JOURNAL OF MARINE SCIENCES & ENVIRONMENTAL TECHNOLOGIES
Vol. 1, Issue No. 2 (December-2015)

ISSN: 2413-5267

Effects of Biochar on Soil Biogenic Gases Activities

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تأثير إضافة الفحم النباتي على فاعليات الغازات الحيوية في التربة

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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 N_2O 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, N_2O.*

الملخص

تأثير إضافة الفحم النباتي (بمعدلات إضافة 2% و10% على أساس الوزن الجاف) وتأثير زمن التلامس بين التربة–الفحم النباتي على إنتاج غاز أكسيد النيتروز (نشاط عكس النترجة) وأكسدة غاز الميثان تمت دراسته في أنواع مختلفة من الترب. الدراسة أظهرت اختلافات في مقدار الاستجابة لإضافة الفحم النباتي، والتي نسبت إلى اختلاف في خواص الترب المستخدمة. معدل إنتاج غاز أكسيد النيتروز كان أقل في الترب الغير المزدة بالمواد المغذية والداعمة، بالإضافة إلى ذلك، نمط إنتاج غاز أكسيد النيتروز كان مختلفا عن الترب المزدة بالمواد المغذية والداعمة، ولا يبدو هناك أي تأثير قوى أو دائم للفحم النشط على إنتاج غاز أكسيد النيتروز وأكسدة غاز الميثان. زيادة الأس الهيدروجيني وملوحة التربة بسبب إضافة الفحم النباتي يمكن أن يكون له تأثيرات سلبية على نشاط الأحياء الدقيقة في التربة الرملية. بالإضافة إلى ذلك، أن تأثير زمني التلامس بين التربة–الفحم النباتي على إنتاج غاز أكسيد النيتروز وأكسدة غاز الميثان غير معنوي إحصائيا.

الكلمات الدلالية: الفحم النباتي، التربة، حيوية، نزع النترات، أكسيد النيتروز.

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

ISSN: 2413-5267

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 CO_2, N_2O and CH_4 from biochar-amended soils have been reported in the literature. Some researchers found a reduction in emissions and others showed no effects or increased N_2O and CH_4 emissions. Cumulative fluxes of CH_4 from rice paddy soil were significantly decreased from 390 *kg* CH_4-*C* h^{-1} in un amended soil to 160 390 *kg* CH_4-*C* h^{-1} in biochar amended soil (Feng *et al.*, 2012). In pasture soil amended with corn stalk biochar and bovine urine, N_2O flux decreased by 70% in comparison with urine only amended soil (Taghizadeh-Toosi *et al.*, 2011). Cumulative fluxes of N_2O in urine only amended soil were 140.6 *kg* N_2O–*N* h^{-1}. This value was increased to 212.8 *kg* N_2O–*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 N_2O was decreased from 1.99 *kg* N_2O–*N* h^{-1} to 0.98 in Chinese paddy soil amended with biochar (40 *t* h^{-1}), while CH_4 emissions were increased from 69.3 to 104.9 *kg* CH_4-*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 N_2O are agreement with previous result of Yanai *et al.* (2007) and Zhang *et al.* (2012c). Karhu *et al.* (2011) found that the cumulative CH_4 flux from boreal agricultural soil amended with biochar at a rate of 9 *t* ha^{-1} decreased by 96%, while N_2O cumulative fluxes which were decreased from 360 *kg* N_2O–*N* h^{-1} in the control to 387 *kg* N_2O–*N* h^{-1} in birch biochar amended soil. The effect biochar on the N_2O 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* CH_4-*C* h^{-1}. This rate was decreased to 0.11 *kg* CH_4-*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

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methane 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-monooxygenase 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_2O 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_2O 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 (Van Zwieten *et al.*, 2009) or to variable soil and biochar properties affecting N_2O and CH_4 emissions (Spokas and Reicosky, 2009). The aim of this work is to investigate the influences of biochar on N_2O 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

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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 $m^2\ g^{-1}$, 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 $m^2\ g^{-1}$, The bitumen activated carbon was ground to a particle size < 163 μm., and the total organic carbon content was 72.7±0.3%. chemical properties of soils, nutrients contents and Longmuir sorption constants were published in Bushnaf (2015).

