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Influence of pruning waste biochar and oyster shell on N_2O and CO_2 emissions from Japanese pear orchard soil

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Abstract

Two incubation experiments were conducted under controlled moisture and temperature conditions to determine the effects of soil amendment treatments based on pruning waste biochar and oyster shell, on N_2O and CO_2 emissions from an orchard soil. In experiment 1, four treatments were tested including, control (CK), pruning waste biochar at 2% (B2%), at 10% (B10%), and oyster shell (OS), mixed with soil from two different depths, namely, from the 0−5 cm and the 0−10 cm layers. In experiment 2, only the 0−10 cm soil layer was used to study the effect of surface application of pruning waste biochar (B2% and B10%) on soil N_2O and CO_2 emissions. The results showed that soil pH, total C and C: N ratio increased with biochar amendment treatments. Significant reduction in soil NO_3^- content was observed for the B10% treatment. Although OS application increased soil pH, no effect was observed on soil mineral N content, total C or C: N ratio. The rate of N_2O emissions from the 0−5 cm soil layer after B2% and B10% addition, significantly declined by 12.5% and 26.3%,

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respectively. However, only the B10% treatment caused significant reduction in N_2O emissions from the 0−10 cm soil layer and from surface soil, by 15.1% and 13.8%, respectively. Oyster shell application had no effect on either soil N_2O or CO_2 emissions from either soil layer tested. Our results suggest that the addition of pruning waste biochar at a high rate has the potential to mitigate N_2O emissions from orchard soils; while, oyster shell can be used for liming without altering soil N_2O nor CO_2 emissions.

Keywords: Earth sciences, Environmental science, Agriculture

1. Introduction

Japanese pear (*Pyrus pyrifolia* Nakai) is one of the most widely grown fruit trees in Japan. There are currently 12,100 hectares under pear cultivation in the country (MAFF, 2017). Nitrogen plays a major role in plant metabolism and is well known to affect tree vigor, yield, fruit size and quality. Japanese pear orchards are typically fertilized with nitrogen in the spring and summer seasons, with amounts ranging from 200 to 300 kg N ha^{-1}. The application of high rates of N fertilizer can cause many problems, such as soil acidification and emission of high levels of N_2O, which is a potent greenhouse effect gas that can destroy the ozone (O_3) layer in the stratosphere. With a 300-fold greater warming potential, compared to CO_2, N_2O emitted from the soil is a downside of the large productivity increase in agriculture due to synthetic nitrogen fertilizer application (Hüppi et al., 2015). Therefore, it is important to reduce N_2O emissions induced by N fertilizers to reduce greenhouse gas emissions associated with agricultural practice.

Agricultural liming materials increase soil pH, which plays an important role in the regulation of soil processes, such as organic matter mineralization, N transformation, nitrification, and denitrification, all of which, in turn, affect soil N_2O production (Shaaban et al., 2014a). Recently, Shaaban et al. (2015) reported that the change in soil pH in a dolomite-treated soil increased N_2O-reductase activity; thereby, reducing N_2O emissions. In contrast, others have observed that lime-treated soils produced larger N_2O emissions when compared to un-limed soils (Baggs et al., 2010; Higgins et al., 2013). These controversial reports likely result from differences in the liming materials used in each case, and in the particular properties of the specific experimental soils involved.

Recycling oyster shell as liming material is reportedly a promising agricultural practice with beneficial effects on acidic soils, as by and large, they lay piled up on the seashore as fishery waste material in Japan, although a portion of these oyster shells has been effectively used as fertilizer and soil conditioner (Mori, 2014). However, information on the effect of oyster shell application on soil N_2O and CO_2 emissions is scarce.

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Japanese pear trees are usually pruned during winter, while dormant. Winter pruning of nashi pear trees is done to encourage more fruiting buds, fruiting close to main branches and to reduce bud height on the tree. Pruning residues are commonly disposed of by burning or landfilling, and only on very few occasions are they used for composting. Normally, residues are considered useless and they are not returned to the soil, resulting in a general loss of C and an adverse environmental impact due to burning in the open. Thus, it is necessary to find an alternative to field burning of pruning residues in orchards.

Recently, attention has focused on the use of biochar as a soil improver, as well as a carbon sequestration and climate change mitigation strategy. Biochar amendment affects C and N turnover by influencing microbial community structure and biomass (Singla et al., 2014); thereby, altering soil CO_2 and N_2O emissions (Yanai et al., 2007). Many studies have reported significantly reduced soil N_2O emissions by biochar application (Van Zwieten et al., 2010; Cayuela et al., 2013, 2014; Oo et al., 2018). A recent meta-analysis by Cayuela et al. (2014) revealed a statistically significant reduction of 54% in N_2O emissions upon soil amendment with biochar. Biochar addition caused a decrease in N_2O emissions compared to control treatment because of (i) increased soil aeration and decreased soil bulk density (Yanai et al., 2007); (ii) increased N_2OR activity due to an increase in pH (Liu et al., 2010) and, (iii) reduced NO_3^- availability due to microbial N-immobilization during microbial consumption of N-depleted volatile biochar compounds (Ameloot et al., 2013). However, other studies have reported no difference, or even an increase in soil N_2O emissions, after biochar application (Clough et al., 2010; Saarnio et al., 2013). Concomitantly, biochar addition can also markedly affect soil CO_2 emissions (Cross and Sohi, 2011; Zimmerman, 2010; Chintala et al., 2014; Oo et al., 2018). Thus, although the effect of biochar addition on soil CO_2 and N_2O fluxes has been extensively investigated, results have not been consistent, probably, due to the wide variation in biochar properties depending on the biomass source, pyrolysis conditions and application rates. Therefore, the issue remains controversial.

Carbonization of orchard pruning residues and its utilization might be one of the best environment-friendly alternatives to field burning in fruit-tree orchards. Burning the waste from Japanese pear orchards for biochar and returning it to the orchard soil might induce carbon sequestration and reduce soil gas fluxes. However, there is still limited information on the effect of incorporation of pruning residue biochar to orchard soils on N_2O and CO_2 emissions.

In Japanese pear orchard fields, the soil is often not tilled and fertilizers are applied uniformly over the soil surface. However, in some cases, shallow tillage is done for weeding and for mixing applied fertilizers. In this study, we evaluated the effect of three different methods of application of pruning waste biochar and oyster shell on greenhouse gas emissions. These methods included, 1) surface application, 2)

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mixing with top soil (0−5 cm) layer, and 3) mixing with top soil (0−10 cm) layer. Our main objectives were (i) to evaluate the effect of pruning waste biochar and oyster shell application on N_2O and CO_2 emissions from two soil layers and (ii) to compare the methods of treatment application (mixing with soil and surface application) on soil N_2O and CO_2 emissions from a Japanese pear orchard.

2. Materials and methods

2.1. Soil and biochar

The soil used in this study was collected in February 2017 from a 24-year-old Japanese nashi pear orchard at the Horticultural Research Institute of the Agricultural Research Center in Ibaraki Prefecture, Ibaraki, Japan (36° 16′ N, 140° 26′ E). The soil was classified as an Andosol. Lime is applied yearly at 400 kg ha^{-1} at the end of January. Soil samples were collected at depths of 0−5 and 0−10 cm from multiple points in a selected field. Soil samples were mixed and passed through a 2 mm mesh size sieve to obtain a composite sample for the incubation study.

