Effects of Biochar on Soil Biogenic Gases Activities

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Abstract

The effect of biochar amendment (2% and 10% on dry weight basis) and the biochar-soil contact period on the denitrification activity and methane oxidation in different type of soils was investigated. The result shows different soil responses to biochar amendments, that was attributed to the differences in soil properties. The N2O production was lower without supplement treatments, and patterns were different from those observed with supplement treatments. Biochar does not seem to have a strong and consistent impact on denitrification or methane oxidation. Increased soil salinity or soil pH may have negatively affected microorganisms in sandy soil. Furthermore, the results show that the influence of two soil-biochar contact periods on denitrification or methane oxidation activities was not significant.

Keywords: Biochar, Soil, Biogenic, Denitrification, N2O.

1. Introduction

Carbonaceous geosorbents such as biochar are widely used as soil additive material for increase nutrients efficiency in agriculture soils, nutrients source, improve soil properties, enhancement of contaminated soil with organic pesticides or petroleum hydrocarbons, reducing nutrients leaching from soil and mitigation of global warming by decreasing carbon dioxide, nitrous oxides and methane emission from soil. addition of biochar is greatly alter
biological activities, the composition of communities and ratio of bacteria to fungi and the predominant microorganism, due to the changes in the chemical and physical properties of the soil (Thies and Rillig, 2009). Moreover, The porous structure of biochar could provide a suitable habitat for soil microorganisms, increase their population, protect them from natural predators (Saito and Marumoto, 2002; and Warnock et al., 2007), increasing in the interaction between microorganisms, nutrients and organic substrate when these sorb on the surface area of biochar (Ortega-Calvo and Saiz-Jimenez, 1998). One of the positive indirect effects of adding biochar are reduction some of the effects of toxic compounds (Lehmann et al., 2003; and Steiner et al., 2007), and the increase in the pH of acidic soils due to the alkaline nature of biochar.

Over the last few years, the denitrification process and methane oxidation have received much attention due to their roles in global warming. To mitigate greenhouse gases emissions, the addition of biochar to soil has been suggested as a geoengineering approach (Lehmann, 2007; Karhu et al., 2011; Taghizadeh-Toosi et al., 2011; and Zhang et al., 2012a). Varied differences in the flux rates of CO₂, N₂O and CH₄ from biochar-amended soils have been reported in the literature. Some researchers found a reduction in emissions and others showed no effects or increased N₂O and CH₄ emissions. Cumulative fluxes of CH₄ from rice paddy soil were significantly decreased from 390 kg CH₄-C h⁻¹ in un amended soil to 160 390 kg CH₄-C h⁻¹ in biochar amended soil (Feng et al., 2012). In pasture soil amended with corn stalk biochar and bovine urine, N₂O flux decreased by 70% in comparison with urine only amended soil (Taghizadeh-Toosi et al., 2011). Cumulative fluxes of N₂O in urine only amended soil were 140.6 kg N₂O–N h⁻¹. This value was increased to 212.8 kg N₂O–N h⁻¹ in biochar and urine-amended soil, this increases was attributed to poor ability of biochar to sorb nitrate and nitrite (Clough et al., 2010). Some studies in the literature showed an increased emission rate for one of the greenhouse gases and decreased emissions for other gases. Zhang et al. (2012a) reported that the cumulative fluxes of N₂O was decreased from 1.99 kg N₂O–N h⁻¹ to 0.98 in Chinese paddy soil amended with biochar (40 t h⁻¹), while CH₄ emissions were increased from 69.3 to 104.9 kg CH₄-C h⁻¹, and no significant difference was found in soil respiration between soil amended with biochar and the control. The reduction in cumulative fluxes of N₂O are agreement with previous result of Yanai et al. (2007) and Zhang et al. (2012c). Karhu et al. (2011) found that the cumulative CH₄ flux from boreal agricultural soil amended with biochar at a rate of 9 t ha⁻¹ decreased by 96%, while N₂O cumulative fluxes which were decreased from 360 kg N₂O–N h⁻¹ in the control to 387 kg N₂O–N h⁻¹ in birch biochar amended soil. The effect biochar on the N₂O cumulative fluxes differences was not statistically significant (Karhu et al., 2011).

