

Effect of biochar addition on short-term N₂O and CO₂ emissions during repeated drying and wetting of an anthropogenic alluvial soil

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Abstract Agricultural soils are an important source of greenhouse gases (GHG). Biochar application to such soils has the potential of mitigating global anthropogenic GHG emissions. Under irrigation, the topsoils in arid regions experience repeated drying and wetting during the crop growing season. Biochar incorporation into these soils would change the soil microbial environment and hence affect GHG emissions. Little information, however, is available regarding the effect of biochar addition on carbon dioxide (CO₂) and nitrous oxide (N₂O) emissions from

agricultural soils undergoing repeated drying and wetting. Here, we report the results of a 49-day aerobic incubation experiment, incorporating biochar into an anthropogenic alluvial soil in an arid region of Xinjiang Province, China, and measuring CO₂ and N₂O emissions. Under both drying–wetting and constantly moist conditions, biochar amendment significantly increased cumulative CO₂ emission. At the same time, there was a significant reduction (up to ~20 %) in cumulative N₂O emission, indicating that the addition of biochar to irrigated agricultural soils may effectively slow down global warming in arid regions of China.

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Introduction

Agricultural soils are a major source of CO₂ and N₂O emissions to the atmosphere (Liu et al. 2006; Lv et al. 2014; Mosier et al. 2005). Agriculture accounts for the yearly emissions of 5.1–6.1 Pg CO₂-equivalents, contributing 10–12 % to the concentration of anthropogenic greenhouse gases (GHG) in the atmosphere (Trenberth and Caron 2001). Thus, reducing GHG emissions is an important strategy in managing global climate change (Wang et al. 2011). In this respect, the incorporation of biochar into soil, produced by the

thermal decomposition (pyrolysis) of organic material under a limited supply of oxygen (O_2) and at a moderate temperature ($<700\text{ }^\circ\text{C}$), is potentially effective (Chan et al. 2008; Fowles 2007; Glaser et al. 2002; Knoblauch et al. 2011; Lehmann et al. 2006, 2009; Singla et al. 2014b). The effect of biochar addition on soil CO_2 and N_2O fluxes has been extensively investigated, but the results have not been consistent. Some studies showed that biochar application promoted short-term release of CO_2 from soil (Jones et al. 2011; Kasozi et al. 2010; Smith et al. 2010), while others found no such effect (Wang et al. 2011), or even a reduction in soil CO_2 emissions (Liu et al. 2011), depending on soil or biochar type. The observed inconsistency may be related to the concentration of dissolved organic carbon (DOC) in the soil (Zhang et al. 2012). N_2O is another greenhouse gas contributing to global warming and stratospheric ozone depletion (Wang et al. 2011).

Denitrification and nitrification, the two major sources of N_2O in soil (Bremner 1997; Delwiche 1981), are largely controlled by the availability of oxygen (Firestone and Davidson 1989) which, in turn, is influenced by soil moisture status (Bruun et al. 2011). Some studies have shown that biochar addition to soil can reduce N_2O emissions by decreasing soil bulk density, increasing soil aeration (Yanai et al. 2007), and enhancing soil microbial activity as well as nitrogen immobilization (Bruun et al. 2011; Cayuela et al. 2013). On the other hand, other workers (Clough et al. 2010; Scheer et al. 2011; Wang et al. 2011) have reported that biochar amendment has no effect on N_2O fluxes, nor does it decrease soil N_2O emissions. This apparent inconsistency may be ascribed to differences in the types and properties of the soils used.

We should also add that most of the results, especially those derived from indoor incubation experiments, were based on the assumption that soil moisture content was invariant throughout the course of the trial (Ameloot et al. 2013; Bruun et al. 2011; Wang et al. 2011). In reality, however, topsoils experience large fluctuations in water content when dry periods are interrupted by occasional rainfall or irrigation events (Chowdhury et al. 2011; Mavi et al. 2012). Soils in arid areas where sprinkler and drip irrigation is practiced experience repeated drying and wetting. As the soil dries, water availability decreases and the water potential becomes more negative (Harris 1981). At the same time, the thickness of the water film

surrounding soil aggregates is reduced, and microbial activity becomes substrate-limited (Stark and Firestone 1995). Multiple drying and wetting cycles also promote soil organic matter decomposition and N mineralization (Miller et al. 2005; Schimel et al. 2011; Xiang et al. 2008).

Biochar application is known to increase the water-holding capacity of soil, enhancing its capacity for absorbing water and resisting drought (Asai et al. 2009; Jones et al. 2010; Kammann et al. 2011). By influencing microbial community structure and biomass (Nocentini et al. 2010; Pietikainen et al. 2000; Samonin and Elikova 2004; Singla et al. 2014a; Warnock et al. 2007), biochar amendment can also affect C and N turnover, and hence alter CO_2 and N_2O emissions from soil (Yanai et al. 2007).

Xinjiang province in northwest China has an arid climate and a large area of agricultural soils under irrigation. As such, the topsoil in this region experiences multiple drying and wetting cycles during cropping (Rajkovich et al. 2012). Here, we assess the effect of biochar addition to an anthropogenic alluvial soil in Xinjiang on CO_2 and N_2O emissions under repeated drying and wetting (DW) and constantly moist (CM) conditions.