2.2. Methods

2.2.1. Nitrous Oxide Production Experiments

Nitrous oxide production from denitrification was examined by monitoring N_2O concentrations in 10 *ml* crimp-top vials (H×Ø= 46×23), 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 KNO_3 (200 $\mu g\ NO_3^-\text{-}N\ g^{-1}$ 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 N_2O 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 N_2O quantification. To study the effects of the biochar-soil contact period on N_2O production, another set of experiments was set up using soils incubated 30 *days* at room temperature prior to the measurements. Denitrification enzyme activity (DEA) ($\mu g\ N_2O\ h^{-1}\ g^{-1}$ dry soil) was then determined from the slope of the linear regression of plots of N_2O production ($\mu g\ N_2O\ g^{-1}$ 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.mv. in the headspace) in 10 *ml* crimp-top vials (H×Ø= 46×23), 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

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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 CH_4, CO_2 and O_2 quantification. To study the effects of the biochar-soil contact period on CH_4 oxidation, another set of experiment was set up using soils incubated 30 *days* at room temperature. CH_4 oxidation rates ($\mu g\ CH_4\ h^{-1}\ g^{-1}$ dry soil) were then determined from the slope of the linear regression of plots of CH_4 concentration ($\mu g\ CH_4\ g^{-1}$ dry soil) against sampling times. CH_4 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^oC. 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. CO_2, O_2, N_2O and SF_6 Quantification

GC-MS analysis of CO_2, O_2, N_2O and SF_6 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 500*V*, 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 CO_2, O_2, N_2O (Scientific and Technical Gases, Staffordshire, UK) and SF_6 (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).

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3. Results and Discussion

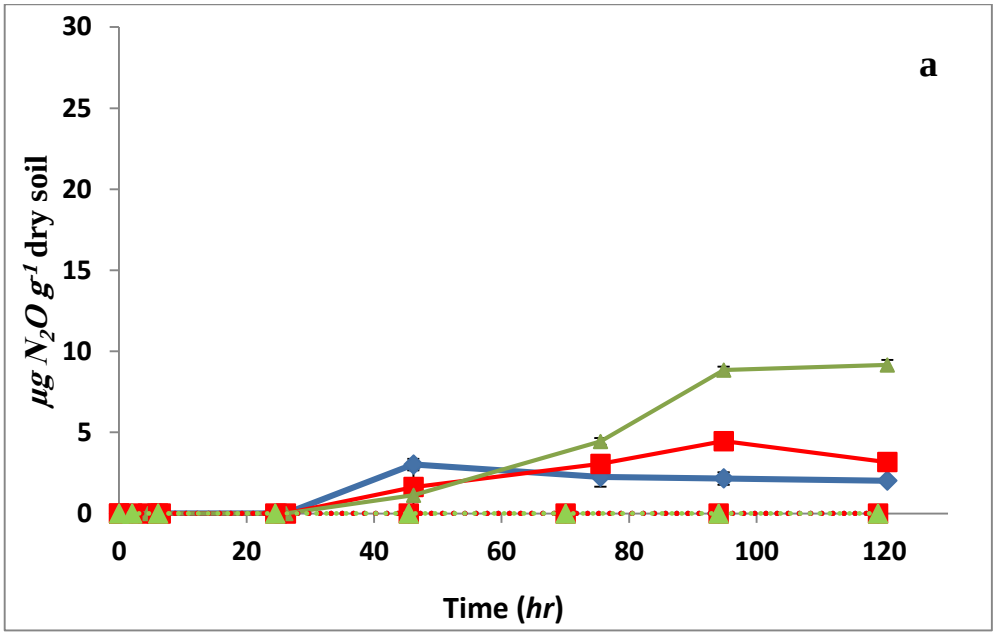
3.1. Effects of Biochar on Nitrous Oxide Production Without Nutrient Supplements

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).

