Effect of biochar origin and soil pH on greenhouse gas emissions from sandy and clay soils

Di Wu[a], Mehmet Senbayram[b,c,*], Huadong Zang[d], Ferhat Ugurlar[b], Salih Aydemir[b], Nicolas Brüggemann[a], Yakov Kuzyakov[d], Roland Bol[a], Evgenia Blagodatskaya[d]

[a] Institute of Bio- and Geosciences, Agrosphere (IBG-3), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany
[b] Institute of Plant Nutrition and Soil Science, University of Harran, Osmanbey, 63000 Sanliurfa, Turkey
[c] Thünen Institute of Climate-Smart Agriculture, Federal Research Institute for Rural Areas, Forestry and Fisheries, Bundesallee 50, 38116 Braunschweig, Germany
[d] Department of Agricultural Soil Science, Büsgen-Institute, University of Göttingen, 37007 Göttingen, Germany

ABSTRACT

Emissions of greenhouse gases (GHGs), such as carbon dioxide (CO_2) and nitrous oxide (N_2O) have great impact on global warming and atmospheric chemistry. Biochar addition is a potential option for reducing GHGs emissions through carbon (C) sequestration and N_2O mitigation. However, the influences of biochar on C and nitrogen (N) transformations in soil are still unclear, resulting in a poor understanding of the mechanisms of N_2O mitigation effects of biochar. Here we carried out two soil incubation experiments to investigate the influence of two common biochars addition (corn cob and olive pulp) with ammonium sulfate on CO_2 and N_2O emissions from two contrasting soil types (acidic sandy and alkaline clay soil). Furthermore, four extracellular enzymes activities that related to C and N cycling, i.e. cellobiohydrolase, chitinase, xylanase and β-glucosidase, were analyzed to gain insights into the underlying mechanisms of biochar's effects on CO_2 and N_2O evolutions. Contrasting effects of two biochars on CO_2 and N_2O emissions were observed in the two different soils. The corn biochar addition had no significant effect on CO_2 and N_2O emissions in the alkaline clay soil, but significantly decreased CO_2 emissions by 11.8% and N_2O emissions by 26.9% in the acidic sandy soil compared to N-fertilizer only treatment. In contrast, olive biochar addition showed no significant effect on CO_2 emissions but decreased N_2O emissions by 34.3% in the alkaline clay soil, while in the acidic sandy soil addition of olive biochar triggered about a twofold higher maximum CO_2 emission rate and decreased N_2O emissions by 68.4%. Up to 50–130% higher specific CO_2 emissions (per unit of C-related enzyme activity: cellobiohydrolase, chitinases and β-glucosidase) were observed after addition of olive biochar compared to corn biochar addition in the acidic sandy soil. We concluded that biochar's effects on N_2O and CO_2 emissions are more pronounced in acidic soils. Alkaline biochar's N_2O mitigation potential in acidic soils seems to be dependent on soil NO_3^- content as drastically higher N_2O emissions were measured in early phase of the experiment (where soil NO_3^- was high) and significantly lower N_2O fluxes were obtained in later phases (with lower soil NO_3^- content).

1. Introduction

Carbon dioxide (CO_2) and nitrous oxide (N_2O) are important long-lived greenhouse gases (GHGs). The global warming potential (GWP) of N_2O is 298 times the GWP of CO_2 when calculated over a 100-year period (IPCC, 2013). The N_2O concentration has increased since pre-industrial times through human activities (Bouwman et al., 2002). Soils are considered to be the largest source of N_2O emissions, while biochemical nitrogen (N) transformations such as nitrification and denitrification are considered as the major sources of N_2O (Baggs, 2011; Butterbach-Bahl and Dannenmann, 2011).

Biochar, which is obtained from thermo-chemical conversion of biomass under oxygen-deficient conditions (Sohi et al., 2010), has been frequently reported to be an effective solution to mitigate CO_2 and N_2O emissions (Cayuela et al., 2014; Lehmann et al., 2011; Yanai et al., 2007). However, large variation in the GHG mitigation effect of biochar has been reported for different kinds of biochar and different soil types (Clough et al., 2010; Taghizadeh-Toosi et al., 2011). The effect of biochar amendment on soil CO_2 evolution, which is known as biochar priming effect, has been found to be positive, neutral and negative. For example, Chintala et al. (2014) found that biochar had a negative priming effect on mineralization of carbon and reduced CO_2 emission,