Biochar was produced from branches pruned off the Japanese nashi pear trees in the orchard. Two weeks after pruning in December 2016, all pruned branches were collected and weighed before burning for biochar. Overall, the tree pruning residues from the nashi orchard amounted to about 7.6 t ha^{-1} on a dry weight basis. Nashi pear biochar was produced from carbonization of pruning waste residues under an open fire using a 534 L open burn kiln (Fig. 1), 150 cm in diameter and 43 cm

Fig. 1. Making biochar in an open burn kiln using pruning residues of Nashi pear orchard.

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Table 1. Properties of soil and biochar.

	Total N (%)	Total C (%)	Bulk density (g cm^{-3})	pH (KCl)	EC (ms cm^{-1})	CaO	MgO	K_2O	P_2O_5	Sand	Silt	Clay
						(mg 100 g^{-1})						
Soil (0–20 cm)	0.54	6.15	0.76	6.2	0.18	689.5	102.1	119.5	156.35	26.7	50.4	22.9

	Total N (g kg^{-1})	Total C (g kg^{-1})	pH (H_2O)	C:N ratio	Surface area (BET) (m^2 g^{-1})	Ash (%)	Volatile matter (%)	Fixed C (%)	Bulk density (g cm^{-3})
Biochar	5.7	374	10.3	65.6	83.9	9.8	9.7	80.5	0.53

high. The open fire kiln is an auto-thermal process that burns part of the feedstock material to heat the rest of the material and turn it into char. Pruning-residues feedstocks were placed inside the chamber of the open burn kiln and ignited. Carbonization of the feedstocks occurred beneath the flames, where oxygen is absent, because the flames consume all of it; thus, creating a pyrolysis zone. The lack of oxygen prevents combustion and so, the biomass smolders but does not release flames or smoke. Instead, much of it is transformed into high-carbon charcoal, oil, and gas. Pyrolysis temperature at pyrolysis zone was approximately 500–600 °C with this method. Feedstocks were added continuously until the kiln was filled up and then quenched with water. Due to the resulting high water-content of feedstocks (36.8%), char production took 2 hours. Biochar yield was about 30% on a dry weight basis. Biochar was air-dried and ground to pass a 2-mm mesh sieve. Properties of the soil and biochar are shown in Table 1.

2.2. Incubation experiment

The effects of biochar and oyster shell application on N_2O and CO_2 emissions were tested by means of an incubation experiment. Immediately after collecting and sieving soil samples, we adjusted soil moisture content to 80% water-filled pore space (WFPS) by adding deionized water. The use of this WFPS soil moisture content was based on actual field measurements, which ranged from 70% to 90% WFPS throughout the whole year in the Nashi pear orchard soil used as experimental location (Fujita et al., 2015). Therefore, polypropylene jars (750 ml) were filled with 177 g moist soil, which corresponded to 100 g oven-dried soil.

Experiment 1: Orchard soil samples obtained from 0–5 and 0–10 cm depths were used to study the effect of pruning waste biochar and oyster shell application on soil N_2O and CO_2 emissions. Commercially available oyster shell was used as liming material, composed of 48% Ca, 0.21% N, 0.18% P_2O_5, 0.03% K_2O and 0.43% Mg. The treatments used in this study were 1) biochar 2% (B2%), 2) biochar 10% (B10%), 3) oyster shell (OS), and 4) control (CK). The 2% and 10% biochar

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application rates were equivalent to 20 t and 100 t ha^{-1}, respectively, based on 10 cm incorporation depth in the field. Oyster shell was applied at 1.3 g kg^{-1} soil, which was equivalent to 3 t ha^{-1}. Biochar and oyster shell were thoroughly mixed with the soil to obtain a completely homogeneous mixture. For all the treatments, N fertilizer (ammonium sulfate) was applied with thorough mixing at a rate of 89 mg kg^{-1} dry soil, which is equivalent to 200 kg N ha^{-1}.

Experiment 2: Orchard soil from a depth of 0−10 cm was used to study the effect of surface application of pruning waste biochar on soil N_2O and CO_2 emissions. Treatments included, 1) control (CK), 2) biochar 2% (B2%), and 3) biochar 10% (B10%). For biochar treatment, biochar was uniformly spread on the soil surface. N fertilizer was applied at a rate of 89 mg kg^{-1} dry soil for all soil amendment treatments.

Experiments were laid out in a completely randomized design with three replications. The jars were incubated aerobically for 71 days at a constant temperature of 25 °C in an incubator (Model: LP-260, Nippon Medical and Chemical Instruments Co., LTD., Osaka, Japan). Aluminum sheets were placed over the top of each jar to prevent moisture loss, and pinholes were pierced to allow gas exchange. Soil moisture content was maintained at 80% WFPS throughout the experiment by weighing the jars twice a week and adding deionized water as required. After 71 days of incubation, we stopped the incubation experiment, since N_2O emissions were relatively low and no comparable emissions were observed among the treatments.

2.3. Gas sampling and analysis

During the incubation period, air samples were collected on days 0, 1, 2, 3, 4, 6, 9, 14, 18, 23, 29, 36, 43, 50, 57, 64, and 71. Before sampling, the jars were thoroughly flushed with ambient air and left opened for approximately 30 min to equilibrate with the atmosphere. The jars were then sealed for 30 minutes using lids that had a rubber septum for gas sampling. These lids were only used during gas sampling and were replaced with the aluminum sheet for the rest of the experiment. Gas samples were drawn from the incubation jar using a 50-ml syringe. The air inside the jar was thoroughly mixed by flushing the syringe three times before collecting the gas samples. Sampled gasses were then transferred to 15 ml vacuum glass vials sealed with butylene rubber stoppers. The concentration of N_2O and CO_2 were analyzed by a gas chromatograph (GC 2014, Shimadzu Corporation, Kyoto, Japan) equipped with an electron capture detector (ECD) and a thermal conductivity detector (TCD) for the determination of each gas, respectively. The difference in gas concentrations between the atmosphere and the samples was used to determine total emissions. Cumulative gas emissions from each jar were calculated by integrating emissions over the 71 days of incubation.

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2.4. Soil and biochar analysis

Soil and biochar total N and total C contents were analyzed by a NC analyzer (Sumigraph NC-80; Sumika Chemical Analysis Service Co., Tokyo, Japan). Soil and biochar pH were measured in the supernatant suspension of 1:5 soil: KCl and 1:5 soil: H_2O solution using a pH meter (Mettler Toledo), respectively. Soil electrical conductivity (EC) was measured in the supernatant suspension of 1:5 soil: H_2O solution using a conductivity meter (CM-40s, TOA CM). Soil particle analysis was performed using the pipette method (Gee and Bauder, 1986). Bulk density was determined by the core method (Blake and Hartge, 1986). Concentrations of CaO, MgO, and K_2O were determined by atomic absorption spectroscopy (AA-6300, Shimadzu, Japan). Soil P_2O_5 was determined after Truog (1930). Soil mineral N contents (NO_3^- and NH_4^+) were determined from 10 g of fresh soil samples extracted with 50 ml 1 M KCl and analyzed using a QuAAtro Auto Analyzer (BLTEC, Tokyo, Japan). Specific surface area of biochar was determined by N adsorption isotherms at 77.3 K interpreted by the BET equation (Brunauer et al., 1938) (Autosorb-1 series, Quantachrome Instruments, USA). The ash, volatile matter and fixed carbon concentrations in biochar were measured in accordance with JIS M 8812., 2004.