ISSN: 2413-5267



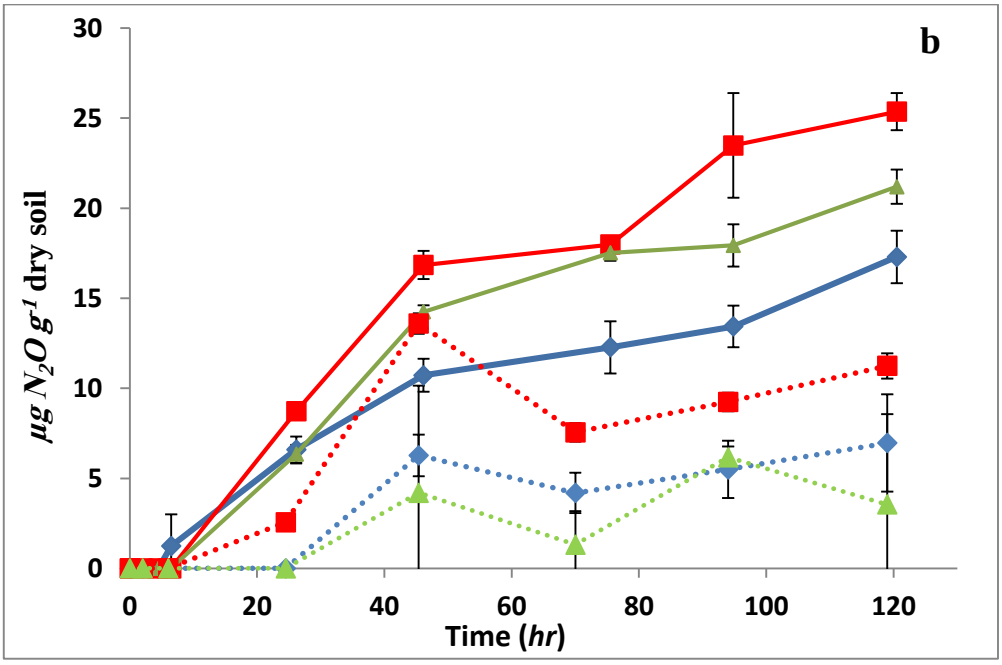


Figure 1. Influence of biochar amendment and contact time on nitrous oxide production in soil (,), soil & 2% biochar (,), and soil & 10% biochar (,), for one day contact (lines) and 30 *days* contact (broken lines), comparing (**a**) sandy soil and (**b**) clayey loam soil. Error bars: ± 1 standard deviation (SD, n=3).

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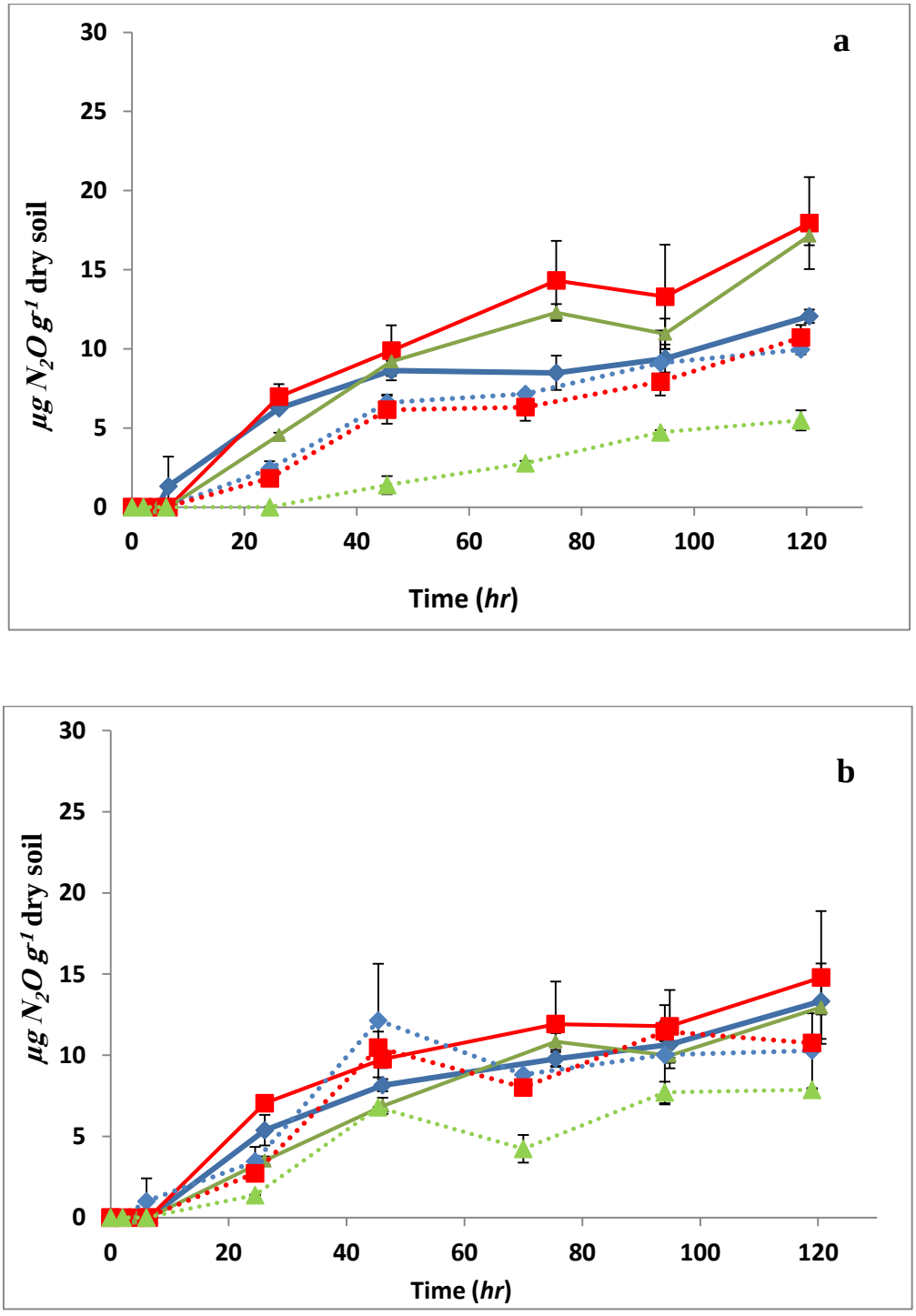

Figure 2. Influence of biochar amendment and contact time on nitrous oxide production in soil (,), soil & 2% biochar (,), and soil & 10% biochar (,), for one day contact (lines) and 30 *days* contact (broken lines), comparing (**a**), loamy soil and (**b**) sandy loam soil. Error bars: ± 1 standard deviation (SD, *n*=3).