Microbial methane oxidation in soil is the main process contributing to a reduction of methane emissions to the atmosphere. The methane oxidation rate in semiarid soil was 3.9 kg CH₄-C h⁻¹. This rate was decreased to 0.11 kg CH₄-C h⁻¹ in the same soil amended with wheat straw biochar (40 t h⁻¹) (Zhang et al., 2012a). Spokas and Reicosky (2009) reported that The
methylene oxidation rate was increased from 0.1 kg CH$_4$C h$^{-1}$ in Minnesota agriculture soil to 4.1 kg CH$_4$C h$^{-1}$ after amended soil with biochar (10% w/w). Many factors affect the activity of methanotrophic microorganisms, such as soil pH (Hütsch et al., 1994), the availability of nitrate and ammonium ions (Castro et al., 1994; and Hütsch et al., 1994), and soil texture (Hütsch et al., 1994). Methanotrophic microbial communities are sensitive to the acidification condition. However, methane oxidation was observed in soil at a soil pH of about 3.2.

It is reported that there is an inverse relationship between the availability of nitrogen and methane oxidation (Steudler et al., 1989). Nitrogen fertilization reduces the methane oxidation of soil (Castro et al., 1994). Castro et al. (1994) found a reduction in methane oxidation rate of 5-20 times in fertilized soils. The extent of nitrogen effects on methane consumption depend on the chemical form of the nitrogen (Mochizuki et al., 2012). Low concentrations of nitrate can suppress methane oxidation in forest soil to a great degree (Mochizuki et al., 2012). The influence of ammonium on methane oxidation is attributed to competition between methane and ammonium at the methane-monoxygenase enzyme (Nesbit and Breitenbeck, 1992; and Castro et al., 1994) and or a transfer of the CH$_4$ oxidizing activity towards nitrification (Nesbit and Breitenbeck, 1992; and Castro et al., 1994).

It appears that factors such as biochar properties, soil type, fertilization and water management regime influence greenhouse gases fluxes (VanZwieten et al., 2009). One of the explanations for the mitigation of N$_2$O emissions from soil amended with biochar is the reduced availability of ammonium and nitrate ions via sorption (Karhu et al., 2011). Biochar increases nitrogen utilization efficiency (Karhu et al., 2011) and facilitates liming, all of which can reduce the activity of denitrifying communities and/or reduce rewetting of the soil to 73% of water-filled pore space (Yanai et al., 2007). However, Clough et al. (2010) attributed no effects of biochar on N$_2$O emission to the poor ability of this particular biochar to sorb ammonium and nitrate ions, and this biochar did not reduce the available nitrogen to denitrifying microorganisms. The reductions in CH$_4$ emissions were attributed to the sorption of dissolved organic carbon by biochar surfaces (Thies and Rillig, 2009), or to improving the soil porosity and soil aeration by biochar which could facilitate CH$_4$ oxidation in soil (VanZwieten et al., 2009) or to variable soil and biochar properties affecting N$_2$O and CH$_4$ emissions (Spokas and Reicosky, 2009). The aim of this work is to investigate the influences of biochar on N$_2$O emissions or methane oxidation by microorganisms in different soils.

2. Materials and Methods

2.1. Materials
The sandy soil was obtained from the Newcastle Law School building construction site on the Newcastle University campus in the U.K. The clayey loam soil, loamy soil and sandy loam soil was obtained from the Cockle Park farm (Newcastle University farm) in Morpeth, Northumberland. Soil was stored at 3°C in the cold room until usage. The biochar was obtained from the Faculty of Marine Resources, Alasmarya Islamic University, Libya.
produced by Environmental Power International EPI (Wiltshire, UK), from wood chips by fast pyrolysis at high temperature. The biochar particle size was < 163 μm, and the total surface area of the biochar was 928 m² g⁻¹, and the total organic carbon content was 83.9%. A bitumen activated carbon (Chemviron Carbon Ltd, Lancashire, UK) was also used in this study. It had a measured surface area of 1012 m² g⁻¹, and the total organic carbon content was 72.7±0.3%.