2.5. Statistical analysis

The effects of biochar and oyster shell application on soil properties and cumulative gas emissions were tested by analysis of variance (ANOVA) using CropStat 7.2 statistical software program. Treatment mean comparisons were done at 5% level of probability by Tukey's HSD test using XLSTAT Version 2016 (Addinsoft).

3. Results

3.1. N_2O emissions

Initial soil N_2O emissions before treatment application ranged from 0.2 to 0.8 μg N kg^{-1} h^{-1} in both experiments (Fig. 2). N_2O emissions dramatically increased following N fertilizer application, and the greatest N_2O flux occurred between days 0 and 3 of incubation and then sharply decreased over the following days, after which, N_2O emissions remained steady with small fluxes in the later part of the incubation period. After 71 days of incubation, soil N_2O emissions were not affected by OS application in experiment 1 (Fig. 3). Mean cumulative soil N_2O emissions for OS and CK treatments were 2.92 and 3.19 mg N kg^{-1} of soil for the 0−5 cm soil layer, and 2.52 and 2.51 mg N kg^{-1} of soil for the 0−10 cm soil layer, respectively. The rate of N_2O emission from OS treated 0−5 cm soil layer samples declined by 8.5% with respect to CK treatment; however, such reduction was not significant. Biochar addition significantly decreased cumulative N_2O emissions, compared

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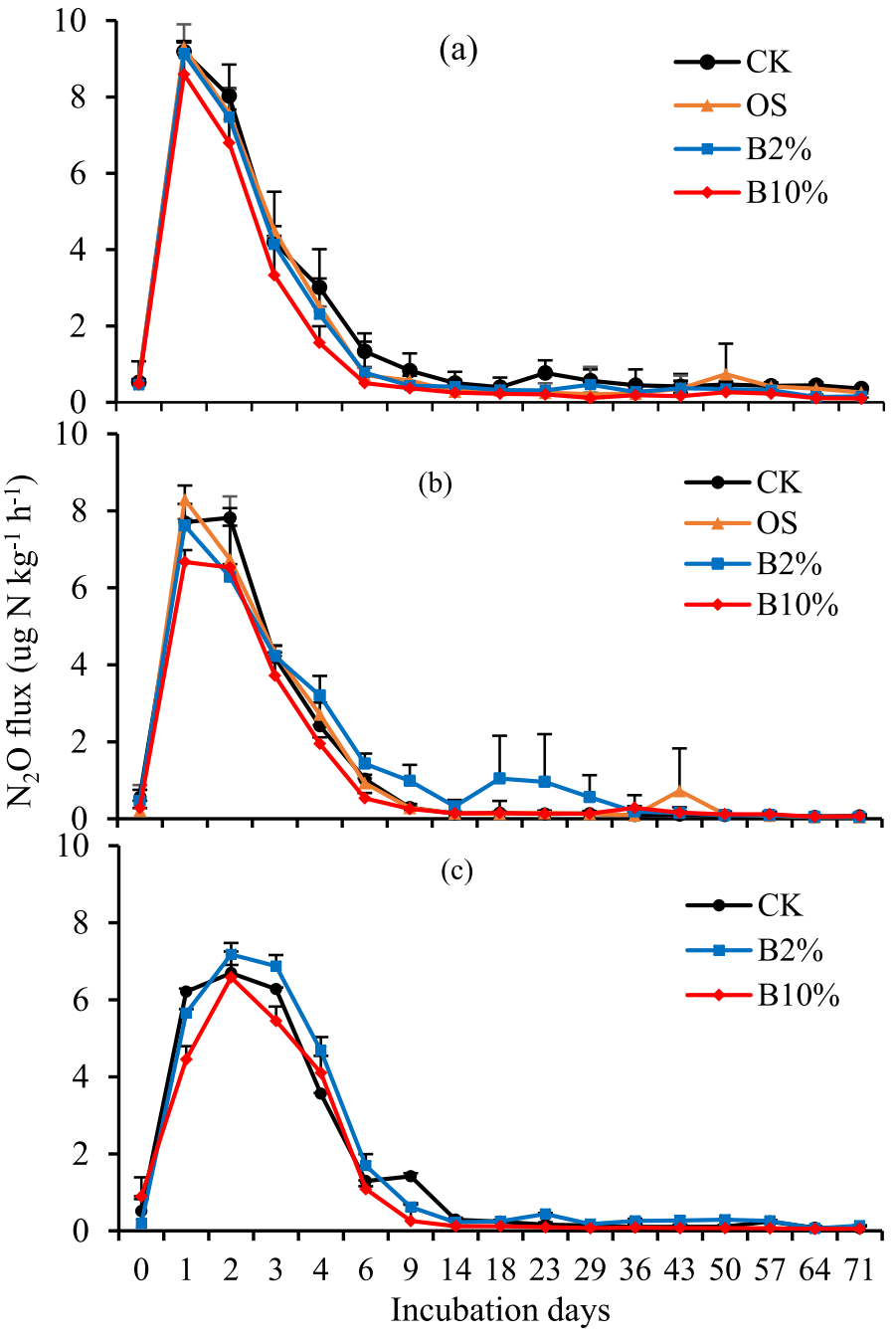

Fig. 2. Soil N_2O emissions during 71 days incubation period; (a) 0−5 cm soil layer, (b) 0−10 cm soil layer and (c) surface application. Error bars indicate standard deviation. CK − control, OS − oyster shell, B2% − biochar 2%, B10% − biochar 10%.

with CK treatment, for both soil layers, except for the B2% treatment in the 0−10 cm soil layer. The rates of emission for B2% and B10% from the 0−5 cm soil layer significantly declined by 12.5% and 26.3%, respectively, relative to CK treatment. On the other hand, only the B10% treatment significantly reduced N_2O emissions

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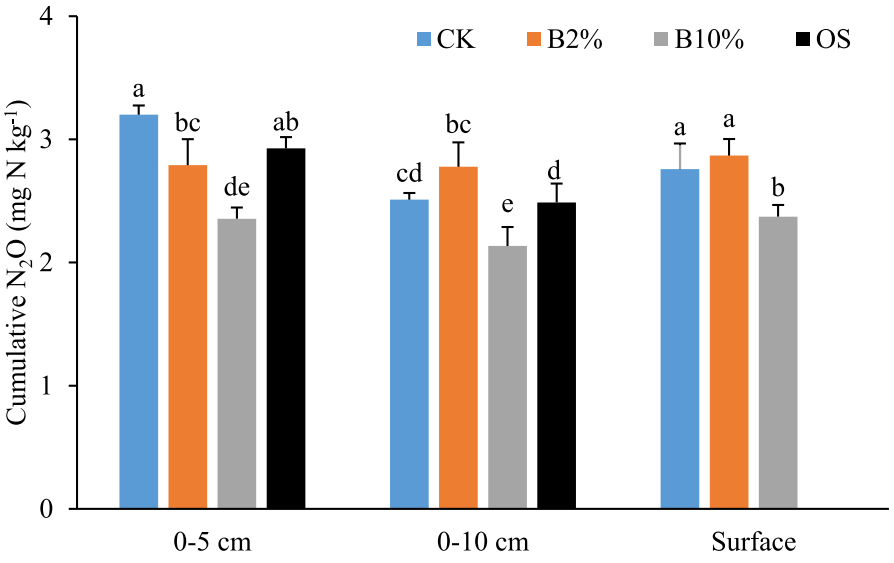

Fig. 3. Cumulative N_2O emissions after 71 days of incubation. Error bars indicate standard deviation. The same letter above or within bars for the 0−5 and 0−10 cm soil layers, and above the bars for surface application indicates that bar mean values are not significantly different at the 5% level by Tukey's HSD test. CK − control, OS − oyster shell, B2% − biochar 2%, B10% − biochar 10%.

by 15.1%, compared with CK, from the 0−10 cm soil layer. However, at the low biochar application rate (B2%), a relative increase (10.4%) in N_2O emissions was observed, compared with CK. When comparing the two biochar application rates tested here, the B10% treatment showed a significant reduction in N_2O emissions compared with the B2% treatment, for both soil layers tested. For the surface application in experiment 2, similar results were observed; i.e., only B10% biochar application significantly reduced N_2O emissions by 13.8%, compared with CK. No significant difference in N_2O emissions was observed between B2% and CK treatments.