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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±15 $\mu g\ N_2O\ g^{-1}$, while the peak for a clayey loam soil without substrate supplements was 17.3±1.5 $\mu g\ N_2O\ g^{-1}$ 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 3.a). 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±0.03 and 2.2±0.04 $\mu g\ N_2O\ h^{-1}\ g^{-1}$ dry soil for 1 and 30 *days* prior contact time in sandy soil respectively, while the rates were 0.01±0.003 and 0.00±0.00 $\mu g\ N_2O\ h^{-1}\ g^{-1}$ dry soil in the same soil without supplements.

ISSN: 2413-5267

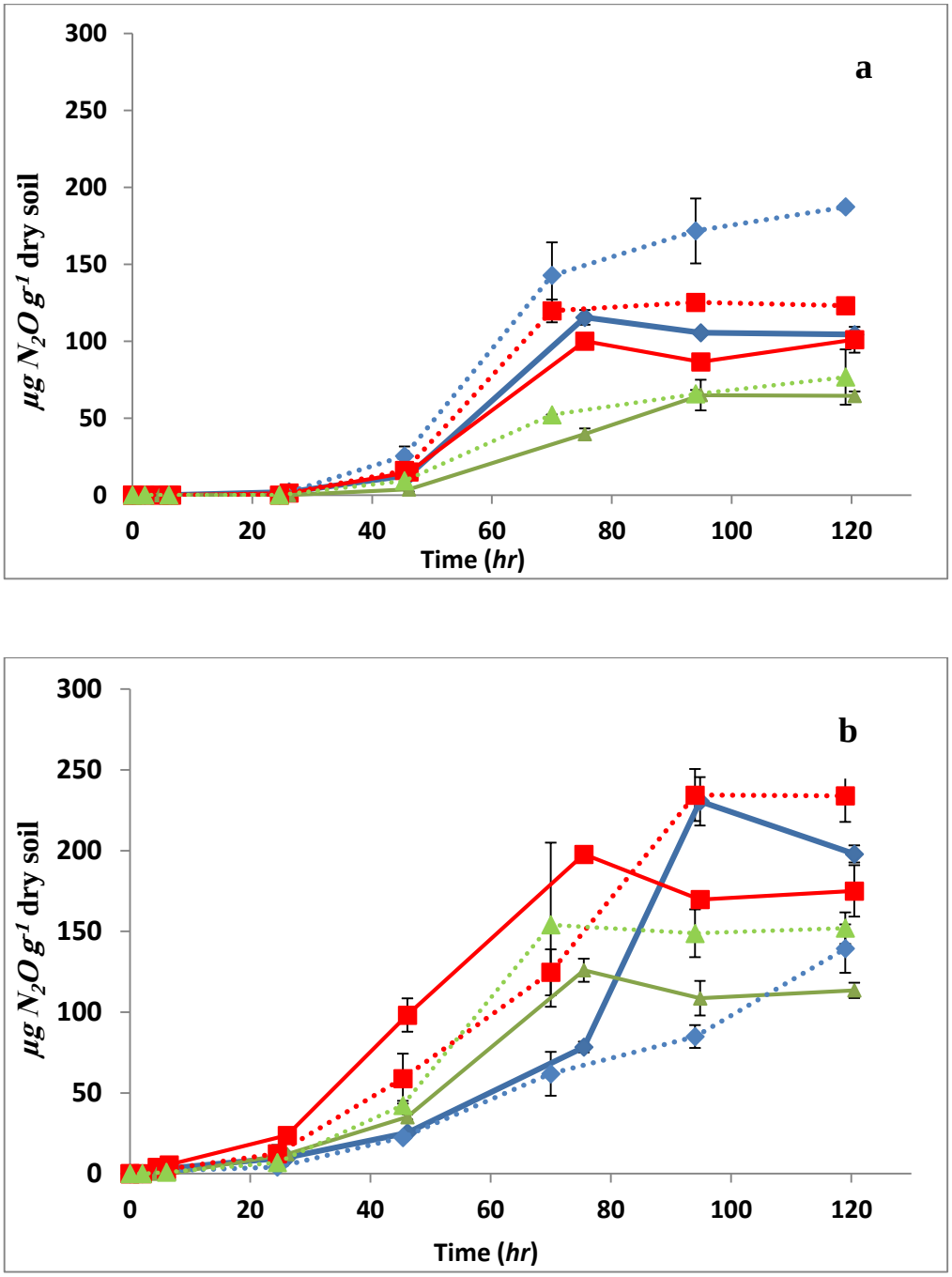

Figure 3. Influence of biochar amendment and contact time on nitrous oxide production in nutrients supplemented soil (—◆—, ···◆···), soil & 2% biochar (—■—, ···■···), and soil & 10% biochar (—▲—, ···▲···), for one day contact (lines) and 30 *days* contact (broken lines), comparing (**a**) sandy soil and (**b**) clayey loam soil. Error bars: ± 1 standard deviation (SD, n=3).

