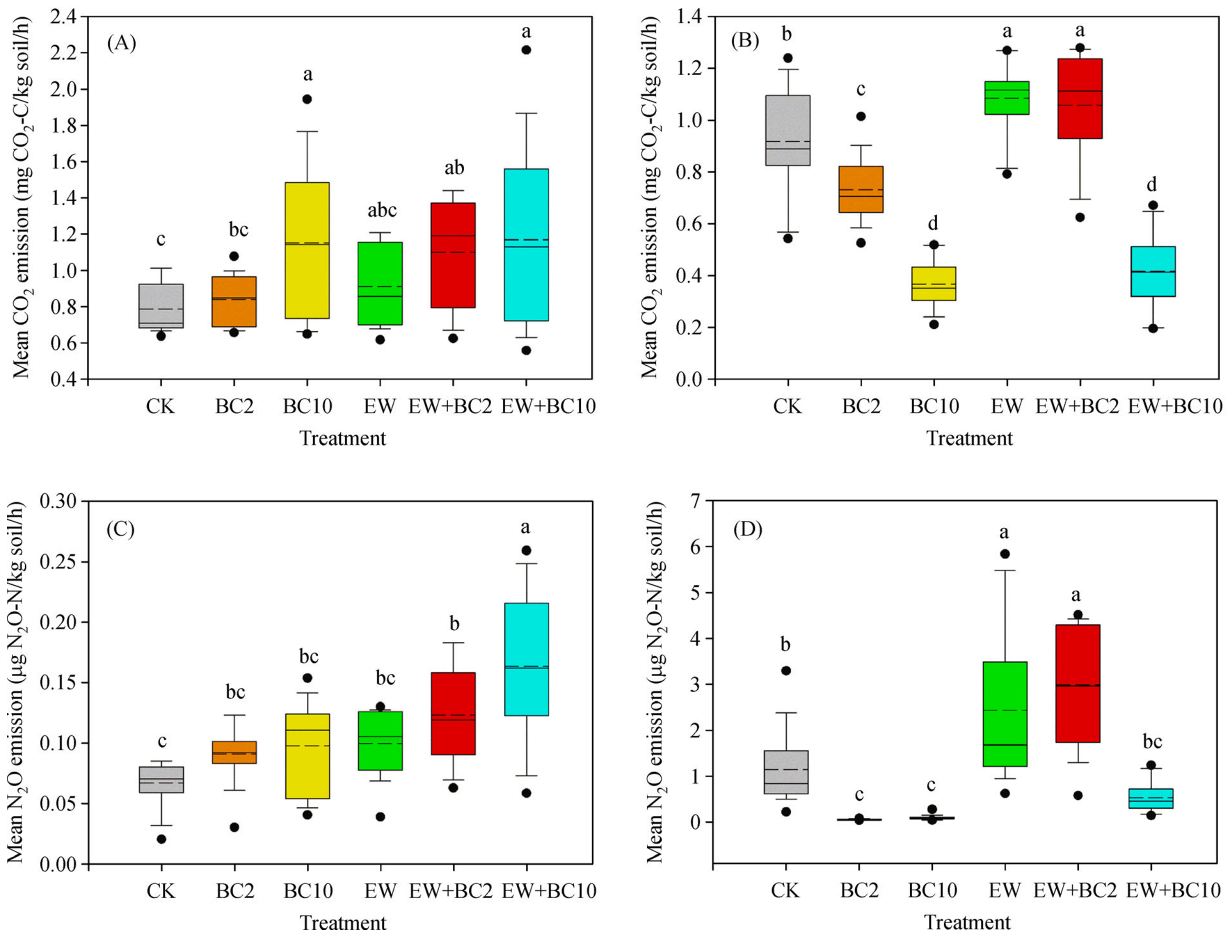

Fig. 3 Boxplots of the effect of biochar and earthworm treatments on soil CO_2 and N_2O emissions in the agricultural and forest soils during the incubation period. (A) CO_2 emitted from the agricultural soil, (B) CO_2 emitted from the forest soil, (C) N_2O emitted from the agricultural soil, (D) N_2O emitted from the forest soil. Box and whisker plots show first to third quartile range (boxes), outliers (circles), median (line) and mean (Discontinuous line) marker values (n = 4). Different lowercase letters on bars indicate significantly different means at $P < 0.05$ based on the Tukey's test.