3.2. CO_2 emissions

Average initial soil CO_2 emission was 4.6 mg C kg^{-1} h^{-1} before treatment application in both experiments (Fig. 4). CO_2 emission increased sharply following application of N fertilizer, peaking on day 1 of incubation, and then decreased over the following days. Subsequently, CO_2 emissions remained steady with low fluxes in the later part of the incubation period. After 71 days of incubation, soil CO_2 emission was not affected by OS application for either soil layers tested in this experiment 1 (Fig. 5). Mean cumulative CO_2 emission in OS and CK treatments were 3,612 and 3,820 mg C kg^{-1} of soil for the 0−5 cm soil layer, and 2,453 and 2,359 mg C kg^{-1} of soil, for the 0−10 cm soil layer, respectively. Addition of different rates of biochar showed no effect on cumulative CO_2 emission from the 0−5 cm soil layer. Mean CO_2 emissions in CK, B2% and B10% treated soil samples were 3,820, 3,869 and

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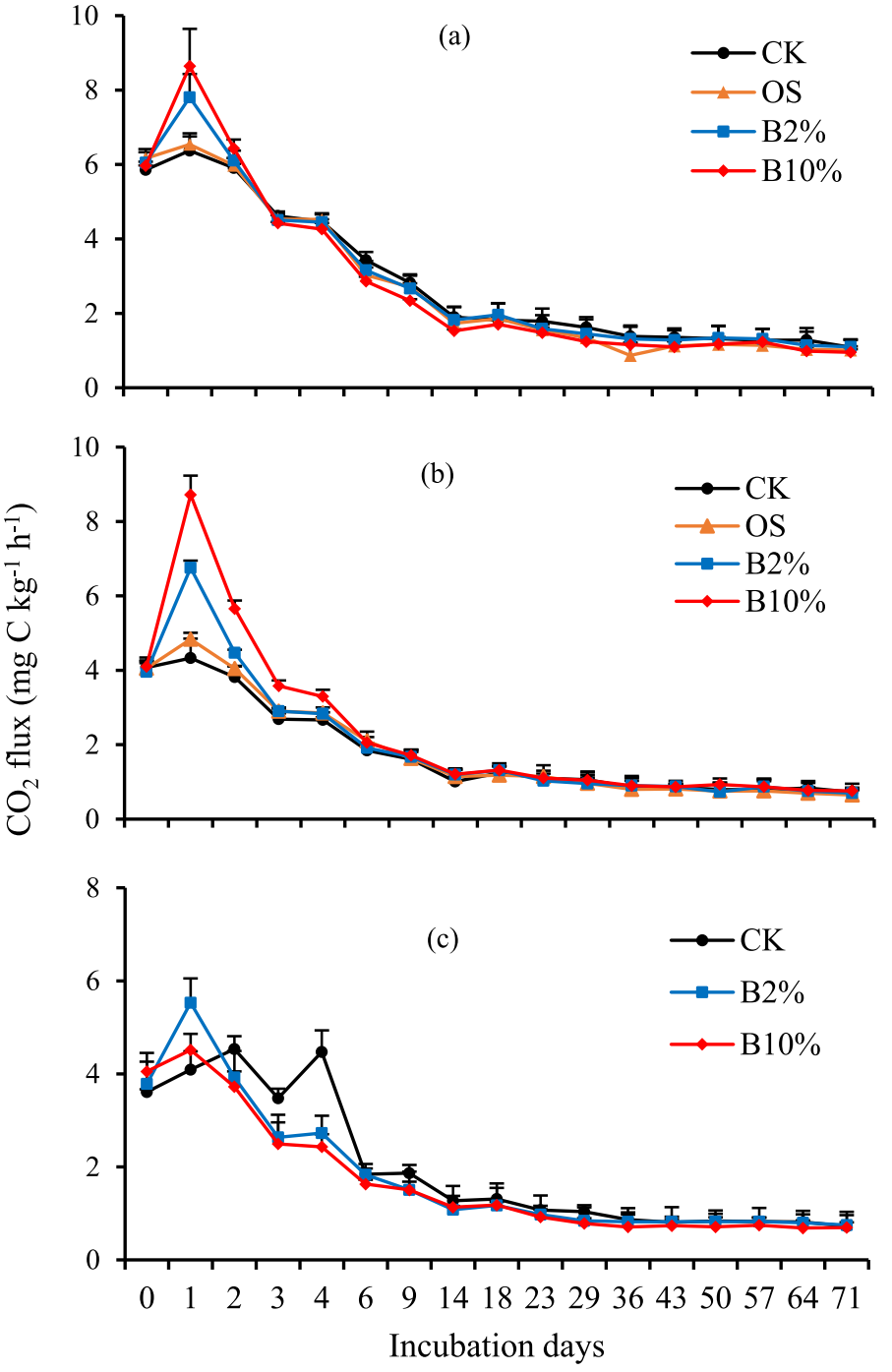

Fig. 4. Soil CO_2 emissions during 71 days incubation period; (a) 0−5 cm soil layer, (b) 0−10 cm soil layer and (c) surface application. Error bars indicate standard deviation. CK − control, OS − oyster shell, B2% − biochar 2%, B10% − biochar 10%.

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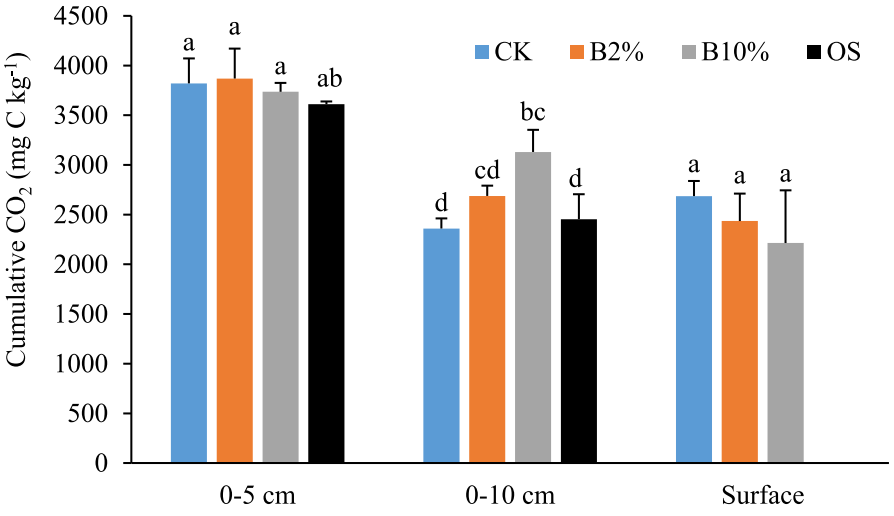

Fig. 5. Cumulative CO_2 emissions after 71 days of incubation. Error bars indicate standard deviation. The same letter above or within bars for the 0−5 and 0−10 cm soil layers, and above the bars for surface application indicates that bar mean values are not significantly different at the 5% level by Tukey's HSD test. CK − control, OS − oyster shell, B2% − biochar 2%, B10% − biochar 10%.