Materials and methods

Soil and biochar preparation

An anthropogenic alluvial soil and cotton stalks were collected from a farm ($39^\circ23'45''\text{N}$, $75^\circ58'43''\text{E}$) in the Kashgar agricultural technology promotion center, Xinjiang Uyghur Autonomous Region, China. The soil was sampled from the top (cultivated) layer (0–20 cm), air-dried, and ground to pass a 1-mm sieve. The cotton stalks were sectioned into pieces of <2 cm in length and 5 mm in diameter, dried at $105\text{ }^\circ\text{C}$ for 24 h, and milled. The milled material was placed in a stainless steel rectangular box ($27 \times 19 \times 10$ cm) covered with a fitting lid and paralyzed in a muffle furnace (Tianjin Taisite Instrument Co., Ltd, SX-12-10) under a limited supply of oxygen. The temperature, heating rate, and hold time were $550\text{ }^\circ\text{C}$, $18\text{ }^\circ\text{C min}^{-1}$, and 30 min, respectively. Some physicochemical properties of the soil and biochar are listed in Table 1.

Table 1 Physicochemical characteristics of the soil and biochar

	pH	EC _{1:5} (dS m ⁻¹)	C (%)	N (%)	C:N _{ratio}	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	DOC (mg kg ⁻¹)	Olsen P (mg kg ⁻¹)	MBC (mg kg ⁻¹)	Sand (%)	Silt (%)	Clay (%)
Soil	8.01	1.64	2.92	0.09	27.83	38.18	3.95	4.39	26.5	2.41	34.2	52.6	13.2
Biochar	10.11	3.40	64.27	1.52	36.31	19.29	3.70	160.30	919.6				

Experimental design

The incubation experiment was carried out with 50 g soil (dry mass) and three replicates per treatment. The six treatments consisted of three groups of biochar addition at a biochar/soil ratio (w/w) of 0 % (0), 5 % (5) and 10 % (10), and two water content treatments, namely, repeated drying and wetting, denoted by DW0, DW5 and DW10, and constantly moist conditions, denoted by CM0, CM5, and CM10.

The air-dry soils were wetted with distilled water to a predetermined water content, corresponding to 40 % water-holding capacity (WHC), and pre-incubated at 25 °C for 7 days in a constant temperature and humidity oven (HWS-430, Ningbo Jiangnan Instrument Factory) so as to stabilize microbial activity (Wang et al. 2011). Throughout the pre-incubation and subsequent measurement periods, distilled water was added to the soils every 12 h (by which time the moisture decreased by less than 3 %) in order to maintain the target water content. Following pre-incubation, biochar and urea (equivalent to 200 mg N kg⁻¹ soil) (Wang et al. 2011) were added to the soils with thorough mixing. Then, 50 g of the biochar–urea amended soil was placed in PVC cores (3.8 cm in diameter, 5 cm in height), provided with a nylon mesh base (25 µm in diameter), and packed to the bulk density of the soil in the field (1.2, 1.0 and 0.8 g cm⁻³ for DW0, DW5, and DW10, respectively). The cores, subjected to either repeated drying and wetting treatments, or kept constantly moist, were separately placed in two constant temperature and humidity ovens (temperature, 25 °C and humidity, 95 %) and incubated for 7 days at 70 % of WHC before the start of the first drying cycle. During the drying period, the soils were dried by lowering the oven air humidity from 95 to 50 % for 7 days after which the soils were rewetted to 70 % of WHC (Fig. 1). This process was repeated three times (Fig. 2). Separate set of cores for each treatment were analyzed for dissolved organic carbon, microbial biomass carbon, NO₃⁻-N, and NH₄⁺-N at each sampling time.

Soil and biochar analysis

The basic physicochemical properties of the soil and biochar used, measured by the standard method (Pansu and Gautheyrou 2007), are listed in Table 1. The pH of the soil (1:5 soil/water, w/w) and biochar (1:20

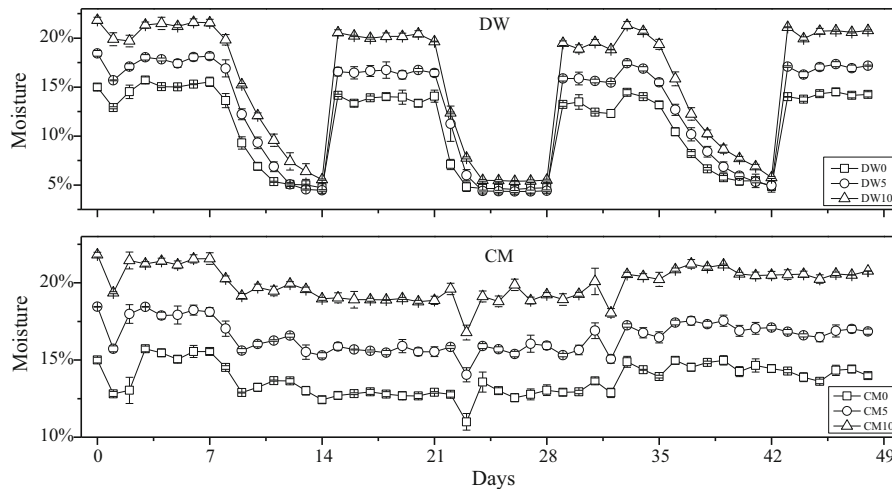
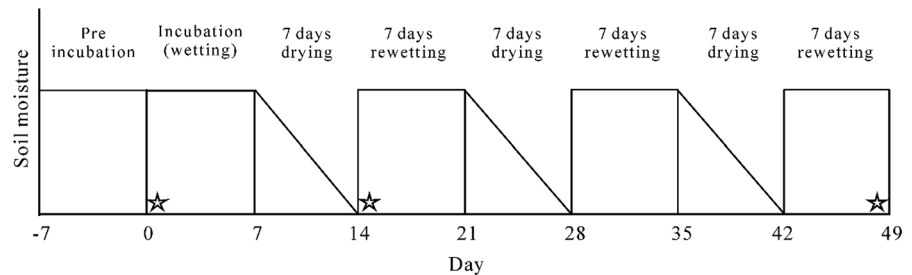