emissions in this study reflect different potential mechanisms of biochar in the agricultural and forest soils with different soil properties. Previous studies (Senbayram et al., 2019) have shown that biochar has an adverse effect on CO_2 emissions in soils with different pH values, in which CO_2 emission from soil treated with biochar was increased in acidic soil conditions, but was reduced in alkaline soil conditions. The mechanism involved is as follows:1) the lower pH in acidic soil may reduce the stability of biochar and thus induce higher CO_2 emission than that in the alkaline soil by accelerating biodegradation of labile biochar components or abiotic release of biochar-C which inorganic carbonates preferred to form CO_2 (Ameloot et al., 2013; Brassard et al., 2018); 2) in acid soil, biochar decreased the substrates limitation by increasing pH and bioavailability of SOC, thus to increase the growth and activities of copiotrophic bacteria, as well as CO_2 emissions. This mechanism was supported by results of the redundancy analysis in this study, which showed that the cumulative CO_2 emissions of the agricultural soil were highly and positively correlated with the pH (Fig. 4(A)). However, in the alkaline soil, adsorption of SOC on biochar was more conducive to the growth of oligotrophic bacteria and lessened the emission of CO_2 (Sheng and Zhu, 2018). In this study, the soil pH was in acid range (5.28–6.65) for all biochar amended treatments in agricultural soil (Table 2). On the contrary, the soil pH was in the alkaline range (7.05–7.79) for all biochar amended treatments in forest soil. Thus, contradictory results on CO_2 emissions after biochar application could be due to the different pH values in the agricultural and forest soils. Lu et al. (2014) also suggested that lower N availability in soil will lead to reductions in microbial activities, which in turn reduce CO_2 emissions. So, lower NO_3^--N and DON content in BC10 than control (Table 3) may also result in lower CO_2 emissions in this study in forest soil. And the redundancy analysis proved that the cumulative CO_2 emissions were positively correlated with the organic NO_3^--N and DON in this study (Fig. 4(B)).

Our results indicated that earthworm enhanced CO_2 emissions in both agricultural and forest soils (Fig. 1), consistent with a recent meta-analysis conducted by Lubbers et al. (2013). First, earthworms emit CO_2 through respiration (Luo et al., 2008). Second, earthworms not only affect soil physic structure (e.g., improved aeration) by their burrowing and feeding activities, but they also boost

Table 2 Effects of biochar amendment, earthworm addition and their interaction on the chemical properties of an agricultural at the end of the incubation experiment (n = 4)

Treatments	pH	TC (g/kg)	TN (g/kg)	C/N	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	DOC (mg/kg)	DON (mg/kg)
Soil before incubation	4.90±0.08	28.63±1.79	2.84±0.06	9.53±0.80	4.09±0.25	50.79±0.49	678.7±7.9	377.9±15.7
CK	4.81±0.03c	27.45±0.53b	2.80±0.04b	9.81±0.30c	3.16±0.17b	86.58±1.47c	642.7±7.0b	621.2±7.7d
BC2	5.28±0.01b	29.50±0.40b	2.98±0.09ab	9.93±0.20bc	2.77±0.04b	95.93±0.25bc	627.2±10.6b	617.7±12.7d
BC10	6.51±0.09a	34.18±0.70a	3.05±0.06ab	11.22±0.32a	2.48±0.12b	101.27±3.35ab	751.4±11.8a	700.2±13.7cd
EW	4.76±0.02c	29.15±0.72b	2.90±0.07ab	10.07±0.33bc	8.55±1.16a	96.99±2.28bc	635.9±5.0b	719.2±17.9bc
EW + BC2	5.25±0.02b	28.45±0.57b	2.90±0.04ab	9.81±0.13c	2.86±0.49b	110.57±5.70a	600.5±14.1b	816.1±32.9a
EW + BC10	6.65±0.05a	34.48±0.66a	3.15±0.03a	10.94±0.11ab	3.58±0.17b	107.75±0.89ab	773.4±7.5a	802.0±15.6ab

Notes: Data are means±standard error, n = 4. Values in the same column followed by the same letters indicate no significant differences within each soil type at the $P < 0.05$ level according to the Tukey HSD test.