whereas Zimmerman et al. (2011) reported both positive and negative priming effects for different types of biochar amendment. Furthermore, although biochar is relatively stable, it still can be partially mineralized via biological and chemical reactions in soil (Kuzyakov et al., 2009; Luo et al., 2011), which makes it more difficult to investigate the effect of biochar addition on soil CO_2 emission. Contradictory results on the suppression effects of biochar application on N_2O emissions were also observed in both laboratory and field studies (Cayuela et al., 2014; Chang et al., 2016; Nelissen et al., 2014). Although no consensus has yet been reached, several hypotheses have been proposed for the N_2O mitigation mechanism of biochar, such as its effect on increasing soil aeration and soil pH, absorbing N in soil, and modifying the soil microorganisms that are involved in N cycle process (Cayuela et al., 2013; Lehmann et al., 2011). The inconsistent findings and explanations from different studies emphasize the need to compare the impact of different biochars on GHGs emissions for different types of soil to reveal the underlying mechanism.

The extracellular enzymes in soil catalyze the initial, rate-limiting step of decomposition and nutrient mineralization, and can be considered as good markers of soil biological processes such as C and N turnover (Allison and Vitousek, 2005). Biochar, as a soil amendment, has been reported to be able to both increase and decrease extracellular enzymes activities, causing either positive or negative priming effect (Zimmerman et al., 2011).

The objective of this incubation study was to investigate the influence of application of two different biochars on CO_2 and N_2O emissions from two soil types (low pH sandy soil and high pH clay soil). In order to gain an insight into the responses of microorganisms to biochar addition, activities of four hydrolytic enzymes (cellobiohydrolase, chitinase, xylanase and β-glucosidase) related to carbon and nitrogen cycles were also analyzed.

2. Material and methods

2.1. Properties of biochar and soil

Two contrasting types of soil (acidic sandy soil and alkaline clay soil) were investigated in this study. The acidic sandy soil (sand 81.8%, silt 14.8%, clay 3.5%) was collected from a farmland close to Gifhorn, Lower Saxony, Germany (52° 34′ 9.5″ N, 10° 45′ 26.6″ E). The soil contained 1.5% total C, 0.09% total N and had a pH of 5.3. The alkaline clay soil (sand 17.8%, silt 26.2%, clay 56.0%) was collected from the experimental farm of Harran University, Sanliurfa, Turkey (37° 07′ 27.7″ N, 38° 48′ 58.1″ E). The soil contained 2.4% total C, 0.11% total N and had a pH of 7.9. Arable crops (oilseed rape, wheat, barley, potato) had been grown prior to soil sampling. The upper 2 cm of soil and roots were removed and the 10–15 cm soil horizon beneath was collected. Before use, both of the soils were air dried and sieved < 4 mm. Two common agricultural wastes/plant residues (corn cob and olive pulp) from South-East Turkey were used as biochar. Biochars were produced by carbonization methods at 250 °C for 2 days. The soil and biochar properties are listed in Table 1 and Table 2, respectively.

2.2. Incubation experiments and gas measurement

The first incubation experiment (Exp. 1) was carried out over

Table 1
The characteristics of the two soils (acidic sandy soil and alkaline clay soil).

Soil	pH	Total N (%)	Total C (%)	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})
Acidic sandy soil	5.3	0.11	2.34	0.50	1.41
Alkaline Clay soil	7.9	0.09	1.52	1.91	9.86

Table 2
The characteristics of the two biochar (olive pulp biochar and corn cub biochar).

Materials	pH	EC (dS m^{-1})	C %	N %	K %	Mg %	P %
Corn cub biochar	8.46	0.88	78	0.67	1.3	0.8	0.5
Olive pulp biochar	9.24	1.06	49	0.64	1.2	0.6	1.1

62 days with a fully automated incubation system at the Institute of Applied Plant Nutrition, University of Gottingen, Germany, as described by Wu et al. (2017). The two soils were subjected to four different treatments ($n = 3$), i.e. i) non-amended control, ii) soil amended with N fertilizer (ammonium sulfate) only (AS), iii) soil amended with olive pulp biochar and N fertilizer (Olive + AS) and iv) soil amended with corn cub biochar and N fertilizer (Corn + AS). In the biochar treatments 19.9 g olive pulp biochar or 12.6 g corn cob biochar was thoroughly mixed with soil, equivalent to 9.8 g C addition. Soil moisture was adjusted to 70% water holding capacity (WHC). Each vessel was packed with 1.5 kg dry soils with a bulk density of 0.9 g cm^{-3}. Ammonium sulfate ($(NH_4)_2$ SO_4) was used as mineral N fertilizer and applied at a rate of 150 kg N ha^{-1} (equivalent to 2.2 g per pot). The headspace of each vessel was continuously flushed with ambient air (about 20 ml air min^{-1}). For the gas concentration analysis of N_2O and CO_2 with the automated incubation system, samples from each incubation vessel's outlet was directed to a gas chromatograph sequentially via two multi-positional valves with electric actuator controlled by a software (Trilution, Gilson Inc., Middleton, WI, USA) and an interface module. The gas chromatograph (450-GC, Bruker, Germany) was equipped with a thermal conductivity detector (TCD) for the quantification of CO_2, and an electron capture detector (ECD) for N_2O. The concentrations were measured about three times per day. The outlet flux rate for each incubation vessel was measured every day manually with a portable gas flow meter (GFM Pro Gas Flowmeter, Thermo Fisher Scientific, Waltham, MA, USA).