Fig. 1 Change in soil moisture content during 49 days incubation

Fig. 2 Experimental design. *Star symbol* indicates sampling time for DOC, MBC, NO_3^- -N, and NH_4^+ -N on day 1, 15 and 49 of the incubation period



biochar/water, w/w) was determined after 1 h end-over-end shaking, using a PHS-3CT pH Meter (Shanghai WeiYe instrument), while the electrical conductivity (EC) was measured using a conductivity meter (HANNA HI9033). The total C and N contents of the soil and biochar were determined by dry combustion analysis using an Elementar instrument (vario MACRO CNS; Elementar, Germany). The water-holding capacity (WHC), determined by the Welcox method (Klute 1986), of DW0, DW5, and DW10 was 20.0, 24.6, and 29.1 %, respectively. Bulk density was measured by the cutting-ring method (Carter and Gregorich 2006). The contents of sand, silt, and clay in the soil were measured by a laser particle size analyzer (APA-2000). Inorganic N (NH_4^+ and NO_3^-) in the soil and biochar samples was extracted with 1 M KCl solution, and the concentration of NH_4^+ -N and NO_3^- -N in the extract was analyzed by a standard colorimetric procedure (Keeney and Nelson 1982). Olsen P was determined

by the sodium bicarbonate (NaHCO_3) extraction method (Olsen et al. 1954). Extracts were analyzed for HPO_4^{2-} using a UV-Vis spectrophotometer (Thermo AquaMate, Thermo Electron Ltd, Cambridge, UK). Dissolved organic carbon (DOC) was extracted in the same manner as inorganic N and analyzed using a Total Organic Carbon Analyzer (multi N/C[®]2100; Analytik Jena AG, Germany).

Soil microbial biomass carbon (MBC) was measured using the chloroform-fumigation extraction technique (Vance et al. 1987). Following Jenkinson et al. (2004), MBC was calculated from the difference in extracted carbon between fumigated and non-fumigated samples, and applying a K_{EC} factor of 0.45. Briefly, 4 g samples of soil were taken from each replicate treatment on a specified date; one sample was immediately extracted with KCl, as described above, and another sample was vacuum-fumigated with chloroform (24 h) prior to extraction. The concentration of DOC in the suspensions was measured as described above.

CO₂ and N₂O sampling and measurements

For the CM treatments, the air samples were collected in all 3 replicates on days 1, 2, 3, 4, 5, 6, 7, 10, 15, 20, 25, 30, 35, 40, 45, and 49 after incubation. For the DW treatments, the air was sampled after 4, 14, 24, 48, 72, 108, and 168 h by a gas-tight syringe (20 ml) for each drying–wetting cycle. Before sampling, the containers (crisper, 850 ml, lock and lock) were thoroughly flushed with ambient air for 5 min using a hairdryer. Then, the soil column was put into the crisper which was immediately closed with a lid equipped with a sample connector and a small circulation fan. The concentrations of CO₂ and N₂O in the headspace were simultaneously measured at 0, 18, 36, and 54 min after closing the lid (Bruun et al. 2011), using a gas chromatograph (Agilent 7890A, USA), equipped with two detectors. N₂O was detected by an electron capture detector (ECD, 300 °C), and CO₂ by a hydrogen flame ionization detector (FID, 250 °C). Flux rates were derived from linear regression, using only measurements with a correlation coefficient (R^2) ≥ 0.95 .

Statistical analysis

Statistical procedures were carried out using the SPSS 16.0 software. Mean separation analysis of cumulative CO₂ and N₂O emissions, and of DOC, MBC, NO₃⁻-N, and NH₄⁺-N concentrations, was carried out based on one-way ANOVA using the Tukey test at a significance level of $P < 0.05$. All figures were drawn using Origin 8.0 software.

Results

Dynamics and cumulative CO₂ emissions

In the case of the constantly moist (CM) treatments, there was a gradual decline in CO₂ flux over time, with the highest CO₂ flux (up to 5.40 mg CO₂-C kg⁻¹ soil h⁻¹ for CM10) occurring within the first 3 days after incubation (Fig. 3a). The CO₂ flux increased significantly as the rate of biochar addition was increased over the entire period of incubation. Correspondingly,