Table 3 Effects of biochar amendment, earthworm addition and their interaction on the chemical properties of a forest soil at the end of the incubation experiment (n = 4)

Treatments	pH	TC (g/kg)	TN (g/kg)	C/N	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	DOC (mg/kg)	DON (mg/kg)
Soil before incubation	6.73±0.01	42.45±1.76	3.95±0.12	11.04±0.58	3.00±0.14	42.39±1.47	703.5±19.5	281.0±6.8
CK	6.87±0.07b	40.10±0.52b	3.85±0.09a	10.42±0.13b	0.86±0.03c	90.67±5.69b	689.6±8.9b	629.8±13.3cd
BC2	7.05±0.07b	40.70±0.92b	3.83±0.06a	10.61±0.09b	0.75±0.07c	97.51±1.19ab	764.2±28.3b	675.1±32.8bc
BC10	7.79±0.02a	44.60±0.35a	3.84±0.03a	11.59±0.18a	1.97±0.14b	49.03±7.94c	907.4±40.8a	536.0±30.8d
EW	6.58±0.07c	39.55±0.44b	3.80±0.04a	10.41±0.07b	0.89±0.03c	115.77±2.79a	736.0±4.2b	756.2±10.7ab
EW + BC2	7.11±0.03b	40.10±0.37b	3.83±0.03a	10.46±0.05b	0.71±0.08c	116.45±2.52a	768.6±7.1b	818.4±41.2a
EW + BC10	7.77±0.06a	44.00±0.58a	3.85±0.03a	11.43±0.06a	2.84±0.13a	81.72±1.71b	869.6±9.1a	532.9±12.0d

Notes: Data are means±standard error, n = 4. Values in the same column followed by the same letters indicate no significant differences within each soil type at the $P < 0.05$ level according to the Tukey HSD test.

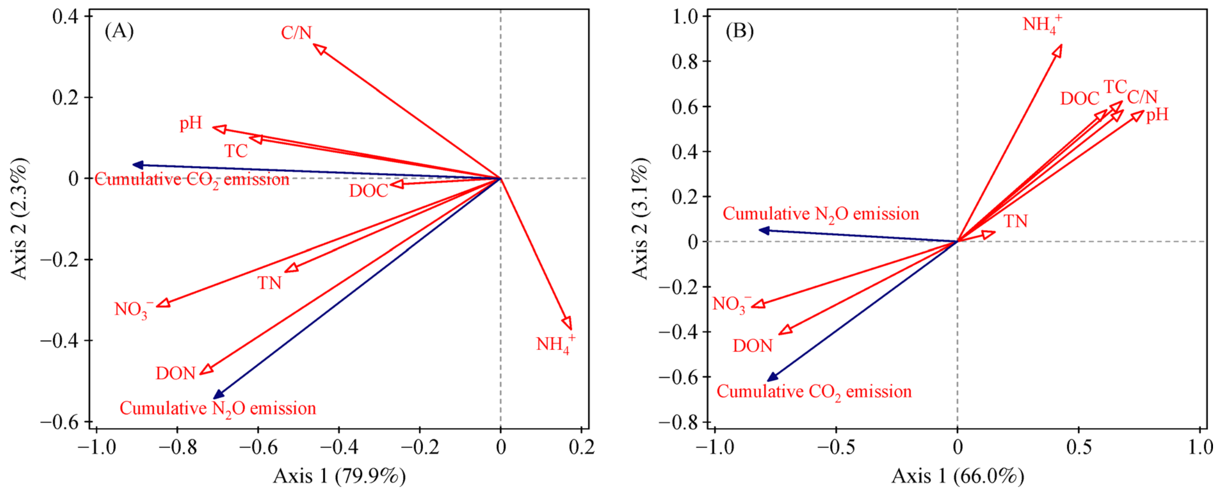

Fig. 4 Redundancy analyses (RDA) of the relationships between soil properties and soil CO_2 and N_2O emissions in (A) agricultural soil and (B) forest soil.