3,736 mg C kg^{-1} of soil, respectively. However, 10% biochar amendment significantly increased CO_2 emissions by 27.8%, from the 0−10 cm soil layer, compared with CK. Relative increase of 13.9% in CO_2 emissions for B2% treatment, compared with CK, was also registered. When comparing B2% and B10%, high CO_2 emissions were observed for B10% treated soil samples, although not statistically significant. In biochar surface application (experiment 2), CO_2 emissions declined by 9.3% and 17.5% in B2% and B10%, respectively, compared with CK; however, the result was not statistically different.

3.3. Soil properties

In experiment 1, initial pH was 6.7 and 6.6 for the 0−5 cm and 0−10 cm soil layers, respectively (Fig. 6). Soil pH significantly increased on day 4 of incubation in both, biochar and oyster shell treatments for both soil layers; no further significant change occurred. Soil pH increased by 0.24 and 0.21 units in the 0−5 cm and the 0−10 cm soil layers, respectively, at the end of incubation following application of oyster shell. Similarly, biochar amendment significantly increased soil pH in a dose-dependent manner, which was pronounced in both soil layers, with the highest value observed in B10% treated soil samples. Increase in soil pH for B2% and B10% treatments were 0.37 and 0.84 units for the 0−5 cm soil layer, and 0.34 and 1.1 units for the 0−10 cm soil layer, respectively.

Total N content was not affected by either OS or biochar application in either soil layer tested in experiment 1 (Table 2). Neither was there any significant difference

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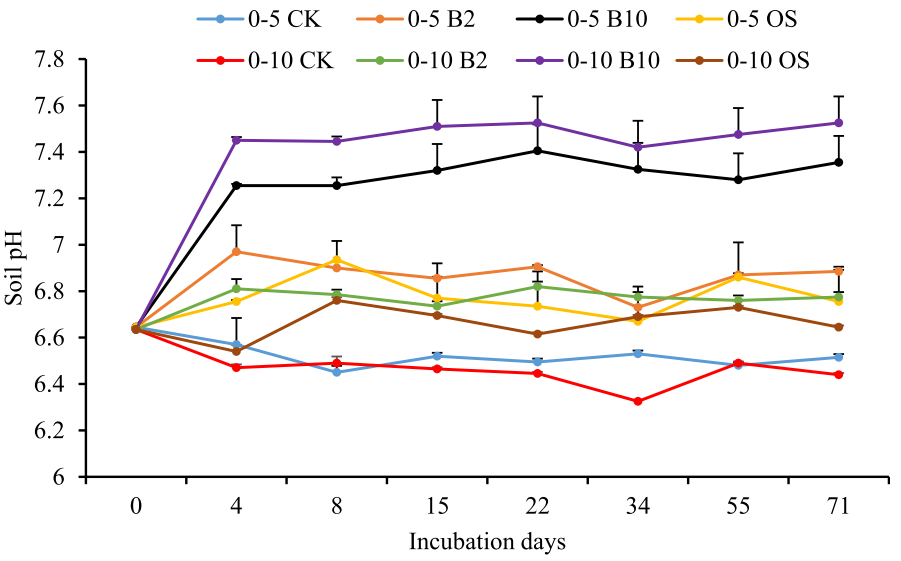

Fig. 6. Changes in soil pH during 71 days incubation period. Error bar indicates standard deviation. 0−5 to 0−5 cm soil layer, 0−10 to 0−10 cm soil layer, CK − control, OS − oyster shell, B2% − biochar 2%, B10% − biochar 10%.

in total N content upon soil surface application of different rates of biochar in experiment 2.

Biochar amendment significantly affected soil total C content in both experiments. Mean total C content for CK, B2% and B10% treated soil samples were 87.6, 99.2, and 121.0 g kg^{-1} of soil for the 0−5 cm soil layer, and 72.4, 83.0, and 101.2 g kg^{-1} of soil for the 0−10 cm layer, respectively. Soil surface application of biochar also increased soil total C content significantly, by 12.4% and 38.2% in B2% and B10% treatments, respectively, compared with CK. In contrast, soil total

Table 2. Soil total N, total C, C/N ratio after 71 days of incubation.

Treatment		Total N (g kg^{-1})	Total C (g kg^{-1})	C:N ratio
0−5 cm	CK	9.5 a	87.6 bc	9.2 e
	OS	9.3 a	85.1 cd	9.2 e
	B2%	9.5 a	99.2 b	10.4 cd
	B10%	8.7 ab	121.0 a	13.9 b
0−10 cm	CK	7.5 bc	72.4 e	9.7 de
	OS	7.2 c	72.5 e	10.1 cd
	B2%	7.7 bc	83.0 d	10.8 c
	B10%	6.8 c	101.2 b	15.0 a
Surface application				
	CK	6.7 a	65.7 c	10.0 b
	B2%	6.6 a	73.9 b	10.6 b
	B10%	6.3 a	90.8 a	14.3 a

Means followed by the same letters are not significantly different at the 5% level by Tukey's HSD test.

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C content was not affected by OS application. Soil C: N ratio was only affected by biochar amendment. High soil C: N ratios were observed in all biochar treatments, compared with CK treatment.

Mean concentration of soil NH_4^+ in the control treatment was 10.0 and 6.2 mg kg^{-1} of soil for the 0–5 cm and 0–10 cm soil layers, respectively, after 71 days of incubation, in experiment 1 (Fig. 7). Although biochar amendment decreased soil NH_4^+ content by 8% and 22% in B2% and B10% treatments in the 0–5 cm soil layer, respectively, the effect was significant only for B10%. On the other hand, no significant differences in soil NH_4^+ content for different rates of biochar amendment were detected for the 0–10 cm soil layer. Neither was there any significant difference in NH_4^+ content observed between OS and CK treatments for any of the soil layers under study. Conversely, a decrease in NH_4^+ content was observed for the OS treatment by 13.4% and 13.6% for the 0–5 and the 0–10 cm soil layers, respectively, in comparison to controls. Further, significant reduction in NH_4^+ content was observed in B10% under soil surface application (experiment 2).

Soil NO_3^- concentration in controls was 180 and 128 mg kg^{-1} of soil for the 0–5 and the 0–10 cm soil layers, respectively, after 71 days of incubation, in experiment 1 (Fig. 8). Although biochar amendment decreased NO_3^- contents by 8% and 16% in B2% and B10%, respectively, in the 0–5 cm soil layer, the effect was significant only in the latter case. Similarly, a significant reduction in NO_3^- content was observed for B10%, compared with CK, in the 0–10 cm soil layer.