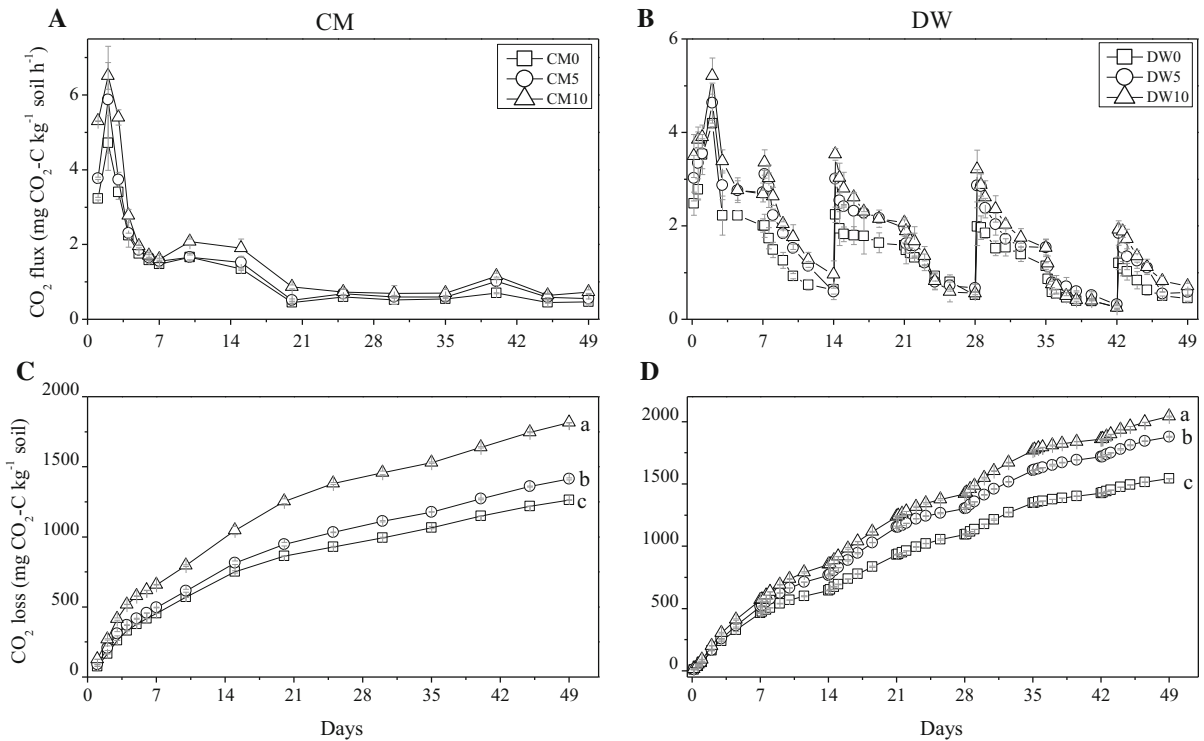


Fig. 3 a, b Soil CO₂ fluxes; c, d Net cumulative CO₂ loss during 49 days incubation. Left column shows the constantly moist (CM) treatment and right column shows drying–wetting

(DW) treatments. Small letters show significant differences (after 49 days) according to the Tukey test ($P < 0.05$)

the mean cumulative CO₂ emissions from CM0, CM5, and CM10 at the end of the incubation period (49 days) were 1264.23, 1415.27, and 1815.49 mg CO₂-C kg⁻¹ soil, respectively, with significant differences ($P < 0.01$) being found for all three treatments (Fig. 3c). With reference to CM0, the cumulative CO₂ emissions increased by 11.95 % for CM5 and 43.60 % for CM10.

On the other hand, the CO₂ flux for the drying-wetting (DW) treatments fluctuated with changes in soil water content (Fig. 3b). Biochar addition promoted CO₂ emission from soil, the largest CO₂ flux being observed for DW10 (5.21 mg CO₂-C kg⁻¹ soil h⁻¹, on day 3). During the three drying periods, there was a gradual decline in CO₂ flux as soil moisture decreased whether or not biochar was added. Compared with DW0 at first drying cycle, DW5 and DW10 produced a higher CO₂ flux (Fig. 3b) during the whole cycle (7 days). During the second and third drying cycles, however, no significant difference in CO₂ flux was observed for DW0, DW5, and DW10 when the soil moisture content was very low (after day 3 of the drying cycle). Rewetting the soil after 7 days of drying

incubation produced a flush in CO₂ flux within 4 h for the three rewetting cycles, after which the CO₂ flux gradually declined. The size of the flush decreased with an increase in the number of DW cycles. The largest flush in CO₂ flux (3.53 mg CO₂-C kg⁻¹ soil h⁻¹) was found for DW10 at the first wetting event. By the third rewetting cycle, the flush decreased to 1.20 mg CO₂-C kg⁻¹ soil h⁻¹. The mean cumulative CO₂ emissions for DW0, DW5, and DW10 during 49 days of incubation were 1542.96, 1879.82, and 2042.16 mg CO₂-C kg⁻¹ soil, respectively. These values are significantly different at $P < 0.01$ (Fig. 3d), the amount for DW5 and DW10 being 21.83 and 32.35 % higher, respectively, than that for DW0.

Dynamics and cumulative N₂O emissions

In the CM treatments, the N₂O flux declined gradually over time. The flux reached a maximum (8.28 μg N₂O-N kg⁻¹ soil h⁻¹ in CM0) on day 5 after which it gradually declined, reaching a steady state after 15 days of incubation (Fig. 4a). There was a clear tendency for the N₂O flux to decrease as the rate of