microbial activities by their excrements that are ideal microhabitats for microorganisms and consequently lead to stimulated CO_2 emissions (Namoi et al., 2019). Specially, in the EW + BC10 treatment applied to the forest soil, the negative effects of biochar far outweigh the positive effects of earthworm. In contrast to our results, Hawthorne et al. (2017) found that wood chip biochar application at high (10%, w/w) application rates increased CO_2 emissions in a forest soil. Deng et al. (2020) reported that spent mushroom substrate biochar addition (5% w/w) increased both CO_2 emission rates and cumulative CO_2 emissions from moso bamboo forest soil. Our findings therefore proved that cattle manure biochar addition at certain rates may reduce soil CO_2 emissions despite the co-application of earthworm in forest soil. The potential mechanisms of biochar and earthworm amendment on soil CO_2 from agricultural soil and forest soil in this study are shown in Figs. 5(A) and 5(B).

3.2 Soil N_2O emissions

In the agricultural soil, all treatments also showed a similar change trend, increased over time during the initial stage of incubation, then gradually decreased until the end of the experiment despite some fluctuations (Fig. 1(C)). The average N_2O emission rates were higher in all treatments containing earthworm than CK (Fig. 3(C)). The total cumulative N_2O emissions were significantly affected by the biochar ($P = 0.002$) and earthworm ($P < 0.001$) but not their interaction ($P = 0.154$) (Table 1). EW, EW + BC2 and EW + BC10 increased cumulative N_2O emissions by 60.0%, 82.3%, and 125.7%, respectively. In addition, cumulative N_2O emissions in EW + BC10 was significantly higher than in EW (Fig. 6(A)).

In the forest soil, the N_2O emission rates in CK, EW, EW + BC2 and EW + BC10 significantly increased over time with maximum emission rates observed on days 24, 30, 30 and 27, respectively, thereafter the rates decreased dramatically (Fig. 1(D)). The CK had lower average N_2O emission rates than EW and EW + BC2 (Fig. 3(D)). The N_2O emission rates from BC2 and BC10 was negligible during the entire process. The total cumulative N_2O emissions was significantly affected by biochar ($P < 0.001$), earthworm ($P < 0.001$) as well as their interaction ($P < 0.001$) (Table 1). The cumulative N_2O emissions was significantly reduced in BC2 and BC10 treatments than in CK. In contrast, cumulative N_2O emissions was significantly enhanced in EW and EW + BC2 than in CK. No significant difference in cumulative N_2O emission was found between EW + BC10 and CK (Fig. 6(B)).

In the present study, we found that the effect of biochar addition on N_2O emissions was clearly dependent on the soil type. Biochar addition increased N_2O emissions in the agricultural soil. Conversely, in the forest soil, the BC2 and BC10 treatments significantly decreased N_2O emission rate and cumulative N_2O emissions. Wang et al. (2018b) suggested that biochar addition causes a rise in soil pH could raise N_2O reductase activity within denitrifier microorganisms, and hence favoring the further reduction of N_2O to N_2 in alkaline soil. While the soil pH increased after biochar application probably stimulated the nitrification and denitrification under high ammonia condition, thus induced N_2O emissions in acid soil. Therefore, the inconsistent effect of the addition of biochar on N_2O emissions in this study could be due to the difference in soil pH (Tables 2 and 3). Soil N_2O emission has also been reported to be significantly affected by the pool size of labile N forms, including NO_3^--N, microbial nitrogen and water-soluble organic nitrogen, etc. (Song et al., 2019). In this study, NO_3^--N and DON concentrations were significantly higher in 10% biochar treated soil than the