2.2. Methods

2.2.1. Nitrous Oxide Production Experiments

Nitrous oxide production from denitrification was examined by monitoring N₂O concentrations in 10 ml crimp-top vials (HxØ= 46x23), closed with grey butyl rubber stoppers, and capped with aluminium crimp caps (Sigma–Aldrich, Dorset, UK) containing 2 g (as dry weight) of sandy soil with and without biochar (2%, 10% as dry weight of soil), clayey loam soil with and without biochar (2%, 10% as dry weight of soil), loamy soil with and without biochar (2%, and 10% as dry weight of soil) or sandy loam soil with and without biochar (2%, and 10% as dry weight of soil). To avoid substrate limitation the following procedures described by Smith and Tiedje (1979) were used: Soils were saturated with a solution containing KNO₃ (200 μg NO₃⁻-N g⁻¹ dry soil, VWR, Leicestershire, UK), glucose (0.5 mg C g⁻¹ dry soil, VWR, Dorset, UK) and glutamic acid (0.5 mg C g⁻¹ dry soil, Sigma-Aldrich, Gillingham, UK). To simulate a more natural situation, a second set of crimp-top vials were set up in which soils were saturated with deionised water, and no substrates were added. The vial’s gas phase was exchanged by flushing with nitrogen gas (BOC, Guildford, UK), which was then replaced with a 1% acetylene in nitrogen blend (CK gases, Hampshire, UK) to inhibit N₂O reductase activity. The experiments were carried out for 14 days in duplicate. Head space gas samples (60 µl) were taken every day using a 100 µl Hamilton gastight syringe to inject samples into the GC-MS for N₂O quantification. To study the effects of the biochar-soil contact period on N₂O production, another set of experiments was set up using soils incubated 30 days at room temperature prior to the measurements. Denitrification enzyme activity (DEA) (μg N₂O h⁻¹ g⁻¹ dry soil) was then determined from the slope of the linear regression of plots of N₂O production (μg N₂O g⁻¹ dry soil) against sampling times. DEA was calculated by using three linear time points (Smith and Tiedje, 1979).

2.2.2. Methane Oxidation Experiments

Batch microcosm experiments were set up by injection of 1 ml of 10% methane standard gas (Scientific and Technical Gases, Staffordshire, UK) (to get an initial concentration of 10,000 p.p.m.v. in the headspace) in 10 ml crimp-top vials (HxØ= 46x23), closed with grey butyl rubber stoppers, and capped with aluminium crimp caps (Sigma–Aldrich, Dorset, UK) containing 2 g (as dry weight) of sandy soil with and without biochar (2%, 10% as dry weight...
of soil), clayey loam soil with and without biochar (2%, 10% as dry weight of soil), loamy soil with and without biochar (2%, and 10% as dry weight of soil) or sandy loam soil with and without biochar (2%, and 10% as dry weight of soil). The experiments were carried out for 10 days in triplicate. The head space of the vials was sampled every day with a 100 μl Hamilton gastight syringe to inject 60 μl into a GC for CH₄, CO₂ and O₂ quantification. To study the effects of the biochar-soil contact period on CH₄ oxidation, another set of experiment was set up using soils incubated 30 days at room temperature. CH₄ oxidation rates (μg CH₄ h⁻¹ g⁻¹ dry soil) were then determined from the slope of the linear regression of plots of CH₄ concentration (μg CH₄ g⁻¹ dry soil) against sampling times. CH₄ oxidation rates were calculated by using three linear time points.

2.2.3. Methane Quantification

GC-FID analysis was performed on a Carlo Erba HRGC 5160 mega series Gas Chromatography. The sample (60 μl) of headspace gas was injected with a 100 μl Hamilton gastight syringe. The separation was performed on a capillary HP-Plot-Q phase column (30 m x 0.320 mm i.d) coated with 20 μm film thickness (Agilent Technologies, Palo Alto, USA). The injection port used a split ratio of 10 and was heated to 200°C. The GC was held isothermally at 36°C with hydrogen as the carrier gas (flow rate of 30 ml min⁻¹, initial pressure 55 kPa). Instrumental quantification was calibrated using standard methane gas (Scientific and Technical Gases, Staffordshire, UK) for a five-point calibration.

2.2.4. CO₂, O₂, N₂O and SF₆ Quantification

GC-MS analysis of CO₂, O₂, N₂O and SF₆ was performed on a Fisons 8060 Gas Chromatograph linked to a Fisons MD800 MS (electron voltage 70 eV, filament current 4A, source current 800 μA, source temperature 200°C, multiplier voltage 500V, interface temperature 150°C). The sample (60 μl) was injected in split mode with a 100 μl Hamilton gastight syringe. The separation was performed on a HP-PLOT-Q capillary column (30 m x 0.32 mm i.d) packed with 20 μm Q phase (Agilent Technologies, Palo Alto, USA). The GC was held isothermally at 35°C with helium as the carrier gas (flow rate of 30 ml min⁻¹, initial pressure 65 kPa, split at 100 ml min⁻¹). The instrument was calibrated using standard CO₂, O₂, N₂O (Scientific and Technical Gases, Staffordshire, UK) and SF₆ (Sigma –Aldrich, Dorset, UK) gases for a five-point calibration.