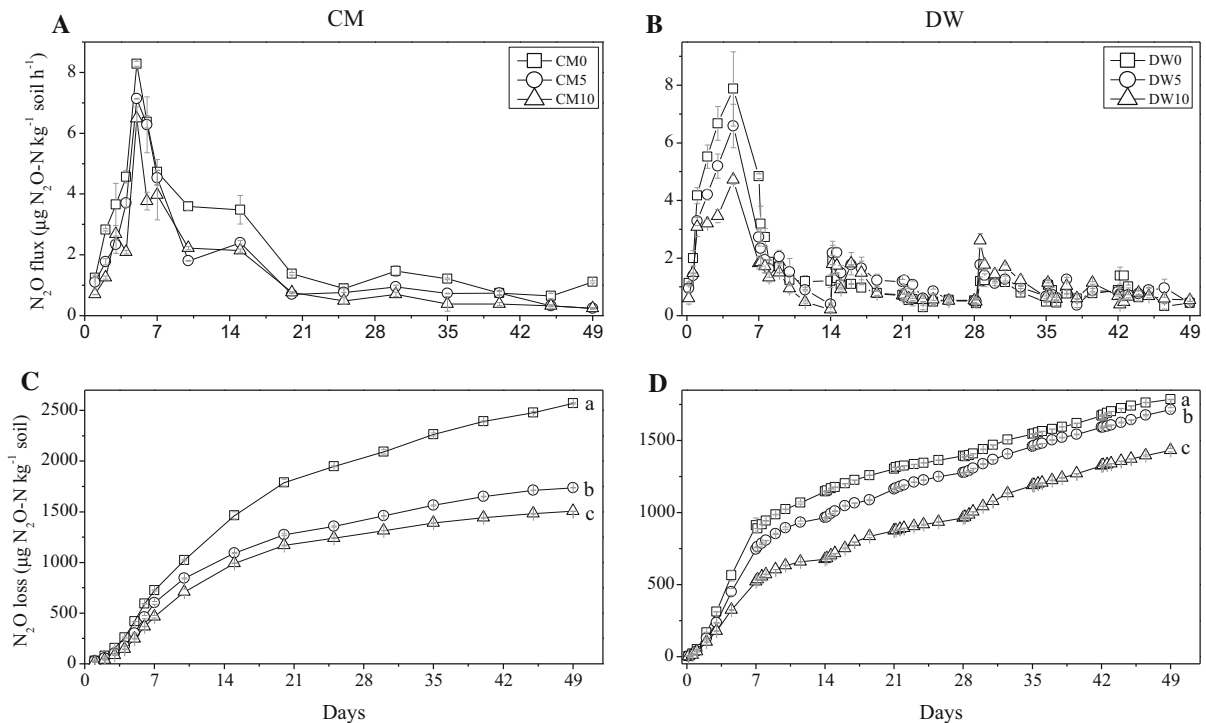


Fig. 4 a, b Soil N₂O fluxes; c, d Net cumulative N₂O loss during 49 days incubation. Small letters show significant differences (after 49 days) according to the Tukey test ($P < 0.05$)

biochar addition increased throughout the whole incubation period ($P < 0.05$). The mean cumulative N_2O emissions for CM0, CM5, and CM10 at the end of the incubation period (49 days) were 2572.44, 1738.91, and 1506.54 $\mu\text{g } N_2O\text{-N } \text{kg}^{-1}$ soil, respectively, while the rate of emission for CM5 and CM10 declined by 32.40 and 41.44 %, respectively, relative to CM0. The difference was significant at $P < 0.01$ (Fig. 4c).

In the DW treatments, the N_2O flux fluctuated slightly with changes in water content (Fig. 4b). Biochar amendment led to a decrease in N_2O emission, the highest N_2O flux being recorded for DW0 (7.88 $\mu\text{g } N_2O\text{-N } \text{kg}^{-1}$ soil h^{-1} , on day 5). During the first 7-day drying period, a clear tendency for the N_2O flux to decrease was found as the rate of biochar addition increased ($P < 0.05$). However, during the second and third drying cycles, the N_2O flux was relatively low and stable, and no significant difference was found among DW0, DW5, and DW10, irrespective of soil moisture. Rewetting after 7 days of drying incubation resulted in a slight flush of N_2O flux within 4 h after which the flux gradually decreased. DW5 had a high flush of N_2O flux (2.19 $\mu\text{g } N_2O\text{-N } \text{kg}^{-1}$ soil h^{-1}) during the first DW cycle. The largest flush of 2.61 $\mu\text{g } N_2O\text{-N } \text{kg}^{-1}$ soil h^{-1} was found for DW10 during the second rewetting event. This amount was well above the value recorded during the third

rewetting flush for DW0 (0.89 $\mu\text{g } N_2O\text{-N } \text{kg}^{-1}$ soil h^{-1}). The mean cumulative N_2O emissions for DW0, DW5, and DW10 for the entire incubation period (49 days) were 1786.84, 1717.22, and 1434.64 $\mu\text{g } N_2O\text{-N } \text{kg}^{-1}$ soil, respectively, and there were significant differences among the three groups ($P < 0.05$) (Fig. 4d). Compared with DW0, the cumulative N_2O emissions decreased by 3.90 % for DW5 and by 19.71 % for DW10. Irrespective of biochar addition, DW treatments led to lower mean cumulative N_2O emissions in comparison with CM treatments although the differences were not significant.

Dissolved organic carbon (DOC) and microbial biomass carbon (MBC)

The DOC and MBC concentrations, measured on day 1, reflected the amounts contained in soil and biochar (0, 5 and 10 %) before urea addition (Fig. 5), while the concentrations, measured on day 15, 43, and 49, reflected the effects of biochar and urea application. For both CM and DW treatments, DOC measured 1 day after the first rewetting increased with increasing addition of biochar. On day 1 before adding urea, the DOC content of DW10 was significantly higher than that of DW0 ($P < 0.01$), presumably because the biochar contained more DOC (160.30 $\text{mg } \text{kg}^{-1}$) than