ISSN: 2413-5267

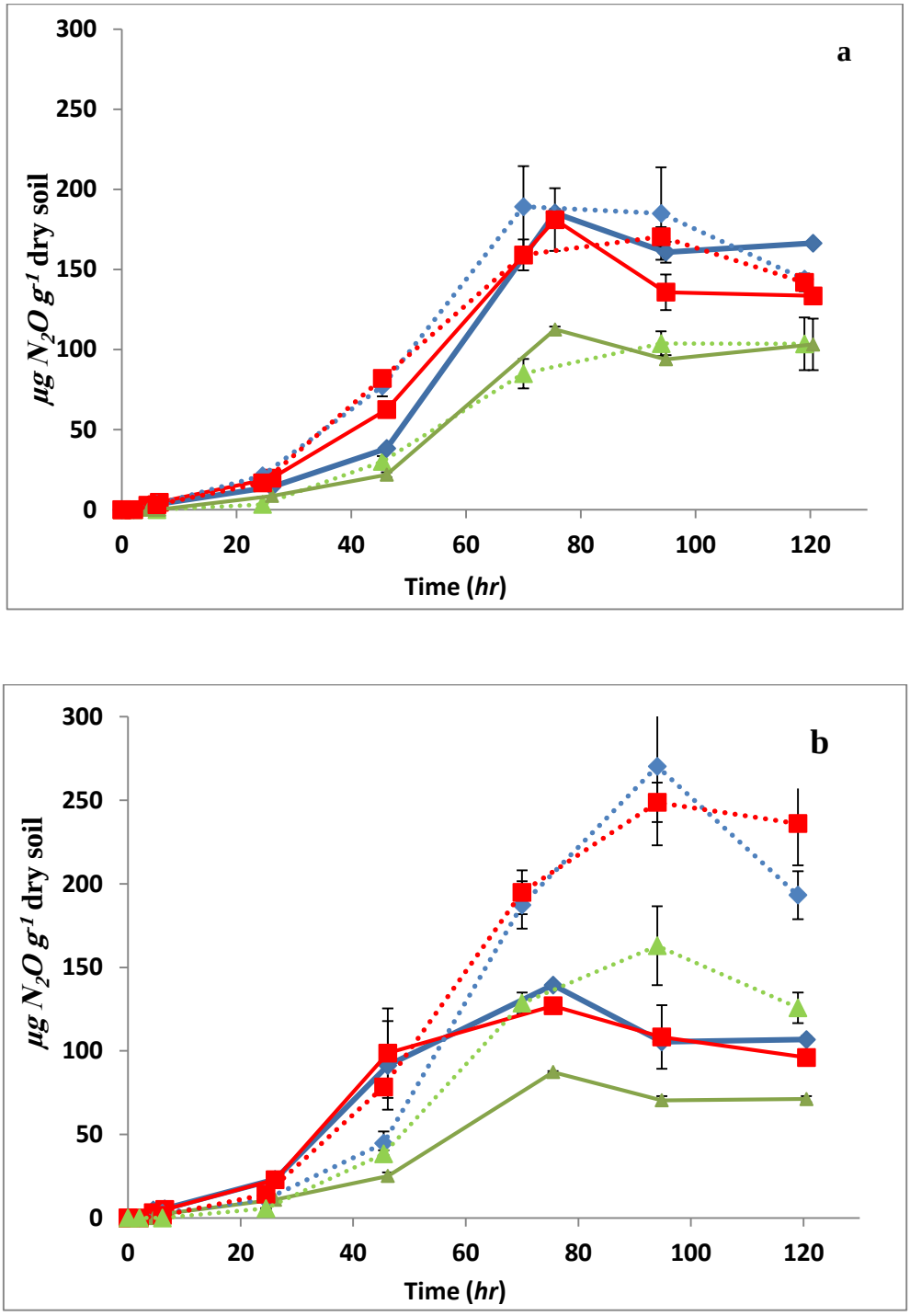

Figure 4. Influence of biochar amendment and contact time on nitrous oxide production in nutrients supplemented soil (—◆—, ···◆···), soil & 2% biochar (—■—, ···■···), and soil & 10% biochar (—▲—, ···▲···), for one day contact (lines), and 30 days contact (broken lines), comparing (**a**), loamy soil and (**b**), sandy loam soil. Error bars: ± 1 standard deviation (SD, n=3).