2.3. Statistical Analysis

The data were statistically analysed using Minitab for Windows (Version 16). Significant effects of biochar amendment rate, contact time, on the denitrification rate and methane oxidation rate were evaluated through the use of ANOVA using the Fisher’s multiple-comparisons test for means (P < 0.05). Correlation between denitrification enzyme activity rates or methane oxidation rates and soil properties were analysed using SPSS for Windows (Version 19).
3. Results and Discussion

3.1. Effects of Biochar on Nitrous Oxide Production Without Nutrient Supplements

The effect of biochar on nitrous oxide production under anaerobic conditions without substrate supplements is shown in Figure (1), for sandy soil and clayey loam soil and Figure (2), for loamy and silty loam soil. High variability was observed in the $N_2O$ emitted from soils treated with different amounts of biochar. With only one-day prior contact in the case of soil amendment with biochar, $N_2O$ production in sandy soil (Figure 1a) started slowly without and with biochar addition (2%, and 10%) then rose gradually to reach a peak of 2.3±0.3, 4.5±0.4 and 9.2±0.3 $\mu g\ N_2O\ g^{-1}$ dry soil, respectively on the fourth day. The $N_2O$ production was below the detection limit in the sandy soil with or without biochar after a 30 days prior contact period, which may be attributed to the decreased available concentration of nitrate and phosphate in 30 days contact period treatments. The effect of soil biochar contact time and biochar amended rate on the $N_2O$ production was statistically significant for sandy soil $P<0.02$ and $P<0.000$, respectively (ANOVA-Fisher’s test).

The $N_2O$ productions in clayey loam soil without and with biochar (2%, and 10%) rose sharply within two days and continued gradually to reach a peak on the sixth day, and the $N_2O$ productions in clayey loam soil were higher in comparison with sandy soil for both contact periods (Figure 1b). For example, the 1-day incubation treatment produced nearly double the production of $N_2O$ in sandy soil. The difference between these two soils could be attributed to difference in availability of nitrate and phosphate and soil pH. For example, concentrations of available nitrate were 666±13 $\mu g\ NO_3^-\ g^{-1}$, 632±16 $\mu g\ NO_3^-\ g^{-1}$ and 392±13 $\mu g\ NO_3^-\ g^{-1}$ in clayey loam soil without and with biochar (2%, and 10%) respectively, while these concentration were 33±2 $\mu g\ NO_3^-\ g^{-1}$, 28±2 $\mu g\ NO_3^-\ g^{-1}$ and 24±1 $\mu g\ NO_3^-\ g^{-1}$ in sandy soil without and with biochar (2%, and 10%) respectively. Moreover, soil pH value in sandy soil without and with biochar (2%, and 10%) were 7.43±0.04, 7.83±0.03 and 7.99±0.02 respectively. The sandy soil pH was higher in comparison with the optimum soil pH for denitrification which is between 5.5 - 6.0 (Dalal et al., 2003), while in clayey loam soil with and without biochar (2%, and 10%) pH values ranged between 5.70±0.02 to 6.62±0.05.

The influence of biochar on the $N_2O$ production in loamy and sandy loam soil is illustrated in Figure (2a) and Figure (2b). From these figures, it can be seen that the concentration of $N_2O$ increased gradually. However, the effect of soil biochar contact time on the $N_2O$ production was not statistically significant for sandy loam soil $P<0.28$ (ANOVA-Fisher’s test). This result may be attributed to the differences in availability of nitrate between the two incubation periods also not being significant in sandy loam soil. The $N_2O$ production increased in loamy soil with biochar (2%, and 10) compared to loamy soil without biochar (Figure 2a). This may be attributed to the increases in soil pH. However, the increase in contact time resulted in decreases in the $N_2O$ production $P<0.011$ (ANOVA-Fisher’s test).
Figure 1. Influence of biochar amendment and contact time on nitrous oxide production in soil (\(\mu g\ N_2O/ g\) dry soil), soil & 2% biochar (■ ■ ■ ■), and soil & 10% biochar (■ ■ ■ ■ ■ ■), for one day contact (lines) and 30 days contact (broken lines), comparing (a) sandy soil and (b) clayey loam soil. Error bars: ± 1 standard deviation (SD, n=3).
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**Figure 2.** Influence of biochar amendment and contact time on nitrous oxide production in soil. (---•---), soil & 2% biochar (-----•-----), and soil & 10% biochar (-----•-----), for one day contact (lines) and 30 days contact (broken lines), comparing (a), loamy soil and (b) sandy loam soil.