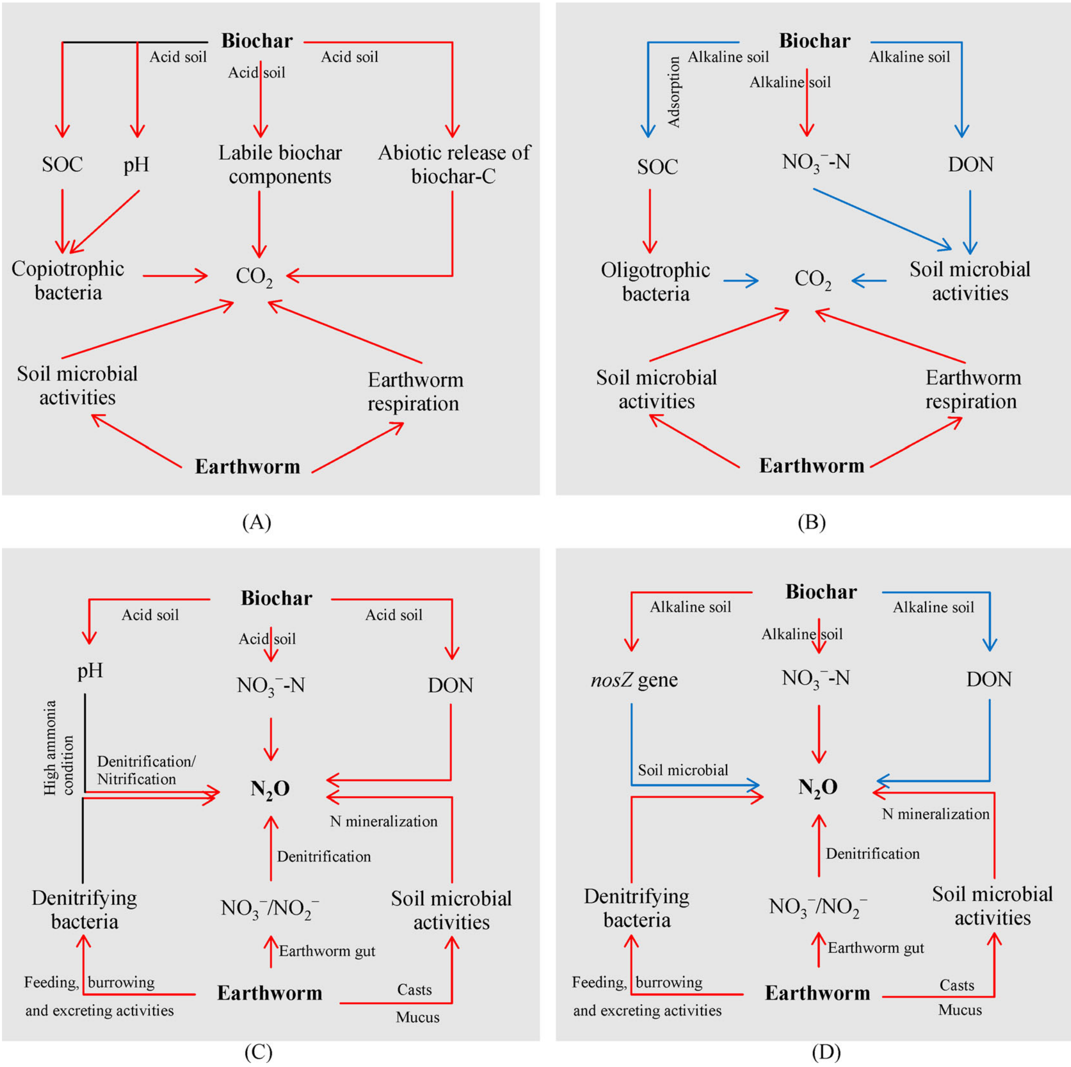

Fig. 5 Potential mechanisms of biochar and earthworm amendment on soil CO_2 from (A) agricultural soil and (B) forest soil, and N_2O emissions from (C) agricultural soil and (D) forest soil. The red line and blue line represent the positive and negative regulations, respectively.