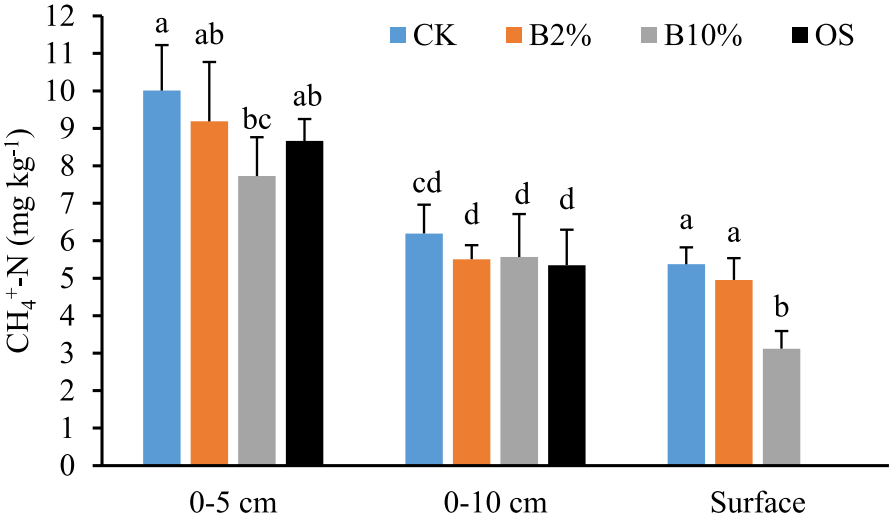

Fig. 7. Soil CH_4^+-N content at the end of incubation. Error bars indicate standard deviation. The same letter above or within bars for the 0–5 and 0–10 cm soil layers, and above the bars for surface application indicates that bar mean values are not significantly different at the 5% level by Tukey's HSD test. CK – control, OS – oyster shell, B2% – biochar 2%, B10% – biochar 10%.

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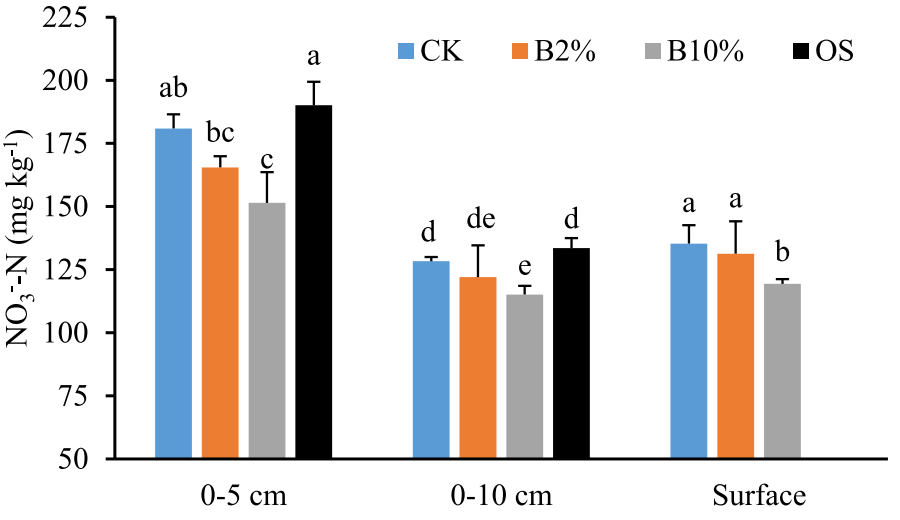

Fig. 8. Soil NO_3^--N content at the end of incubation. Error bars indicate standard deviation. The same letter above or within bars for the 0−5 and 0−10 cm soil layers, and above the bars for surface application indicates that bar mean values are not significantly different at the 5% level by Tukey's HSD test. CK − control, OS − oyster shell, B2% − biochar 2%, B10% − biochar 10%.

Soil NO_3^- content was not affected by OS application in any of the soil layers tested. Significant reduction (12%) in NO_3^- content was observed in the B10% treatment, while there was no difference in NO_3^- content between CK and B2% under soil-surface treatment application (experiment 2).

4. Discussion

4.1. N_2O emissions

Our results showed that addition of a nitrogen fertilizer, such as ammonium sulfate, caused an initial sharp increase in N_2O emissions that subsequently declined gradually towards the end of the incubation period in both experiments (Fig. 2). The short-lived increase in N_2O flux suggested that N fertilizer and decomposition of crop residues can provide a temporary abundance of C and N to microorganisms, resulting in a rapid increase in N_2O emission (Azam et al., 2002). The activity of microorganisms and substrate N decreased with C and N consumption over a certain period, with N_2O then falling to background levels (Gao et al., 2016).

In this study, the effect of biochar amendment on soil N_2O emission depended largely on biochar application rate and depth of soil layer (Fig. 4). For the 0−5 cm soil layer, B2% and B10% treatments significantly reduced N_2O emissions, compared with CK. However, only B10% showed significant reduction in N_2O emission for the 0−10 cm soil layer (experiment 1) and surface application (experiment 2).

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Surface application of 2% biochar only partially covered the soil surface; thus, applied N fertilizer may have still directly reached into the soil; thereby, favoring the observed increase in N_2O emission. At a higher biochar application rate, such as B10%, the soil was completely covered with a thin layer of biochar. Surface application of N fertilizer onto the biochar continuous layer (B10% treatment), likely reduced N_2O emission significantly, since fresh biochar does not naturally possess microbial population for nitrification and denitrification. Reduction in N_2O emission from the soil surface covered by B10% might partly be due to increased ammonia volatilization. Schomberg et al. (2012) reported that application of biochar with high pH increased ammonia volatilization, compared with the control treatment. They proposed that this was likely due to an accelerated high-pH ammonification process; thereby, increasing ammonia volatilization. Alternatively, the same authors suggested that that biochar materials can promote adsorption of ammonia.

Decreased soil N_2O emission has been observed in several studies focused on the effect of biochar on such emission, although other studies have reported no effect or even increased N_2O emission upon biochar amendment (Table 3). This indicates that the extent to which soil N_2O emission responds to biochar amendment likely depends on a complex interaction between soil type, soil pH, soil microbe population, biochar feedstock, pyrolysis temperature and biochar application rate. The results from this study clearly indicated that, with respect to the effect of different application rates of pruning waste biochar on N_2O emissions, only the higher rate of biochar application, B10%, consistently resulted in a reduction in N_2O emission. This finding might partly be explained by the reduction of the availability of soil mineral N (NH_4^+ and NO_3^-) content; whereby, the soil inorganic-N pool for N_2O production may have been reduced (Figs. 7 and 8). Reduction in soil NH_4^+ content was observed at different rates of biochar application, but the result was not consistent for all application methods tested here (Fig. 7). Singh et al. (2010) and Angst et al. (2013) have proposed that biochar might enhance soil adsorption of NH_4^+; thus, reducing N availability for microbes, with the net result of suppressed N_2O emission.

Spokas et al. (2010) reported that biochar contained microbial inhibiting compounds which could hinder or even suppress the formation of NO_3^- and N_2O. However, Nelissen et al. (2014) argued that, if nitrification or denitrification are suppressed by microbial inhibiting compounds contained in biochar, then logically higher mineral N concentrations may be expected upon biochar addition, compared to controls. Therefore, microbial inhibition could not be the cause of the reduced N_2O emission observed in this study upon biochar amendment, since we recorded a lower mineral N content, compared to control treatment. Taghizadeh-Toosi et al. (2011) observed lower NO_3^- concentration and N_2O emission after pine biochar addition (30 t ha^{-1}) in a field trial, after applying a high amount of urine. Decreased soil NO_3^- content associated with biochar treatment has been reported repeatedly (Nelissen et al.,

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Table 3. Overview of studies investigating biochar`s effect on N_2O emissions from acidic soil.