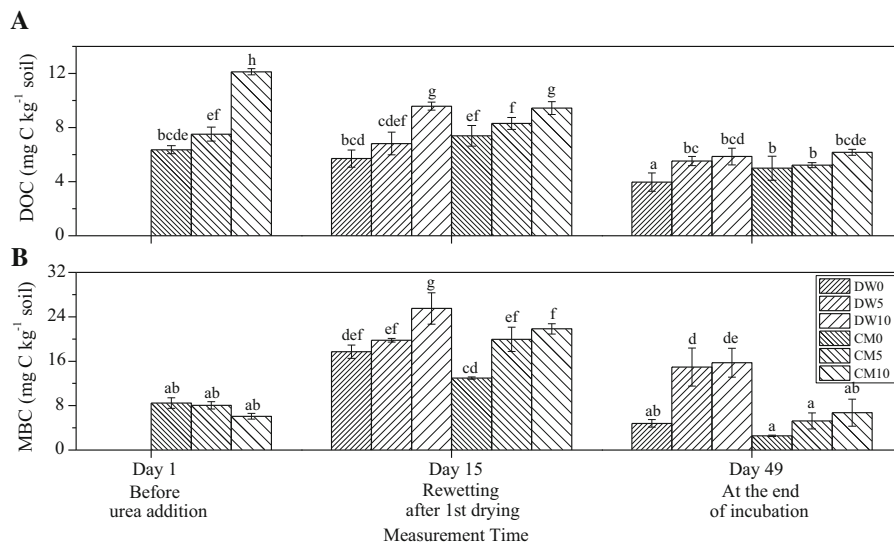


Fig. 5 a Dissolved organic carbon (DOC) and b Soil microbial biomass carbon (MBC) during the 49-day incubation period. Small letters show significant differences according to the Tukey test ($P < 0.05$)

the soil (4.39 mg kg^{-1}) (Table 1). One day after the first rewetting (day 15), DOC concentrations for DW0 and DW5 were lower than those for CM0 and CM5, respectively. The DOC concentrations for DW10 and CM10 were significantly higher than for DW0, CM0, DW5, and CM5 and there was no significant difference between DW0 and DW5, nor between CM0 and CM5. At the end of incubation (day 49), less DOC was released after 3 DW cycles and the DOC concentrations for CM and DW were not significantly different. Compared with the initial DOC concentration (day 1), the DOC concentrations of CM0, CM5, CM10, DW0, DW5, and DW10 decreased by 21.54, 30.63, 49.09, 37.58, 26.46, and 51.65 %, respectively.

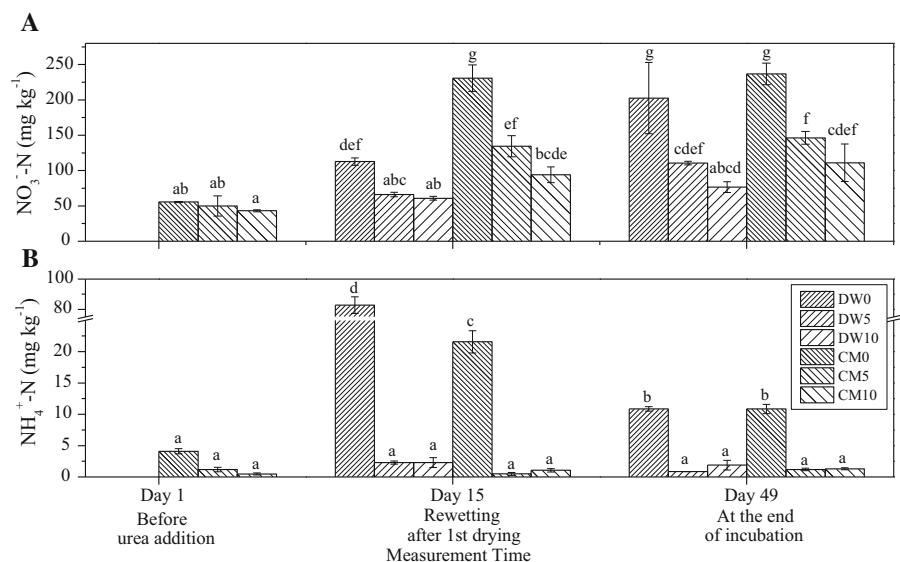
The MBC concentrations for DW0, DW5, and DW10, measured on day 1, were low ($6.09\text{--}8.47 \text{ mg C kg}^{-1}$ soil) and showed no significant differences (Fig. 5b). One day after the first rewetting (day 15), MBC concentrations for DW0 and DW5 were slightly higher than the corresponding values for CM0 and CM5, but the MBC concentration for DW10 was significantly higher than that for CM10. Moreover, MBC concentrations had a tendency to increase with biochar addition. At the end of incubation (day 49) and after three DW cycles, the MBC concentrations for all six groups were significantly lower than the corresponding values, measured on day 15 ($P < 0.05$). The MBC concentrations for DW were significantly higher than for CM ($P < 0.05$), while the values for DW5 and DW10 were significantly higher than those for DW0, CM0, CM5, and CM10 ($P < 0.01$) (Fig. 5b).

Extractable N (NO_3^- -N and NH_4^+ -N)

At the beginning of incubation and before urea addition (day 1), the NO_3^- -N concentrations were low, reflecting the initial values for soil and biochar ($43.18\text{--}55.46 \text{ mg kg}^{-1}$) (Fig. 6a). On day 1 after the first rewetting period (day 15) and at the end of incubation (day 49), the NO_3^- -N concentrations for CM0 and DW0 ($112.70\text{--}236.87 \text{ mg kg}^{-1}$) were both significantly higher than the corresponding values for CM5, CM10, DW5, and DW10 ($60.97\text{--}111.08 \text{ mg kg}^{-1}$) ($P < 0.01$), but no significant differences were found between CM5 and CM10, nor between DW5 and DW10. Moreover, the NO_3^- -N content for CM was higher than that for DW, and the content measured on day 49 was slightly higher than that on day 15, but the difference was not significant.