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Table 1. Denitrification rates ($\mu g\ N_2O\ h^{-1}\ g^{-1}$ dry soil). The error range is the standard deviation of duplicated bottles.

Soil	Without supplement solution		With supplement solution	
	1 day incubation	30 days incubation	1 day incubation	30 days incubation
Sandy soil	0.01 ±0.003	0.00±0.00	1.3±0.03	2.2±0.04
Sandy soil & 2% Biochar	0.04±0.004	0.00±0.00	1.2±0.05	1.5±0.02
Sandy soil & 10% Biochar	0.11±0.02	0.00±0.00	0.8±0.06	0.9±0.15
Clayey loam soil	0.10±0.01	0.05±0.02	2..4±0.2	1.2±0.2
Clayey loam soil & 2% Biochar	0.17±0.01	0.08±0.002	1.6±0.05	2.4±0.02
Clayey loam soil &10 % Biochar	0.14±0.01	0.05±0.00	1.2±0.07	1.7±0.12
Sandy loam soil	0.08±0.02	0.03±0.06		1.8±0.7
Sandy loam soil & 2% Biochar	0.07±0.04	0.07±0.006	0.7±0.2	2.7±0.2
Sandy loam soil & 10% Biochar	0.09±0.01	0.07±0.01	0.7±0.00	1.5±0.2
Loamy soil	0.05±0.003	0.05±0.004	1.8±0.00	1.5±0.2
Loamy soil & 2% Biochar	0.11±0.04	0.08±0.002	1.3±0.03	1.4±0.01
Loamy soil & 10% Biochar	0.11±0.003	0.06±0.002	1.1±0.1	1.2±0.1

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 to7.99±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 N_2O production. The reduced N_2O 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

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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)**Figure 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 $\mu g\ CH_4\ h^{-1}\ g^{-1}$ 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 $\mu g\ CH_4\ h^{-1}\ g^{-1}$ 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 CH_4 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 differences in lag phase period could be attributed to competition between methane and ammonium at the methane-monooxygenase (Nesbit and Breitenbeck, 1992; Castro *et al.*, 1994; and Sitaula *et al.*, 1995) and a transfer of the CH_4 oxidising activity towards nitrification (Nesbit and Breitenbeck, 1992; Castro *et al.*, 1994; and Sitaula *et al.*, 1995), because, although the ammonium concentration in clayey soil was decreased after a 30 *days* contact period, this concentrations were probably still high enough to inhibit methane oxidation in clayey loamy soil without biochar, while in clayey loamy soil without biochar could be regulated the ammonium.

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 $\mu g\ CH_4\ h^{-1}\ g^{-1}$ dry soil in sandy loam 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 CH_4 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.

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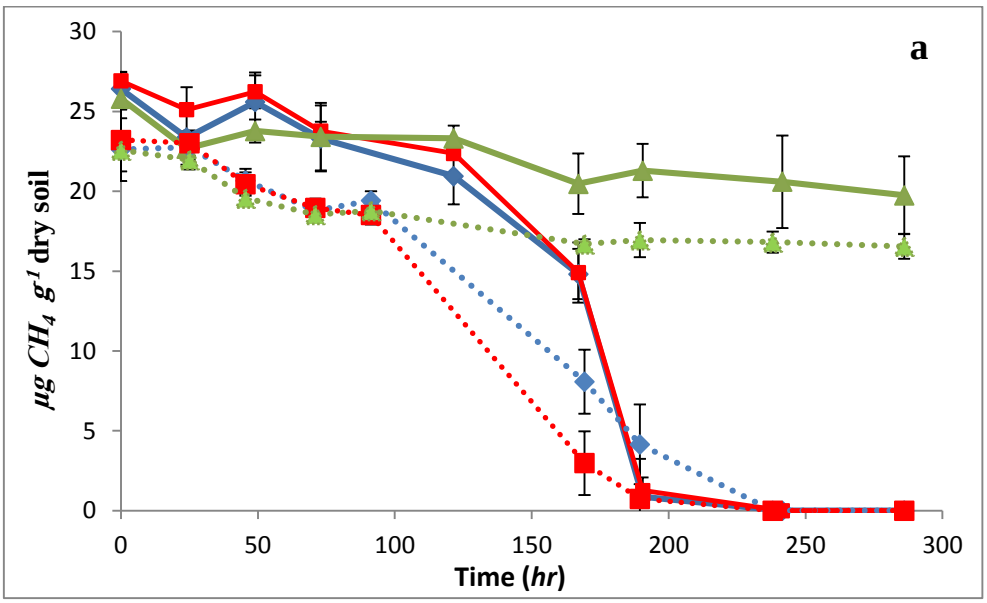