Error bars: ± 1 standard deviation (SD, n=3).
3.2. Effects of Biochar on Nitrous Oxide Production with Nutrient Supplements

The influence of biochar amendments and contact period on nitrous oxide production when substrate is provided is illustrated in Figure (3), for sandy soil and clayey loam soil, and Figure (4), for loamy soil and sandy loam soil. The $N_2O$ production in soils supplied with nitrate, glucose and glutamic acid was ten times higher in comparison with those without substrate supplements. For example, the $N_2O$ concentration in clayey loam soil provided with supplements reached a peak at $230\pm15$ µg $N_2O$ g$^{-1}$, while the peak for a clayey loam soil without substrate supplements was $17.3\pm1.5$ µg $N_2O$ g$^{-1}$ dry soil. The higher $N_2O$ production in substrate supplements treatments is due to provision of soluble organic carbon (glucose and glutamic acid) and nitrate which are the main factors affecting denitrification in soil (Dalal et al., 2003). The batches with substrate supplements therefore measure potential denitrification enzyme activity when substrates are abundantly available. Adding 2% or 10% biochar to sandy soil had slight reducing effects on the $N_2O$ production in comparison with sandy soil without biochar (Figure 3a). However, after 30 days contact period, the $N_2O$ production only increased in sandy soil without or with 2% biochar. The difference in the $N_2O$ production from sandy soil with 10% biochar between two contact periods was not significant (Figure 3a). The one-day contact period results (Figure 3b) show that, the $N_2O$ produced in the clayey loam soil without biochar was higher in comparison to clayey loam soil with 2% biochar and the $N_2O$ production was the lowest in clayey loam soil with 10% biochar. Increasing incubation period from one day to 30 days contact period enhanced the $N_2O$ production in clayey loam soil with biochar (2%, and 10%), while the $N_2O$ production was reduced in clayey loam soil without biochar. These increases in the $N_2O$ production may be attributed to alkalinity effects of biochar which enhanced pH in clayey loam soil. From Figures (4a and 4b), it can be clearly seen that the differences in the $N_2O$ emitted from loamy soil without or with 2% biochar and sandy loam soil without or with 2% biochar were not significant, whereas addition of 10% biochar significantly reduced the $N_2O$ production from loamy soil and sandy loam soil. Increasing the soil biochar contact period from one day to thirty days resulted in increasing $N_2O$ production in sandy loam soil without or with biochar (2%, 10%) $P<0.000$ (ANOVA-Fisher’s test), while the effects of two contact period on the $N_2O$ production in loamy soil was not significant $P<0.502$ (ANOVA-Fisher’s test).