The change in NH_4^+ -N concentrations showed a similar trend to that described above for NO_3^- -N. The concentrations of NH_4^+ -N, however, decreased more steeply with an increase in biochar addition (Fig. 6b). On the first day of incubation and before adding urea, the NH_4^+ -N concentrations for all groups were low. Although the concentration for DW0 (4.70 mg kg^{-1}) was higher than that for DW5 (1.19 mg kg^{-1}) and DW10 (0.47 mg kg^{-1}), the difference was not significant. On day 15 after the first rewetting, the NH_4^+ -N concentrations for DW0 and CM0 ($21.58\text{--}82.83 \text{ mg kg}^{-1}$) were significantly higher than the values for CM5, CM10, DW5, and DW10 ($0.49\text{--}2.29 \text{ mg kg}^{-1}$) ($P < 0.01$), while the concentration for DW0 was significantly higher

Fig. 6 **a** Nitrate nitrogen (NO_3^- -N) and **b** Ammonium nitrogen (NH_4^+ -N) during the 49-day incubation period. Small letters show significant differences according to the Tukey test ($P < 0.05$)



than that for CM0 ($P < 0.01$). At the end of the incubation (day 49), the $\text{NH}_4^+\text{-N}$ concentrations for DW5, DW10, CM5, and CM10 ($0.86 - 1.89 \text{ mg kg}^{-1}$) were significantly lower than those for DW0 and CM0 ($10.85\text{--}10.87 \text{ mg kg}^{-1}$) ($P < 0.01$). The $\text{NH}_4^+\text{-N}$ concentrations of DW0 and CM0 on day 49 were significantly lower than those on day 15 ($P < 0.01$).

Discussion

CO₂ emissions

Several laboratory and field incubation studies (Jones et al. 2011; Smith et al. 2010; Spokas et al. 2009; Zimmerman 2010) have indicated that addition of biochar to soil caused an immediate and short-term increase in CO₂ emission. The biochar-induced increase in soil respiration rate has been attributed to microbial decomposition of some labile components in the biochar (Smith et al. 2010) or to the abiotic release of C (Zimmerman 2010). The concentration of labile components in biochars depends on biomass type and pyrolysis conditions (Bruun et al. 2011; Lehmann et al. 2009).

In our case, the biochar had a higher DOC concentration ($160.30 \text{ mg kg}^{-1}$) than the soil (4.39 mg kg^{-1}), thus the DW/CM5 and DW/CM10 treatments had higher DOC concentrations than DW0 (Fig. 5a). Decomposition of this DOC fraction together with other labile components may explain the higher CO₂ flux for DW/CM5 and DW/CM10, relative to DW/CM0, throughout the incubation period of CM treatment and during the first week of DW treatment (Bruun et al. 2011). After 4 days of incubation, the CO₂ flux for the CM treatments gradually decreased. The flux for CM0 was always lower than that for CM5 and CM10 because of the decrease in soil DOC and MBC concentrations. From day 15 to 49, the DOC concentrations for CM0, CM5, and CM10 decreased by 32.48, 37.23, and 34.64 %, respectively, while the MBC concentrations declined by 80.31, 73.69, and 69.14 %, respectively. This observation would indicate that under CM conditions, the dissolved organic carbon in biochar decomposed quite rapidly giving rise to a short-term increase in CO₂ emission as previously reported (Ameloot et al. 2013; Bruun et al. 2011; Jones et al. 2011).

In case of the DW treatments, respiration rates declined on drying the soils (Fig. 3b), although the extent of this reduction was not significantly different among the three groups. The reduction in respiration rates was most likely due to limited availability of water and substrate to soil microbes when the water potential was low (Franzluebbers et al. 1994; Mavi et al. 2012; Parr et al. 1981; Pulleman and Tietema 1999). During the first drying cycle, the CO₂ fluxes for DW5 and DW10 were higher than for DW0. However, during the second and third drying cycles, the CO₂ flux for all three groups declined, indicating that the impact of biochar addition on CO₂ flux, in the drying phase, diminished with repeated drying and wetting.

Several investigators (Denef et al. 2001; Fierer et al. 2003; Halverson et al. 2000; Kieft 1987) have suggested that the flush in CO₂ flux following rewetting is due to the accumulation of osmolytes during the drying phase and the rapid release of previously protected labile organic matter. Similarly, we observed a flush in CO₂ flux at first rewetting, the magnitude of which was higher for DW10 and DW5 than for DW0 (Fig. 3b), suggesting that more substrates were released in DW10 and DW5 than in DW0 for utilization by soil microbes. This suggestion was further supported by the finding that the concentrations of MBC and DOC, after the first rewetting, were higher for DW10 and DW5 than for DW0 (Fig. 5b).

Because of the high electrical conductivity (EC) of biochar, relative to soil (Table 1), biochar addition would enhance the salinity of DW5 and DW10, reducing the ability of microbes to tolerate a low water potential after multiple DW cycles (Blum et al. 2013). Biochar amendment also increased cumulative CO₂ emission, the level of which was significantly different ($P < 0.05$) among DW0, DW5, and DW10. Regardless of biochar addition, the cumulative CO₂ emission for the DW treatments was higher than that for the CM treatments, indicating that multiple drying and wetting cycles promote the release of CO₂. Nevertheless, biochar amendment can contribute to the long-term sequestration of carbon in soil (Ameloot et al. 2013; Jones et al. 2011).