Soil type and pH	Feedstock, Pyrolysis temp. (°C)	Biochar dose and condition	Effect on N_2O emission	Reference
Gleysol, 6.3	Green waste mainly from tree pruning, slow pyrolysis 650	20 t ha^{-1} biochar, Field	Decrease	Hüppi et al. (2015)
Ferrosol, 4.6	Cattle feedlot waste, 550	10 t ha^{-1}, Field	Decrease or Increase	Scheer et al. (2011)
Silt loam, 5.5	Pine, 350	15 t ha^{-1}, Field	No effect	Taghizadeh-Toosi et al. (2011)
Silt loam, 5.5	Pine, 350	30 t ha^{-1}, Field	Decrease	Taghizadeh-Toosi et al. (2011)
Acrisols, 4.0	Rice husk and cacao shell, 400 and 500 °C	1, 2, 5 and 10%, Incubation	Decrease	Obia et al. (2015)
Luvisol, 6.28	peanut hull; 498 °C	2−14%, Incubation	Increase	Kammann et al. (2012)
Luvisol, 6.28	Wood chip, Maize, Beech, Beet root chip, bark chip, 203−800C	8%, Incubation	Decrease	Kammann et al. (2012)
Alfisol, 6.13 Vertisol, 8.8	Poultry manure, Eucalyptus, 400	0.76%, Incubation	Decrease or Increase	Singh et al. (2010)
Acid Brown Earth, 6.9	Pig manure digestate, Sitka Spruce, 600	0.8%, Incubation	No effect or Increase	Troy et al. (2013)
Albic Argicryoll, 5.68	Wheat straw, 450C	0.3%, Incubation	No effect	Cheng et al. (2012)
Alluvial soil, 8.01	Cotton stalk, 550C	5% and 10%, Incubation	Decrease	Yang et al. (2016)
Aridic Argiustoll, 8.95	oak pellets, 550 °C	10%, Incubation	No effect	Zheng et al. (2012)
Aquic Haplustoll, 6.27	oak pellets, 550 °C	10%, Incubation	No effect or Decrease	Zheng et al. (2012)
Andosol 6.2	Bamboo (500−600 °C), rice husk (500−560 °C), sawdust (800 °C)	0.5, 1 and 2%, Incubation	Decrease	Oo et al. (2018)

2014; Case et al., 2012; Kammann et al., 2012; Oo et al., 2018). Case et al. (2012) and Kammann et al. (2012) showed that lower N_2O emission after biochar addition correlated with lower mineral N availability due to abiotic or biotic N immobilization. In this study, the decrease in N_2O emission by pruning waste biochar amendment might partly be explained by the reduction in the availability of soil NO_3^--N (Fig. 8); whereby, the soil inorganic-N pool for N_2O production may have been reduced. Case et al. (2012) hypothesized that biological or physical immobilization

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of NO_3^- was greater in the 10% biochar treatment, compared to the 0% biochar treatment, removing significant amount of NO_3^- from the extractable pool that consequently, could not be utilized by soil nitrifiers or denitrifiers to produce N_2O. However, more frequent sampling of mineral N is needed in this kind of this experimentation on biochar soil amendment to unequivocally account for the effect of biochar on N cycling processes.

Biochar addition might also reduce soil denitrification via enhanced soil aeration and further increased N_2O-reductase activity due to the characteristic alkalinity of biochar (Clough et al., 2010). In this study, reduction in N_2O emission by biochar amendment might be partly due to increased soil pH (Fig. 6). Van Zwieten et al. (2010) reported that N_2O emission can be decreased relative to control treatment due to an increase in soil pH by biochar amendment. Low pH prevents the assembly of functional N_2O reductase (N_2OR) enzyme, which reduces N_2O to N_2 in the denitrification reactions (Liu et al., 2010). Increased N_2OR activity due to pH rise by biochar amendment might be one of the reasons for reduction in N_2O emission upon treatment.

In this study, the effects of B2% biochar amendment on N_2O emissions from two soil layers were inconsistent, which might be attributed to the relatively small pH increase (0.3−0.4 units) induced. Cayuela et al. (2013) also observed reduced $N_2O/(N_2O + N_2)$ ratios during N_2O peak emission in wet soils amended with brush biochar, but a direct pH effect was not clear, probably because of the small magnitude of the observed pH increase. However, decreased N_2O emission was observed when 10% biochar was used, which increased soil pH by 0.8−1.1 pH units, compared with the control, in both soil layers under study. At high pH levels, bacteria are capable to produce more N_2O-reductase, which is a key enzyme for N_2O reduction in soils (Bakken et al., 2012). Production of N_2 in favor of N_2O, following biochar amendment, was recently demonstrated using stable isotopes (Cayuela et al., 2013), although a biochar related pH change could not be the sole reason behind the induced reduction in N_2O emission, because the same effect was not replicated by addition of calcium carbonate (Cayuela et al., 2014).

In this study, N_2O emission from an orchard soil was not influenced by oyster shell application (Fig. 4). However, previous field and laboratory experiments have shown decrease, increase, or no alteration soil N_2O emission due to liming (Table 4). Zaman et al. (2007) reported that lime application decreased N_2O emissions from urea-treated soil, while no such effect was observed in a urine-treated or in untreated soils. The addition of dolomite reportedly reduced significantly N_2O emissions from acidic paddy rice soils, mainly owing to an induced increase in soil pH (Shaaban et al., 2014a) and the consequent direct effect of pH on microorganisms to reduce N_2O to N_2 (Shaaban et al., 2015). The change in soil pH in dolomite-treated soil increased N_2O-reductase activity and consequently reduced N_2O

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Table 4. Overview of studies investigating lime's effect on N_2O emissions from acidic soil.

Soil, pH	Liming material	Dose and condition	Effect on N_2O emission	Reference
Andosol, 6.2	Dolomite	3.5 g kg^{-1}, Incubation	Decrease	Oo et al. (2018)
Ultisol, 5.52	Dolomite	1, and 2 g kg^{-1}, Incubation	Decrease	Shaaban et al. (2015)
Ultisol, 5.25, 5.52	Dolomite	5, and 15 g kg^{-1}, Incubation	Decrease	Shaaban et al. (2014a)
Clay loam, 5.4 Sandy loam, 5.3	Lime ($CaCO_3$)	2.3, 5.7, 18.9 g kg^{-1}, Incubation	Increase Increase	Higgins et al. (2013)
Mollisol, 5.33	Lime (CaO)	0.4% (w:w), Incubation	Increase	Han et al. (2011)
Gleysol, 6.3	Limestone	pH adjusted application to 6.5, Field	No effect	Hüppi et al. (2015)
Udic Ustochrept, 4.7	Hydrated lime	1.1 to 5.6 g kg^{-1}, Incubation	Decrease or Increase	Clough et al. (2004)
Regosol, 4.71	Hydrated lime	4.49 and 7.30 g kg^{-1}, Incubation	Decrease	Mkhabela et al. (2006)
Orthic gleysoil, 5.6	Lime	47 and 118.5 g 3 kg^{-1}, Incubation	Increase	Zaman et al. (2007)

emission (Shaaban et al., 2015). In contrast, Baggs et al. (2010) and Higgins et al. (2013) observed that lime-treated soil produced larger N_2O emissions, when compared to un-limed soil.