The second incubation experiment (Exp. 2) was designed to determine the effect of soil pH on the influence of the biochars on CO_2 and N_2O emission. The experiment was carried out over 53 days with same automated sampling system as in Exp. 1. The treatments ($n = 3$) were the same as the soil treatments of alkaline clay in Exp. 1 (control, AS, Olive + AS, Corn + AS). Additionally, for the manipulated low pH treatment the soil pH was adjusted to the same value of acidic sandy soil (pH = 5.3) by applying concentrated H_2SO_4.

2.3. Soil sampling and analysis of NH_4^+ and NO_3^-

Soil samples from the upper 10 cm were collected two times (one after four days of incubation (10 g soil) and the other one at the end of incubation) from Exp. 1 and were stored at −80 °C until further analyses. For mineral N analysis the soil samples were extracted with 0.01 M CaCl₂ (1:5 w/v) by shaking for 1 h. The extracts were then filtered through Whatman 602 filter paper and stored at −20 °C until analysis. The concentrations of NH_4^+ and NO_3^- in soil extracts were measured colorimetrically using an autoanalyzer (SKALAR, The Netherlands).

2.4. Soil enzyme activity measurement

To analyze the responses of microorganisms to biochar addition after four days of incubation in Exp. 1, the activities of four hydrolytic enzymes (cellobiohydrolase, chitinase, xylanase and β-glucosidase) related to carbon and nitrogen cycles were analyzed according to the fluorimetric protocol in (Saiya-Cork et al., 2002) with the modification in (German et al., 2012). Four types of artificial fluorogenic substrates based on 4-methylumbelliferone (MUF) were used: 4-Methylumbelliferyl-β-D-glucopyranoside (MUF-G) to detect β-glucosidase activity; 4-

Methylumbelliferyl-β-D-cellobioside (MUF-C) to detect cellobiohy-drolase activity; 4-Methylumbelliferyl-β-D-xylopyranoside (MUF-X) to detect xylanase activity; 4-Methylumbelliferyl-N-acetyl-β-D-glucosami-nide dehydrate (MUF-NAG) to detect chitinase activity. Briefly, a buffer solution composed of 0.1 M MES was prepared for MUF substrates. The substrates were dissolved in dimethyl sulfoxide (DMSO) and further diluted with sterile distilled water as well as MUF buffer for the desired concentrations. One gram of soil (dry weight equivalent) was homo-genized in 50 ml of sterilized distilled water and dispersed for 2 min by an ultrasonic probe at $50 \, J \, s^{-1}$. To determine the background fluores-cence or quenching effects, the soil suspensions and buffer solution were also mixed with eight different volumes (0–120 µl) of 10 µM MUF standards. The solutions were pipetted into deep-well plates using a 50 µl soil suspension, 50 µl MES, and 100 µl of substrate solution. We used a Victor[3] 1420-050 Multilabel Counter (PerkinElmer, USA) at 365 nm excitation and 450 nm emission to measure fluorescence. The enzyme activities were expressed as MUF release in nmol g^{-1} dry soil h^{-1} (Razavi et al., 2015).

2.5. Calculations and statistical analysis

The cumulative gas emissions were calculated by linear interpola-tion between measured daily fluxes. Emission rates were expressed as arithmetic means of the three replicates and ANOVA tests were used to reveal significant pairwise differences among the three treatments at $P < 0.05$. Statistical analyses were conducted using the R software package, version 3.2.2.

3. Results

3.1. Gas emissions

In Exp. 1 the incubation period was divided into three phases re-garding the CO_2 and N_2O emissions patterns (Fig. 1): phase I (0–15 days), phase II (15–30 days) and phase III (30–62 days). In gen-eral, the acidic sandy soil had a lower CO_2 flux rate than the alkaline clay soil. In the acidic sandy soil, application of olive biochar induced about a twofold higher maximum CO_2 emission rate compared to AS and Corn + AS treatments in phase I (Olive + AS 4.0 ± 0.8, AS 2.0 ± 0.1 and Corn + AS 1.7 ± 0.3 mg C kg^{-1} soil day^{-1}). However, the two biochar addition treatments had lower CO_2 emission rates compared to the AS treatment during phase II and phase III. In the acidic sandy soil, corn biochar addition significantly reduced cumula-tive CO_2 emissions by 11.8% during the whole 62 day experiment, compared to AS treatment, whilst no overall significant difference was found between Olive + AS and AS treatment (Table 3).