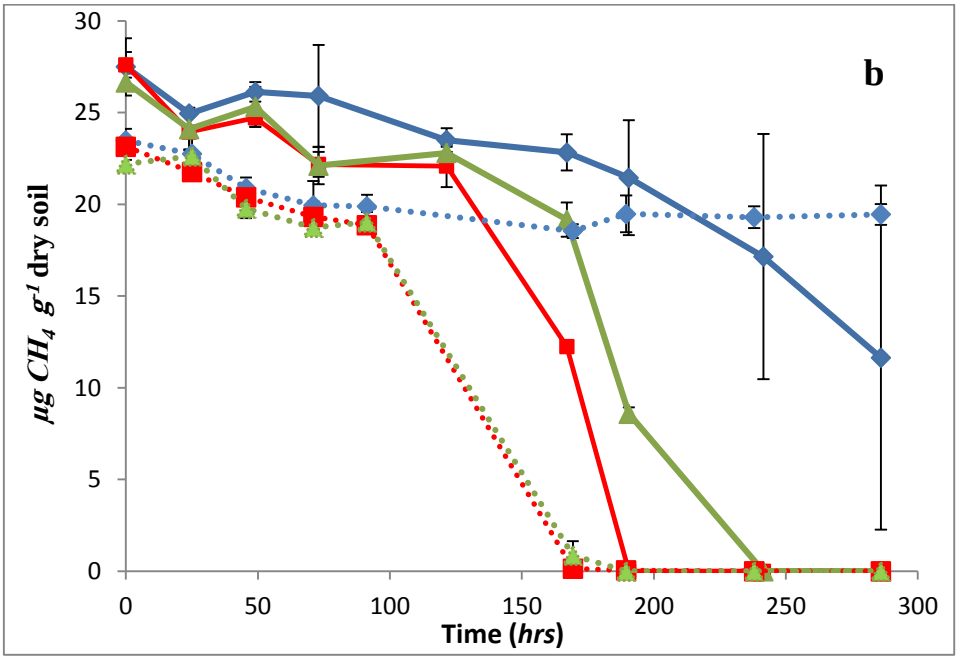

Figure 5. Influence of biochar amendment and contact time on methane oxidation in soil (,), soil & 2% biochar (,), and soil & 10% biochar (,), for one day contact (lines), and 30 days contact (broken lines), comparing (**a**) sandy soil and (**b**) clayey loam soil. Error bars: ± 1 standard deviation (SD, n=3).

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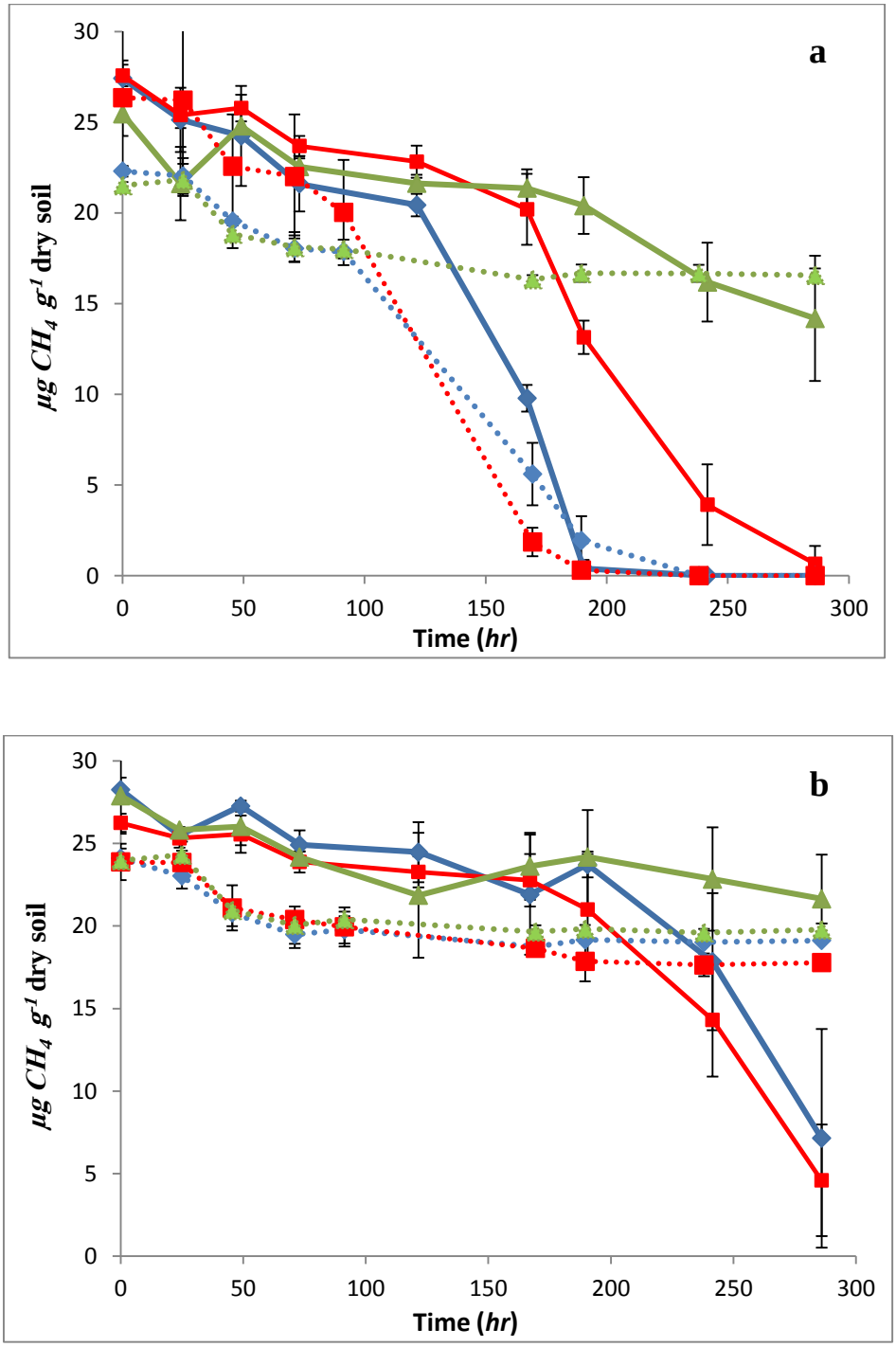