N₂O emissions

Figure 3a, b indicates that the addition of a nitrogen fertilizer, such as urea, can cause an initial sharp increase in N₂O emissions which then slowly declined,

confirming previous observations (Bouwman 1996; Mosier et al. 1998). For both CM and DW treatments, N_2O emissions peaked within 7 days of incubation and then gradually declined. The N_2O flux was higher for CM0 than for CM5 and CM10, indicating that biochar amendment suppressed N_2O emissions (Spokas et al. 2009; Van Zwieten et al. 2009; Yanai et al. 2007). This finding may partly be explained by the reduction of the availability of NO_3^- (Fig. 6), thereby reducing the soil inorganic-N pool for N_2O production. On day 15 and 49 of incubation, the NO_3^- -N concentration for CM0 (230.79–236.87 mg kg^{-1}) was always significantly higher than that for CM5 (134.46–146.33 mg kg^{-1}) and CM10 (94.13–111.08 mg kg^{-1}) ($P < 0.05$). The NO_3^- -N concentration for the three groups did not decrease from day 15 to 49, consistent with the observation by Bruun et al. (2011). The difference in NH_4^+ -N concentration between CM0, CM5, and CM10 (Fig. 6b) was even more significant ($P < 0.01$), indicative of the NH_4^+ -N lost or absorbed after urea and biochar addition. Unlike that of NO_3^- -N, however, the concentration of NH_4^+ -N with respect to DW0 was significantly reduced from day 15 to day 49 of incubation ($P < 0.01$). One possible explanation for these findings was that the urea was converted to ammonium carbonate by soil enzymes in the initial stages of the trial followed by oxidation of $(\text{NH}_4)_2\text{CO}_3$ by nitrobacter under aerobic conditions. As a result, the concentration of NO_3^- -N tended to increase, while that of NH_4^+ -N declined as incubation progressed. Another reason why the NH_4^+ -N reduced after the addition of biochar may be partly explained by prior ammonia volatilization. The hydrolysis of urea could produce ammonium nitrogen, which could result in ammonia volatilization due to higher pH of soil and biochar (Wang et al. 2015).

In the case of DW treatments, N_2O emission during the drying phase was significantly affected by soil water content ($P < 0.05$) (Fig. 1 DW). During the first drying cycle, the N_2O flux for DW0, DW5, and DW10 decreased from 3.20 to 1.20, 2.34 to 0.40, and 1.86 to 0.22 $\mu\text{g N}_2\text{O-N kg}^{-1}\text{soil h}^{-1}$, respectively. The flux for DW0 was higher than for DW5 and DW10, probably because of the availability to microbes of water and substrate was limited at low water potentials (Fig. 6). However, during the second and third drying cycles, the N_2O flux for all three treatments did not appreciably change because the flux at the start of day 21 and day 35 was lower than that at the first drying

cycle (day 7). In other words, the impact of biochar addition on N_2O flux, during the drying phase, has largely disappeared after the first DW cycle.

Although there was a flush in N_2O flux after the first rewetting (day 15), no significant difference in N_2O flux (1.79–2.18 $\mu\text{g N}_2\text{O-N kg}^{-1}\text{soil h}^{-1}$) was observed for DW0, DW5, and DW10. However, compared with the value measured on day 14 (at the end of drying), the N_2O flux for DW0, DW5, and DW10 on day 15 increased by 60.83, 445.00, and 713.63 %, respectively. It would therefore appear that in the case of DW0, more substrates related to N_2O production were released after rewetting and made available to microbes as compared with DW5 and DW10 (Mavi et al. 2012).

At the start of the second rewetting cycle (day 29), the N_2O flux for DW5 and DW10 was higher than for DW0, although the concentrations of NO_3^- and NH_4^+ for DW5 and DW10 were significantly lower than those for DW0 ($P < 0.05$). Nevertheless, the denitrifying microbes in DW5 and DW10 could presumably make better use of the limited substrates available as compared with their counterparts in DW0. Furthermore, N_2O was mainly produced by denitrification when the soil was rewetted to 70 % of WHC (Bollmann and Conrad 1998; Dalal et al. 2003; Firestone and Davidson 1989). By the third rewetting event (day 43), the flush in N_2O flux was very low or absent, probably because of the lack of substrate to sustain microbial growth as indicated by the low MBC content.

Conclusions

Our results indicate that biochar addition to soil enhances CO_2 emissions. In the case of the constant moisture (CM) treatments, the increase in CO_2 flux is related to an increase in dissolved organic carbon (DOC) and microbial biomass carbon (MBC) concentrations. At the same time, there was a reduction in N_2O emissions due to a decrease in NO_3^- -N and NH_4^+ -N concentrations. Rewetting of the soil produced a flush of CO_2 and N_2O which decreased or disappeared as the number of drying–wetting (DW) cycles increased. This observation may be ascribed to a reduction in both substrate availability to microbes and MBC concentration. The flush in CO_2 flux, after rewetting, increased with biochar addition although no

similar increase in N₂O flux was observed. For both CM and DW treatments, biochar amendment led to a significant increase in cumulative CO₂ emissions, and a decline in cumulative N₂O emissions. Thus, biochar amendment of irrigated agricultural soils in arid areas of China has the potential of effectively reducing N₂O emissions and mitigating regional global warming.

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