In the alkaline clay soil, neither olive nor corn biochars showed a significant effect on CO_2 emissions compared to the AS treatment in phase I. However, during phase II and III the CO_2 emission rates in the biochar treatments were slightly higher than the AS treatment. For the whole experimental period there was no significant difference for cu-mulative CO_2 emissions between N-fertilizer only treatments and bio-char amendment treatments (Table 3).

In the acidic sandy soil, the N_2O emission in both N-only treatment and biochar addition treatments sharply increased after onset of the incubation and peaked in phase I or phase II (Fig. 2). The N_2O emission in AS and Corn + AS treatments followed a similar trend, decreased gradually after peaking in phase II and remained higher in phase III. In contrast, in Olive + AS treatment the N_2O emission peak occurred earlier, then drastically decreased to close to background level in phase II and stayed constantly low during phase III. Application of olive biochar and corn biochar application significantly decreased cumula-tive N_2O emission by 68.4% and 26.9% compared to N-fertilizer only treatment, respectively (Table 3).

In the alkaline clay soil, olive biochar application significantly re-duced the cumulative N_2O emission by 34.3%, while corn biochar
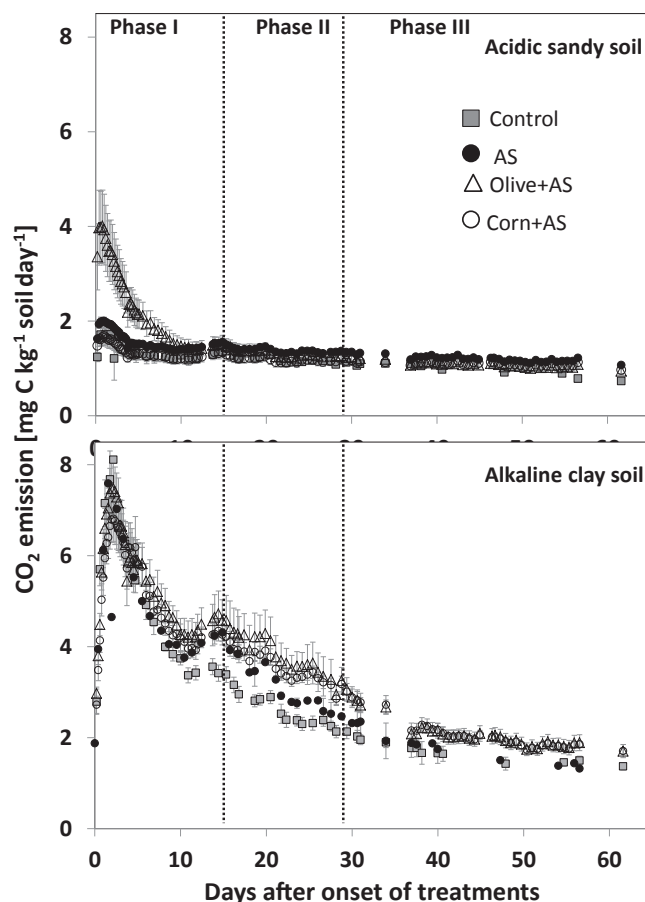

Fig. 1. The CO_2 fluxes from acidic sandy soil and alkaline clay soil in the four treatments. Data presented are the average of soil cores. Error bars show the standard error of the mean of each treatment (n = 3).

Table 3
Cumulative CO_2 and N_2O emissions from sandy and clay soil over 62 days in-cubation period.

Soil type	Treatment	CO_2 (mg C kg^{-1} soil)	N_2O (µg N kg^{-1} soil)
Acidic sandy soil	Control	67.2a	7.98a
	AS	82.7b	82.4c
	Olive + AS	86.0b	26.1a
	Corn + AS	72.9a	60.2b
Alkaline clay soil	Control	156.3a	5.8a
	AS	171.0ab	24.3c
	Olive + AS	185.9ab	16.8b
	Corn + AS	193.2b	20.8c

Letters indicate significant differences at the $p < 0.05$ level between treat-ments.

application had no significant effect on the cumulative N_2O emission compare to AS treatment (Table 3).