Denitrification rates in soils with or without biochar (2%, and 10%) and with or without supplements are presented in Table (1). Denitrification rates should correspond to the denitrification enzyme activity rates for the treatments with substrate supplementation solution. For example, these rates were $1.3\pm0.03$ and $2.2\pm0.04$ µg $N_2O$ h$^{-1}$ g$^{-1}$ dry soil for 1 and 30 days prior contact time in sandy soil respectively, while the rates were $0.01\pm0.003$ and $0.00\pm0.00$ µg $N_2O$ h$^{-1}$ g$^{-1}$ dry soil in the same soil without supplements.
Figure 3. Influence of biochar amendment and contact time on nitrous oxide production in nutrients supplemented soil (---, soil & 2% biochar (--•--•--•--•), and soil & 10% biochar (- - - - - - - -), for one day contact (lines) and 30 days contact (broken lines), comparing (a) sandy soil and (b) clayey loam soil. Error bars: ± 1 standard deviation (SD, n=3).
Figure 4. Influence of biochar amendment and contact time on nitrous oxide production in nutrients supplemented soil (—), soil & 2% biochar (—), and soil & 10% biochar (—), for one day contact (lines), and 30 days contact (broken lines), comparing (a), loamy soil and (b), sandy loam soil. Error bars: ± 1 standard deviation (SD, n=3).
Table 1. Denitrification rates (µg N₂O h⁻¹ g⁻¹ dry soil). The error range is the standard deviation of duplicated bottles.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Without supplement solution</th>
<th>With supplement solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day incubation</td>
<td>30 days incubation</td>
</tr>
<tr>
<td>Sandy soil</td>
<td>0.01 ±0.003</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Sandy soil &amp; 2% Biochar</td>
<td>0.04±0.004</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Sandy soil &amp; 10% Biochar</td>
<td>0.11±0.02</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Clayey loam soil</td>
<td>0.10±0.01</td>
<td>0.05±0.02</td>
</tr>
<tr>
<td>Clayey loam soil &amp; 2% Biochar</td>
<td>0.17±0.01</td>
<td>0.08±0.002</td>
</tr>
<tr>
<td>Clayey loam soil &amp; 10% Biochar</td>
<td>0.14±0.01</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Sandy loam soil</td>
<td>0.08±0.02</td>
<td>0.03±0.06</td>
</tr>
<tr>
<td>Sandy loam soil &amp; 2% Biochar</td>
<td>0.07±0.04</td>
<td>0.07±0.006</td>
</tr>
<tr>
<td>Sandy loam soil &amp; 10% Biochar</td>
<td>0.09±0.01</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Loamy soil</td>
<td>0.05±0.003</td>
<td>0.05±0.004</td>
</tr>
<tr>
<td>Loamy soil &amp; 2% Biochar</td>
<td>0.11±0.04</td>
<td>0.08±0.002</td>
</tr>
<tr>
<td>Loamy soil &amp; 10% Biochar</td>
<td>0.11±0.003</td>
<td>0.06±0.002</td>
</tr>
</tbody>
</table>

The denitrification enzyme activity rates in sandy soil provided with supplement solution decreased with increasing biochar application rate P<0.001 (ANOVA-Fisher’s test), but the difference between 0% and 2% was not statistically significant. That could be related to soil pH which increased from 7.48±0.4 in sandy soil to 7.99±0.02 in sandy soil with biochar (10%). Although the denitrification rates in sandy loam soil and loamy soil with biochar (10%) were lower in comparison with these soils without or with a lower amount of biochar (2%), the differences were not statistically significant P<0.12, and P<0.36 (ANOVA-Fisher’s test) for sandy loam soil and loamy soil respectively. It would seem that in supplement solution experiments, increased biochar application reduced availability of nitrate and soluble organic carbon. However, the available nitrate and soluble organic carbon in these soils were still sufficient to reach high N₂O production. The reduced N₂O production observed for the DEA with supplements solution for the highest biochar dosage was not observed without supplement solution. This indicates that it might be a “method artifact”, because biochar
adsorbs supplements, as discussed here, but this biochar effect may not be so relevant in real soils.

3.3. Effects of Biochar on Methane Oxidation

Result for the methane oxidation from sandy soil, clayey loam soil, silty loam soil and loamy soil without or with biochar (2%, and 10%) are shown in Figures (5 & 6) and the methane oxidation rates and lag phase period are presented in Table (2). The methane concentration decreased sharply after the lag phase period. For example, in clayey loam soil, the methane concentrations were stable in the first 190 hrs of lag phase for clayey loamy soil without biochar and 120 hrs of lag phase for clayey loamy soil with biochar (2%, and 10%) in a 1-day prior contact period experiment. In the experiments following a 1-day contact period, the methane oxidation rates were 0.01±0.003, 0.15±0.001 and 0.15±0.003 μg CH₄ h⁻¹ g⁻¹ dry soil in clayey loam soil without and with biochar (2%, and 10%) respectively, whereas these rates were 0.036±0.01, 0.12±0.004 and 0.12±0.002 μg CH₄ h⁻¹ g⁻¹ dry soil in clayey loam soil without and with biochar (2%, and 10%) following a 30 days contact period. Moreover, although the difference in the CH₄ oxidation rates, between the two contact periods were not statistically significant (P<0.397, ANOVA-Fisher’s test), the lag phase period was increased in clayey loamy soil without biochar (Table 2) while these lag phase periods decreased in clayey loamy soil with biochar (2%, and 10%).