untreated soil in agricultural soil (Table 2), while they were significantly lower in 10% biochar treated soil than the untreated soil in forest soil (Table 3), which may indirectly increase or reduce N_2O emissions. And the redundancy analysis also confirmed that the cumulative N_2O emissions were positively correlated with the organic NO_3^--N and DON in both agricultural and forest soils in this study (Figs. 4(A) and 4(B)). In addition, Wang et al. (2018b) also reported that biochar addition showed no obvious influence on the *nosZ* gene abundance in the acidic soil, while biochar application significantly increased the *nosZ* gene abundance in the alkaline soil. Therefore, the enhanced *nosZ* gene could be responsible for the suppression of N_2O emissions in alkaline soil.

Our results show that earthworms played a strong role in promoting soil N_2O emissions. Similar results have also been reported by Wang et al. (2015) and Zhu et al. (2016). Marhan et al. (2015) revealed that the burrowing, feeding and casting activity of earthworms will expand or enhance the suitable habitat for denitrifying bacteria. Kong et al. (2017) suggested that an anaerobic environment was provided by the earthworm gut where the ingestion of soil organic matter may produce NO_3^--/NO_2^-- and bioavailable organic C. Therefore, earthworms may be a source of N_2O due to the denitrification process that frequently happens in this condition. Besides, casts and mucus produced by earthworms prefer to promote the activities of microorganisms and enzymes responsible for N mineralization, which would both enhance mineral N concentrations and increase N_2O emissions (Paz-Ferreiro et al., 2014).

Unlike the study of Wu et al. (2021), who reported that

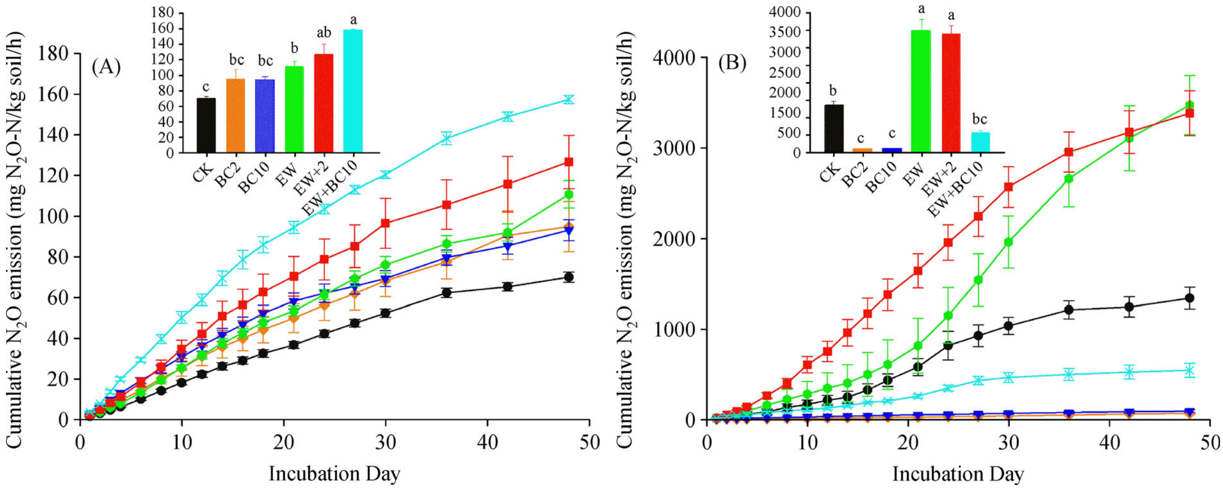

Fig. 6 Changes of cumulative N_2O emission during a 48-day incubation: (A) the cumulative N_2O emission from the agricultural soil and (B) the cumulative N_2O emission from the forest soil. The inserts are the cumulative N_2O emission at the end of 48-day incubation. Values are the mean (n = 4 replicates)±standard errors (bars). Different letters indicate significant differences between treatments at $P < 0.05$ according to the Tukey's HSD test. CK, soil only (control); BC2, soil + 2% biochar; BC10, soil + 10% biochar; EW, soil + one earthworm; EW + BC2, soil + one earthworm + 2% biochar; EW + BC10, soil + one earthworm + 10% biochar.