In Exp. 2, compared to alkaline clay soil, the pH manipulated acidic clay soil had significantly higher cumulative CO_2 emissions and lower cumulative N_2O emissions (Table 4). Olive biochar and corn biochar addition showed no significant effect on CO_2 emissions in the both acidified- and alkaline-clay soil compared to AS treatment. However, olive biochar reduced cumulative N_2O emission by 53.4% and 34.7% in the acidified and alkaline clay soil compared to AS treatment, respec-tively, whilst corn biochar had no significant impact on N_2O emissions in both soils.
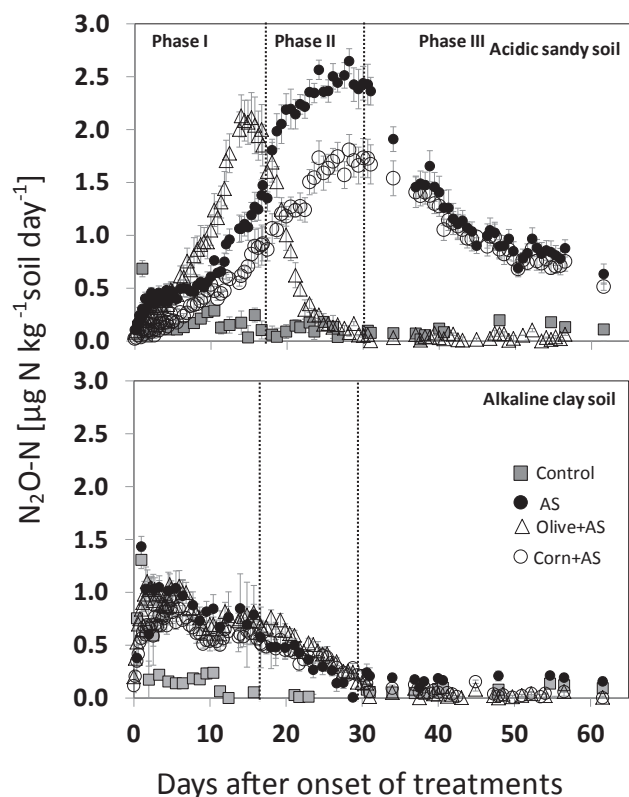
Fig. 2. The N_2O fluxes from sandy soil and clay soil in the four treatments. Data presented are the average of soil cores. Error bars show the standard error of the mean of each treatment (n = 3).

Table 4
Cumulative CO_2 and N_2O emissions in the alkaline and acidic clay soil over 62 days incubation period.

Soil type	Treatment	CO_2 (mg C kg^{-1} soil)	N_2O (µg N kg^{-1} soil)
Alkaline clay soil	Control	122.5 a	15.0 a
	AS	176.2 b	23.8 a
	Olive + AS	179.8 b	15.6 a
	Corn + AS	180.1 b	22.6 a
Acidified clay soil	Control	165.7 a	2.8 b
	AS	225.9 b	11.8 a
	Olive + AS	235.4 b	5.5 b
	Corn + AS	232.5 b	11.4 a

Letters indicate significant differences at the *p* < 0.05 level between treatments.

3.2. NH_4^+ and NO_3^- concentrations in soil

In Exp.1, treatments in the alkaline clay soils had significantly higher NO_3^- content than in the acidic sandy soil at the end of the incubation. In the acidic sandy soil, the two biochar treatments led to relatively lower NO_3^- concentration and higher NH_4^+ concentration compared to N-only treatment (Table 5). In the alkaline clay soil, olive biochar addition was associated with slightly higher NH_4^+ concentration compared to AS treatment, whilst no significant difference was found among AS treatments and the two biochar addition treatments on NO_3^- content.

3.3. Extracellular enzyme activities

In Exp.1, the extracellular enzyme activities were about three times higher in the alkaline clay soil than in the acidic sandy soil (Fig. 3). In the acidic sandy soil, adding N-fertilizer (ammonia sulfate) significantly

Table 5
NH_4^+ and NO_3^- concentrations in sandy and clay soils at the end of incubation depending on mineral N and biochar additions.

Soil type	Treatment	NH_4^+ (mg N kg^{-1} soil)	NO_3^- (mg N kg^{-1} soil)
Acidic sandy soil	Control	0.15 ± 0.02 a	5.02 ± 0.23 a
	AS	0.11 ± 0.01 a	35.85 ± 2.52 b
	Olive + AS	0.23 ± 0.07 a	30.51 ± 0.71 b
	Corn + AS	0.13 ± 0.01 a	30.64 ± 3.19 b
Alkaline clay soil	Control	0.13 ± 0.01 a	7.79 ± 0.40 a
	AS	0.12 ± 0.01 ab	41.74 ± 1.55 b
	Olive + AS	0.07 ± 0.01 c	45.96 ± 1.81 b
	Corn + AS	0.10 ± 0.00 bc	39.43 ± 1.79 b

Letters indicate significant differences at the *p* < 0.05 level between treatments.

increased cellobiohydrolase and chitinase activities. Interestingly, olive biochar amendment more than offset the stimulating effect of N fertilizer, causing significantly lower cellobiohydrolase and chitinase activities compare to AS treatment (*p* < 0.05). No significant influence on enzyme activities was found for corn biochar addition. In the alkaline clay soil, olive and corn biochar amendment significantly increased the activities of C-cycle related enzymes (cellobiohydrolase, xylanase and β-glucosidase), except for olive biochar on β-glucosidase, which was not statistically significantly increased. In contrast, no significant change was observed in the activity of the N-cycle related enzyme (chitinase).