The influence of biochar amendments on methane oxidation may also depend on soil properties. For example, the methane oxidation rates in sandy loam soil were 0.02±0.006, 0.025±0.008 and 0.015±0.002 μg CH₄ h⁻¹ g⁻¹ dry soil in sandy loam soil without or with biochar (2%, and 10%) respectively and there was no statistically significant difference (P<0.842 ANOVA-Fisher’s test). 10% biochar added to sandy soil and sandy loam soil did not affect the methane oxidation rate (Figures 5a, and 6b). However, the methane oxidation rate in sandy soil and loamy soil with biochar (10%) was lower in comparison with soil without and with biochar (2%). Adding biochar (2%) to loamy soil did have effects on the CH₄ concentration. The lag phase period before methane oxidation was decreased in loamy soil without or with biochar (2%) with increased contact period from 1day to 30 days. Moreover, the lag phase period was increased in loamy soil with biochar (10%) from 190 hrs to 300 when contact period increased from a 1day to 30 days.
Figure 5. Influence of biochar amendment and contact time on methane oxidation in soil

(—, ●—•), soil & 2% biochar (□—☆—☆), and soil & 10% biochar (△—☆—☆), for one day contact (lines), and 30 days contact (broken lines), comparing (a) sandy soil and (b) clayey loam soil. Error bars: ± 1 standard deviation (SD, n=3).
Figure 6. Influence of biochar amendment and contact time on methane oxidation in soil (---), soil & 2% biochar (-----), and soil & 10% biochar (-----), for one day contact (lines), and 30 days contact (broken lines), comparing (a) loamy soil and (b) sandy loam soil. Error bars: ± 1 standard deviation (SD, n=3).
Table 2. The methane oxidation rates ($\mu g \, CH_4 \, h^{-1} \, g^{-1}$ dry soil) and the lag phase periods before methane oxidation. The error range is the standard deviation of duplicated bottles.

<table>
<thead>
<tr>
<th>Soil</th>
<th>The methane oxidation Rates</th>
<th>The lag phase period (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>30 days</td>
</tr>
<tr>
<td>Sandy Soil</td>
<td>0.11±0.005</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td>Sandy Soil &amp; 2% Biochar</td>
<td>0.12±0.004</td>
<td>0.15±0.008</td>
</tr>
<tr>
<td>Sandy Soil &amp; 10% Biochar</td>
<td>0.02±0.004</td>
<td>0.018±0.01</td>
</tr>
<tr>
<td>Clayey loam soil</td>
<td>0.10±0.003</td>
<td>0.036±0.01</td>
</tr>
<tr>
<td>Clayey loam soil &amp; 2% Biochar</td>
<td>0.15±0.001</td>
<td>0.12±0.004</td>
</tr>
<tr>
<td>Clayey loam soil &amp; 10% Biochar</td>
<td>0.15±0.003</td>
<td>0.12±0.002</td>
</tr>
<tr>
<td>Loamy soil</td>
<td>0.11±0.004</td>
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</tr>
<tr>
<td>Loamy soil &amp; 2% Biochar</td>
<td>0.15±0.003</td>
<td>0.11±0.008</td>
</tr>
<tr>
<td>Loamy soil &amp; 10% Biochar</td>
<td>0.02±0.004</td>
<td>0.041±0.01</td>
</tr>
<tr>
<td>Sandy loam soil</td>
<td>0.02±0.006</td>
<td>0.09±0.03</td>
</tr>
<tr>
<td>Sandy loam soil &amp; 2% Biochar</td>
<td>0.025±0.008</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>Sandy loam soil &amp; 10% Biochar</td>
<td>0.015±0.002</td>
<td>0.009±0.009</td>
</tr>
</tbody>
</table>