the increased soil N_2O emissions induced by earthworms (*Metaphire guillelmi*) were considerably reduced (about 34%) by corn straw biochar (1% w/w) addition in an agricultural soil. However, our data indicated that 10% w/w cattle manure biochar addition decreased the cumulative N_2O emissions by 100% resulting from earthworm in forest soil, while 10% w/w additional biochar application resulted in a 42.1% increase in cumulative N_2O emissions compared with only earthworm inoculation treatment in agricultural soil. This is also in contrast with the results that emissions of N_2O increased by 72% in the presence of earthworms (*Pontoscolex corethrurus*) in an agricultural soil, and 1% w/w woody biochar (*Croton megalocarpus*) addition suppressed N_2O emissions from soils caused by earthworms (Namoi et al., 2019). Our findings highlight a distinct difference between cattle manure biochar and other types of biochar on N_2O emissions from agricultural and forest soil. The potential mechanisms of biochar and earthworm amendment on soil N_2O from agricultural soil and forest soil in this study are summarized in Figs. 5(C) and 5(D).

3.3 Soil physicochemical properties

In the agricultural soil, the addition of biochar significantly ($P < 0.001$) affected the pH value in soil (Table 4), all biochar addition treatments (BC2, BC10, EW + BC2 and EW + BC10) have significantly higher pH value than the control at the end of the incubation experiment (Table 2). Earthworm addition did not significantly (P = 0.563) change the pH value in soil as compared to control. In the forest soil, biochar ($P < 0.001$) and earthworm ($P = 0.003$) addition significantly affected soil pH, but there is no interaction between them (Table 5). Soil pH was significantly higher in BC10 and EW + BC10 than in CK, whereas soil pH was significantly lower in EW than in CK ($P < 0.05$) (Table 3). The observations of soil pH increased with increasing biochar application rate in both agricultural soil and forest soil might be due to the strong alkalinity of the biochar.

Biochar addition significantly affected TC in the agricultural ($P < 0.001$) and forest soils (P = 0.009) (Tables 4 and 5). Soil TC was higher in BC10 and EW + BC10 than in CK ($P < 0.05$), but was not changed by earthworms in both soils. Soil DOC was only affected by biochar addition in both soils ($P < 0.001$). Soil DOC was higher in BC10 and EW + BC10 than in CK in both soils. In both the agricultural soil and forest soil, soil TN was not affected by biochar, earthworm and their interaction. Soil C/N was only significantly changed by biochar addition in both the agricultural soil ($P < 0.001$) and forest soil (P = 0.007). Soil C/N was observed to be higher in BC10, EW + BC10 than in CK in both soils. In present study, biochar applied at 10%, caused a significant increase in TC and DOC in the agricultural and forest soils, which could be also due to the high C content of the biochar.

Biochar ($P < 0.001$), earthworm ($P < 0.001$) and their interaction ($P < 0.001$) had significantly influence on soil NH_4^+-N concentration in both soils. Soil NH_4^+-N concentration was found higher ($P < 0.05$) in EW than in CK but not different between other treatments and CK in the agricultural soil. Soil NH_4^+-N was higher in BC10 and EW + BC10 than in CK but not different between other treatments and CK in the forest soil. The increased NH_4^+-

Table 4 The results (F value and P value) of two-way ANOVAs on the effects of biochar amendment, earthworm addition and their interaction on the chemical properties of an agricultural at the end of the incubation experiment (n = 4)