In order to demonstrate the C-related metabolism efficiency as influenced by mineral N fertilizer and biochar amendment, the CO_2 emission per unit of enzyme activity was shown in Fig. 4. In the acidic sandy soil, olive biochar addition significantly increased specific CO_2 emissions for chitinase and cellobiosidase, while corn biochar addition showed no significant effect on specific CO_2 emissions. In the alkaline clay soil, both olive and corn biochar addition significantly decreased specific CO_2 emissions for cellobiohydrolase and xylanase, but had no effect on specific CO_2 emissions for chitinase and β-glucosidase.

4. Discussion

4.1. Effect of biochar on CO_2 and N_2O emissions

Biochar amendment can have contrasting effects on CO_2 emission, depending on soil and biochar types (Liu et al., 2011; Zhang et al., 2012). In our study, olive biochar addition induced a significant CO_2 emission peak in the acidic sandy soil as compared to AS treatment, but no effect was observed in the alkaline soil. This could be due to labile substances in olive biochar (Kuzyakov et al., 2009), which specifically mineralized under low soil pH condition. Exp. 2 with pH manipulation was therefore conducted to test this hypothesis. Surprisingly, CO_2 emissions in Olive + AS treatment were similar as to the AS treatment of the acidified clay soil (Table 4). This suggests that the mineralization of labile substances in olive biochar at low soil pH condition is unlikely to be the main reason for the triggered CO_2 emissions in Olive + N treatment in the acidic sandy soil. A likely explanation could be the various interactions between soil organic matter and biochar mineralization, which resulted in "positive" or "negative" priming as observed in several previous studies (Chang et al., 2016; Kuzyakov et al., 2000; Subedi et al., 2016; Zimmerman et al., 2011).

In spite of numerous studies on N_2O mitigation ability of biochar, up to now no consensus has been reached about why and how biochar reduces N_2O emissions (Cayuela et al., 2014). Several plausible mechanisms have been proposed. For example, one is improving soil porosity and aeration (Yanai et al., 2007). However, if this was the case, we would observe a more significant N_2O mitigation effect in clay soils, especially when considering the high soil moisture condition (70% WHC) in our study. Therefore, the present study may confirm the
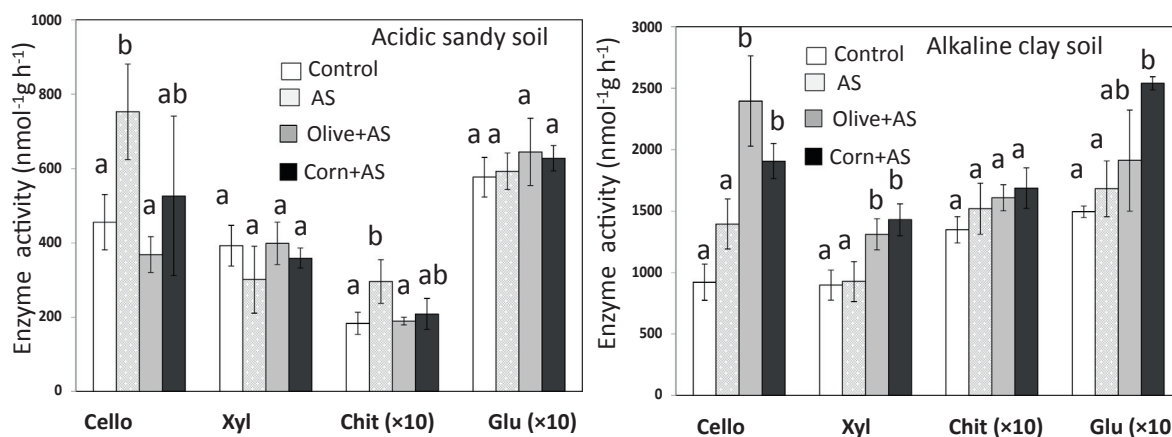
Fig. 3. Activities of cellobiohydrolase (Cello), Xylanase (Xyl), chitinase (Chit) and β-glucosidase (Glu) as influenced by mineral N fertilizer and biochar amendments. Error bars show the standard error of the mean of each treatment (n = 3). Letters indicate significant differences at the p < 0.05 level between treatments. Note, that the values of chitinase and b-Glucosidase activity were divided by ten in order to fit the scale.