The results of the present study demonstrated that, although application of oyster shell to acidic soils increased soil pH compared with CK, no significant difference in soil N_2O emissions was observed in either of the two soil layers evaluated. Contradictory reports could be due to differences in soil type, soil pH, liming materials used and application rate in each case (Table 4).

Biochar amendment increased soil C: N ratio, which might be a key parameter affecting soil N utilization. Increase in soil C: N ratio under biochar amendment suggested that soil C: N ratio also affects N_2O emission (Table 2). In our previous study, a strong negative relation between N_2O emission and soil C/N and C/IN ratios suggested that both, soil C/N and C/IN ratios influence N_2O emission (Oo et al., 2018). Ernfors et al. (2007) also reported that soil C: N ratio was negatively correlated with N_2O emission. Feng and Zhu (2017) stated that, when the soil C: N ratio increases, N demand of microbes increases above N availability and N becomes the limiting factor, relative to C, for nitrification or denitrification; thus, N_2O emission become relatively low. In this study, biochar with a high C: N ratio (66:1) may have reduced the amount of soil mineral N (Figs. 7 and 8), which in turn would have affected soil N_2O emission.

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N_2O emission varies depending largely on pyrolysis temperature (Table 3). During the pyrolysis of biomass, potentially toxic organic compounds, such as phenolic compounds (PHCs) at low temperature (Karagöz et al., 2005), polycyclic aromatic hydrocarbons (PAHs) at relatively high temperature, polychlorinated dibenzo dioxins and furans (PCDDs/Fs) (Hilber et al., 2012) are formed. During the first weeks after soil amendment, the release of these compounds might alter microbial processes and this transient release of toxic compounds after amendment has been postulated as a mechanism contributing to explain the reduction of N_2O emissions (Cayuela et al., 2014). For example, PAHs seem to be a dominant factor for the reduced N_2O emissions for the low temperature biochar (300−400BC), while BC200 contained a relatively large amount of PHCs, and markedly reduced N_2O emissions (Wang et al., 2013). However, biochar used in those studies was produced under a stable and fixed temperature with a specific type of pyrolysis method. In this study, Nashi pear biochar produced in an open burn kiln might also contain toxic compounds that would in turn influence N_2O emission from amended soil. Therefore, it is necessary to analyze the content in toxic organic compounds in pruning waste biochar produced by an open burn kiln under a wide range of pyrolysis temperatures, to better explain the observed reduction in N_2O emission from amended soils.

4.2. CO_2 emissions

An initial sharp increase in CO_2 emission after biochar treatment was followed by a gradual decrease with incubation time in both experiments (Fig. 3). This pattern of emission was due to rapid mineralization of the readily decomposable soil organic carbon (Rochette et al., 2006). Other studies have reported an immediate and short-term increase in CO_2 emission upon addition of biochar to the soil (Smith et al., 2010; Zimmerman, 2010). In this study, the biochar amendment effect on soil CO_2 emission largely depended on the method of application. Neither surface application nor mixing with the 0−5 cm soil layer had any significant effect on CO_2 emission. In our experimental soil, there was high indigenous C content (total C 88 g kg^{-1} of soil) and further addition of biochar did not affect cumulative CO_2 emission from the orchard soil used. However, a significant increase in CO_2 emission was observed when 10% biochar was applied to the 0−10 cm soil layer. Cross and Sohi (2011) argued that the effect of biochar also depends on the condition of the soil to which it was applied, i.e., addition of biochar to a soil with a high C content may not result in any additional change in CO_2 emission. Soil CO_2 emissions have been reported to increase (Smith et al., 2010; Zimmerman, 2010; Oo et al., 2018), decrease (Chintala et al., 2014; Zimmerman et al., 2011), or remain unchanged (Wang et al., 2012) after biochar amendment. Increase in soil CO_2 emission by biochar treatment was attributed to microbial decomposition of some labile components in the biochar (Smith et al., 2010) or to abiotic release of carbon (Zimmerman, 2010). Kuzyakov et al. (2009) reported that CO_2

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emissions remained unchanged in biochar amended soils due to sorption of soil nutrients and organic C onto the biochar. In this study, the significant variation in soil CO_2 emissions observed might be due to differences in soil total C and mineral N (NH_4^+ and NO_3^-) contents between the two soil layers under evaluation (Table 2 and Figs. 5, 7, and 8). In our previous study, the increase in soil CO_2 emissions recorded under biochar amendment was influenced by TC, C/IN and NO_3^--N content, since these factors are highly related with CO_2 emission (Oo et al., 2018). Fang et al. (2010) observed that soil CO_2 flux was positively related to soil NO_3^- content and that the accumulation of soil NO_3^- and NH_4^+ were consistent with increased CO_2 emission.

CO_2 emissions varied significantly between the two tested soil layers (Fig. 5). Decreased CO_2 emission from the deeper layer (0−10 cm layer) compared to the more superficial layer (0−5 cm) was related with low mineral N content and total N and C content in deeper soil layer (Table 2 and Figs. 7 and 8). Rastogi et al. (2002) and Heller et al. (2010) reported that available soil C (labile and non-labile components of soil organic matter) and N contents influence CO_2 production and emission from soils. Increasing soil N content generally leads to higher soil respiration if carbon is not limiting (Niu et al., 2010; Peng et al., 2011).

Lime is considered to improve soil conditions by increasing soil pH, whereby, microbial respiration and loss of soil organic carbon as CO_2 increase (Fuentes et al., 2006). Many studies have reported diverse effects of liming on CO_2 emissions (Valzano et al., 2001; Kemmitt et al., 2006; Shaaban et al., 2017; Oo et al., 2018). Shaaban et al. (2014b) concluded that soil pH played an important role in CO_2 emission through its influence on microbial decomposition of soil organic matter. In this study, although oyster shell application increased soil pH of both the soil layers, there was no difference in soil CO_2 emission between oyster shell and control treatments (Figs. 5 and 6). Shaaban et al. (2017) proposed that the effects of a pH increase on microbial activity and CO_2 production following liming of acidic soils vary with ecosystems and it seems that different soil conditions and ecosystems respond differently to pH alterations. Thus, our results indicate that oyster shell can be used as an alternative liming material without affecting greenhouse gas emissions.

5. Conclusion

Recycling of agricultural wastes is an important step in environmental protection and sustainable agricultural development. Conversion of pruning residues from orchards to biochar is a useful and environment-friendly *alternative* to crop *residue* and biomass *burning*. This study demonstrated the potential of pruning waste biochar return to orchard soils; although the biochar application rate required to reduce soil N_2O emission is relatively high. Surface application of biochar B10% might be a promising method to reduce both, N_2O and CO_2 emissions from orchard soils. To

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our knowledge, this is the first report on N_2O emission after surface application of biochar to an orchard soil. Further, oyster shell could be used as an alternative to liming material without affecting soil N_2O and CO_2 emissions. Our results suggest that the conversion of pruning waste residues to biochar and its application to orchard soils has the potential to mitigate soil N_2O emission. Future research must investigate the effect of pruning waste biochar and OS application on N_2O and CO_2 emissions under field conditions.

Declarations

Author contribution statement

Aung Zaw Oo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shigeto Sudo: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Khin Thuzar Win: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Akira Shibata, Takeru Gonai: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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