Correlations between the denitrification enzyme activity rate or the methane oxidation rate and soil pH, electrical conductivity, availability of nitrate, ammonium and phosphate are illustrated in Table (3). Without regards to type of soil, all soil properties have significant effects on the denitrification enzyme activity. The availability of phosphate and soil electrical conductivity have moderate negative effects on the methane oxidation rate. Correlation coefficients between soil properties and denitrification enzyme activity rate or the methane oxidation rate were different in different soil types. In sandy soil, correlation coefficients between available ammonium and soil electrical conductivity were significantly positive on denitrification enzyme activity rate, whereas in clayey soil and loamy soil only available ammonium has significant positive correlation coefficients, and in sandy loam soil the soil pH and soil electrical conductivity have significant positive effects on denitrification enzyme activity rate (Table 3). From Table 3, it can see that the significant negative correlation coefficient indicate that the methane oxidation rate was sensitive to increase in the soil pH and soil electrical conductivity in sandy soil, sandy loam soil and loamy soil. However, the soil pH and soil electrical conductivity in clayey soil seemingly facilitated the methane oxidation, although this may actually be due to better soil aeration following biochar amendment, while available nitrate and phosphate inhibited the methane oxidation. These
results show that variable soil properties may be the main effects on the biogenic gases activities and biochar amendment effects must be interpreted in the context of biochar impacts on these variable soil properties.

This study has shown that biogenic gases activities depended on soil properties and biochar application rate. Some of study findings are consistent with those of (Feng et al., 2012; Taghizadeh-Toosi et al., 2011; Zhang et al., 2012a; and Karhu et al., 2011) who found reduction in biogenic gases activities or the reduction is not significant. However, the other study findings do not support the previous research. Which could explain the different responses to biochar. However, high biochar application rate (10%) reduced biogenic gases activities except in clayey loam soil amended with biochar (10%) where methane oxidation was increased. Biochar properties, soil properties, fertilization and water management regime influence greenhouse gases fluxes (VanZwieten et al., 2009). The reduction in $N_2O$ emissions from soil amended with biochar could be explained by reduction of availability of ammonium and nitrate ions via sorption (Karhu et al., 2011), and increases nitrogen utilization efficiency (Karhu et al., 2011; and Zhang et al., 2012b) and liming. The reductions in $CH_4$ emissions in biochar amended soil (10%) were attributed to the sorption of dissolved organic carbon by biochar surfaces (Thies and Rillig, 2009; and Knoblauch et al., 2011)

4. Conclusion

This work investigated the impact of biochar (2%, and 10% on dry weight basis) on denitrification activity and methane oxidation in different types of soils. Soil properties have the most significant influence on the $N_2O$ production and methane oxidation and the differences in soil properties could explain different soil responses to biochar amendments (Spokas and Reicosky, 2009; and VanZwieten et al., 2009). Furthermore, the $N_2O$ production was lower without supplement treatments, and patterns were different from those observed with supplement treatments. However, contrary to other reports (Spokas and Reicosky, 2009; Taghizadeh-Toosi et al., 2011; and Zhang et al., 2012a), biochar does not seem to have a strong and consistent impact on denitrification. Increased soil salinity or soil pH may have negatively affected microorganisms in sandy soil. Furthermore, since there were no differences between two soil-biochar contact periods, the results show that this variable did not influence methane oxidation rates.
Table 3. Correlation coefficients and P value of relationships between soils properties and the denitrification rates or the methane oxidation rate.

<table>
<thead>
<tr>
<th></th>
<th>Global</th>
<th>Sandy soil</th>
<th>Clayey soil</th>
<th>Sandy loam soil</th>
<th>Leamy soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Denitrification rate</td>
<td>CH oxidation rate</td>
<td>Denitrification rate</td>
<td>CH oxidation rate</td>
<td>Denitrification rate</td>
</tr>
<tr>
<td>Available Nitrate</td>
<td>Correlation P value</td>
<td>0.38** (0.001)</td>
<td>-0.24 (0.051)</td>
<td>0.25 (0.3)</td>
<td>0.26 (0.3)</td>
</tr>
<tr>
<td>Available ammonium</td>
<td>Correlation P value</td>
<td>0.65** (0.000)</td>
<td>-0.081 (0.5)</td>
<td>0.96** (0.000)</td>
<td>-0.47 (0.048)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Correlation P value</td>
<td>0.34** (0.004)</td>
<td>-0.56** (0.002)</td>
<td>0.08 (0.8)</td>
<td>0.08 (0.78)</td>
</tr>
<tr>
<td>pH</td>
<td>Correlation P value</td>
<td>-0.37** (0.002)</td>
<td>0.13 (0.3)</td>
<td>0.45 (0.06)</td>
<td>-0.65** (0.003)</td>
</tr>
<tr>
<td>EC</td>
<td>Correlation P value</td>
<td>0.59* (0.000)</td>
<td>-0.24* (0.04)</td>
<td>0.51* (0.03)</td>
<td>-0.89** (0.000)</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.
References