Treatments	d.f.	F value and P value	pH	TC (g/kg)	TN (g/kg)	C/N	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	DOC (mg/kg)	DON (mg/kg)
Biochar (B)	1	F	807.26	59.20	9.36	14.78	21.00	11.35	130.51	9.60
		P	< 0.001	< 0.001	0.571	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Earthworm (E)	2	F	0.35	0.41	0.76	0.06	26.19	19.14	0.23	76.89
		P	0.563	0.763	0.108	0.813	< 0.001	< 0.001	0.641	< 0.001
B × E	2	F	2.64	59.20	9.36	14.78	14.44	0.96	3.09	4.72
		P	0.099	0.107	0.326	0.989	< 0.001	0.327	0.779	0.017

Notes: d.f.: degree of freedom.

Table 5 The results (F value and P value) of two-way ANOVAs on the effects of biochar amendment, earthworm addition and their interaction on the chemical properties of a forest soil at the end of the incubation experiment (n = 4)

Treatments	d.f.	F value and P value	pH	TC (g/kg)	TN (g/kg)	C/N	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	DOC (mg/kg)	DON (mg/kg)
Biochar (B)	1	F	192.57	37.45	0.16	61.69	214.73	56.29	36.03	35.16
		P	< 0.001	0.009	0.913	0.007	< 0.001	< 0.001	< 0.001	< 0.001
Earthworm (E)	2	F	11.31	1.61	0.13	1.57	15.52	52.14	0.06	17.10
		P	0.003	0.420	0.225	0.795	< 0.001	< 0.001	0.593	< 0.001
B × E	2	F	2.80	37.45	0.16	61.69	16.23	1.26	1.97	4.63
		P	0.087	0.450	0.902	0.219	< 0.001	0.177	0.484	0.033

Notes: d.f.: degree of freedom.

N content by 10% biochar addition in the forest soil was likely due to the ammonia oxidation (nitrification) was inhibited (Backer et al., 2017).

Biochar and earthworm had significant effect ($P < 0.001$) on soil NO_3^--N concentration in both soils. Soil NO_3^--N was higher in BC10, EW + BC2 and EW + BC10 than in CK in the agricultural soil ($P < 0.05$), was higher in EW and EW + BC2 than in CK but was lower in BC10 and EW + BC10 than in CK in the forest soil ($P < 0.05$). Biochar ($P < 0.001$), earthworm ($P < 0.001$) as well as their interaction ($P < 0.05$) had significant effect on soil DON in both soils. Soil DON was higher in EW, EW + BC2 and EW + BC10 than in CK in the agricultural soil, and higher in EW and EW + BC2 than in CK ($P < 0.05$) but lower in BC10 and EW + BC10 than in the CK in the forest soil. In our observations, biochar applied at 10%, caused contrasting effects on NO_3^--N and DON in the two soils: it decreased NO_3^--N and DON in the forest soil, similar to results reported in Sial et al. (2019), but increased them in the agricultural soil. The increase in NO_3^--N content in the agricultural soil might be caused by biochar stimulating the soil gross nitrification rate (Hu et al., 2014). Earthworm addition significantly increases the content of N compounds, NH_4^+-N, NO_3^--N and DON in both agricultural and forest soils which could be due to production and degradation of organic matter. The positive effect of earthworms addiition on N mineralization mainly resulted from mucus secretion, dead tissue, as well as the improvement of soil physical properties and fragmentation of organic substances caused by earthworm activities (Araujo et al., 2004).

4 Conclusions

Our study demonstrated that cattle manure biochar stimulated CO_2 and N_2O emissions in the studied agricultural soil, on the contrary, reduced their emissions in the forest soil. Earthworm *Aporrectodea turgida* also stimulated CO_2 and N_2O emission in both agricultural and forest soils. Biochar addition at 10% can offset earthworm activity in the forest soils and reduced CO_2 and N_2O emissions, and lowered NO_3^--N and DON concentrations. Therefore, manure biochar application at high rates should be considered as a viable management option to reduce CO_2 and N_2O emissions and improve soil biochemical properties.

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