minimal contribution of increasing soil porosity and aeration to the suppression of N_2O emissions by biochar addition (Case et al., 2012). The mechanism of biochar's mitigation effect on N_2O emission may also be explained by i) toxic effect of biochar (Clough et al., 2010), ii) enhanced N immobilization and iii) NH_4^+/NO_3^- sorption ability of biochars (Clough et al., 2013). However, these mechanisms also failed to explain why the same biochar in our study caused variable N_2O emissions in different soil types, and why increased N_2O emission was observed with biochar amendment in the earlier incubation phase.

Soil pH is one of the most important factors that affect N_2O production and consumption processes in soil. It has also been suggested that biochar's liming effect could decrease N_2O/N_2 ratio and therefore reduce N_2O emissions (Zwieten et al., 2010). In Exp. 2, the N_2O mitigation effect of olive biochar in the alkaline clay soil (pH = 7.9) significantly increased from 35% to 53%, after adjusting the pH to a value close to that of the acidic sandy soil (pH = 5.3). This indicates that the high N_2O mitigation effect of olive biochar might be largely attributed to its liming effect. Cayuela et al. (2013) hypothesized that biochar might facilitate the transfer of electrons to soil denitrifying microorganisms, thus promote the reduction of N_2O to N_2. However, in our Exp. 1 with acidic sandy soil, olive biochar application triggered an earlier N_2O emission peak and much larger N_2O emissions in phase I compared to AS treatment, indicating that N_2O reduction was not always promoted by biochar amendment. This, instead, is likely due to the positive priming effect caused by olive biochar addition, which stimulated labile C mineralization and resulted in higher denitrification rate, as suggested by the observed higher CO_2 and N_2O emissions in

phase I. Interestingly, olive biochar significantly reduced N_2O emission in phase II in acidic sandy soil, compared to AS treatment. This is possibly due to the higher denitrification rate triggered by olive biochar addition in phase I, which led to lower nitrate concentration in phase II, as indicated by the lowest NO_3^- concentration in Olive + AS treatment in the end of incubation. Lower NO_3^- concentration in Olive + AS treatment at phase II possibly led to a faster switch from N_2O-emitting conditions (($N_2O/N_2O + N_2$) ratio close to one) to non-N_2O-emitting condition (($N_2O/N_2O + N_2$) ratio close to zero) (Senbayram et al., 2012). We presume that the contrasting effects of olive biochar addition on N_2O emissions during different phases were likely due to biochar's liming effect, which induced a positive priming effect, increased soil C mineralization and thus affected denitrification rate and denitrification product stoichiometry. We also attributed the higher N_2O mitigation efficiency of olive biochar (pH = 9.2) than corn biochar (pH = 8.5) to its higher liming effect in the acidic soil. Different C:N ratio and porous structures of olive and corn biochar also may be responsible for current findings as reported earlier (Cayuela et al., 2014; Clough et al., 2013), however, further research is needed to test at what extend they effect these parameters.

4.2. Effect of biochar on enzyme activities

At day 4 of the experiment corresponding to the maximal CO_2 emission, a fluorometric assay was applied to measure the activities of four hydrolytic enzymes (i.e. cellobiosidase, chitinase, xylanase, β-glucosidase) in soil. Overall, remarkably higher enzyme activities were
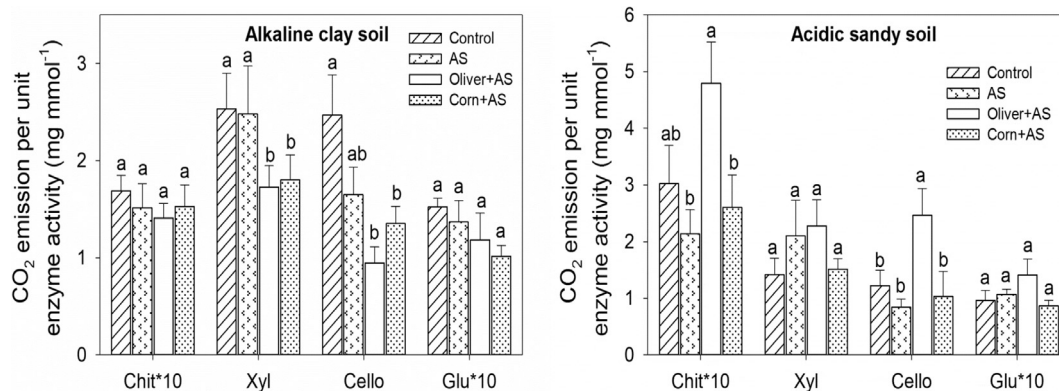

Fig. 4. CO_2 emission per unit of cellobiohydrolase (Cello), Xylanase (Xyl), chitinase (Chit) and β-glucosidase (Glu) activities as influenced by mineral N fertilizer and biochar amendments. Error bars show the standard error of the mean of each treatment (n = 3). Letters indicate significant differences at the *p* < 0.05 level between treatments. Note, that the values of chitinase and β-Glucosidase activity were divided by ten in order to fit the scale.

observed in the alkaline clay soil, which might be attributed to the higher mineral N content and clay content (Turner et al., 2002).

The simultaneous increase in CO_2 emission and cellobiohydrolase activity in acidic sandy soil after N addition may be explained by the N-stimulated decomposition of plant-derived cellulose-like compounds (Fig. 3), which are consistent with previous studies that suggested the production of C acquiring enzymes increases with increasing N availability (Keeler et al., 2009; Saiya-Cork et al., 2002). The decrease of cellobiohydrolase and chitinases activities by olive biochar addition might be due to the co-localization of substrates and microbes on the biochar surfaces, which may reduce the need for enzyme production (Lehmann et al., 2011). Although no significant effect of enzyme activity was observed after corn biochar addition, the CO_2 emission was decreased (Figs. 1 and 3). This might be because of the sorption of substrate by biochar, which was proved by the decrease DOC in previous study (Ahmad et al., 2014; Lammirato et al., 2011). Furthermore, up to 50–130% higher specific CO_2 emissions (per unit of C-related enzyme activity: cellobiohydrolase, chitinases and β-glucosidase) were observed after addition of olive biochar compared to corn biochar addition in the sandy soil (Fig. 4), indicating contrasting properties of the two biochar types and, possibly, less efficient intracellular metabolism of dominating microbial population under olive biochar. This also suggests that the CO_2 after olive biochar addition was produced partly by the decomposition of cellulose- and glucosamine-derived compounds, indicating a specifically enhanced microbial activity (Gärdenäs et al., 2011). Thus, N addition to the sandy soil stimulated enzyme activities and further increased the organic C decomposition. Contrasting biochar effect on specific CO_2 emissions may be due to the difference of olive and corn biochar in basic properties and compounds composition.

In the alkaline clay soil, no effect of N addition was observed neither in enzyme activity nor specific CO_2 emissions ($p > 0.05$; Figs. 3 and 4). Likely, the soil already had a sufficiently high N availability for microbial biomass (Table 1), resulting in a neutral effect of N addition on soil enzymes (Jing et al., 2016). This could be further supported by the lack of change of CO_2 emission after N addition to the clay soil (Fig. 1). However, biochar addition stimulated microorganisms in the alkaline clay soil, leading to a significant increase in the activities of cellobiohydrolase, xylanase and β-glucosidase (Fig. 3). This corresponded well to increased enzyme activities after biochar addition to a silt loam soil (Wang et al., 2016), and was possibly associated with better soil aeration after the formation of larger aggregates stimulated by the biochar addition (Awad et al., 2012). Moreover, decreased specific CO_2 emissions for cellobiohydrolase and xylanase were observed after biochar amendment (Fig. 4), indicating higher efficient intracellular metabolism of dominating microbial population in the biochar treatments. Therefore, neutral effect of N addition on soil enzymes corresponds to the neutral effect on CO_2 emission. No effect of biochar amendment on CO_2 emission may be attributed to the higher efficient intracellular metabolism and the absorption of CO_2 in the alkaline clay soil.

5. Conclusions

Contrasting effects of olive biochar addition on N_2O emissions during different phases were attributed to the biochar's liming effect in acid soil, which induced a positive priming effect, increased soil C mineralization and thus affected denitrification rate and denitrification product stoichiometry. Up to 50–130% higher specific CO_2 emissions (per unit of C-related enzyme activity: cellobiohydrolase, chitinases and β-glucosidase) were observed after addition of olive biochar compared to corn biochar addition in the acidic sandy soil. Therefore, soil and biochar pH was identified as an important factor that controls biochar's effect on N_2O and CO_2 emission. We concluded that biochar's effects on N_2O and CO_2 emissions are clearer in acidic soils. Alkaline biochar's N_2O mitigation potential in acidic soils seems to be dependent on soil NO_3^- content as drastically higher N_2O emissions were measured in

early phase of the experiment (where soil NO_3^- was high) and significantly lower N_2O fluxes were obtained in later phases (with lower soil NO_3^- content).

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