Figure 6. Influence of biochar amendment and contact time on methane oxidation in soil (◆,◆), soil & 2% biochar (■,■), and soil & 10% biochar (▲,▲), for one day contact (lines), and 30 days contact (broken lines), comparing (**a**) loamy soil and (**b**) sandy loam soil. Error bars: ± 1 standard deviation (SD, n=3).

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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

Soil	The methane oxidation Rates		The lag phase period (*hrs*)	
	1 day	30 days	1 day	30 days
Sandy Soil	0.11±0.005	0.15±0.01	120	95
Sandy Soil & 2% Biochar	0.12±0.004	0.15±0.008	120	95
Sandy Soil & 10% Biochar	0.02±0.004	0.018±0.01	300	300
Clayey loam soil	0.010±003	0.036±0.01	190	300
Clayey loam soil & 2% Biochar	0.15±0.001	0.12±0.004	120	95
Clayey loam soil &10 % Biochar	0.15±0.003	0.12±0.002	120	95
Loamy soil	0.11±0.004	0.12±0.01	170	90
Loamy soil & 2% Biochar	0.15±0.003	0.11±0.008	170	70
Loamy soil & 10% Biochar	0.02±0.004	0.041±0.01	190	300
Sandy loam soil	0.02±0.006	0.09±0.03	190	300
Sandy loam soil & 2% Biochar	0.025±0.008	0.08±0.01	190	300
Sandy loam soil & 10% Biochar	0.015±0.002	0.009±0.009	300	300

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

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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.

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Table 3. Correlation coefficients and P value of relationships between soils properties and the denitrification rates or the methane oxidation rate.

		Global		Sandy soil		Clayey soil		Sandy loam soil		Loamy soil	
		Denitrification rate	CH_4 oxidation rate	Denitrification rate	CH_4 oxidation rate	Denitrification rate	CH_4 oxidation rate	Denitrification rate	CH_4 oxidation rate	Denitrification rate	CH_4 oxidation rate
Available Nitrate	Correlation	0.38**	-0.24	0.25	0.26	0.17	-0.51*	-0.46	0.8**	0.03	0.40
	P value	(0.001)	(0.051)	(0.3)	(0.3)	(0.5)	(0.03)	(0.06)	(0.000)	(0.9)	(0.1)
Available ammonium	Correlation	0.68**	-0.081	0.96**	-0.47*	0.65*	0.50*	0.52*	-0.16	0.80**	-0.42
	P value	(0.000)	(0.5)	(0.000)	(0.048)	(0.006)	(0.04)	(0.03)	(0.55)	(0.04)	(0.084)
Phosphate	Correlation	0.34**	-0.36**	0.08	0.08	-0.14	-0.79**	0.28	0.5*	0.15	-0.058
	P value	(0.004)	(0.002)	(0.8)	(0.78)	(0.6)	(0.000)	(0.26)	(0.035)	(0.55)	(0.82)
pH	Correlation	-0.37**	0.13	0.45	-0.65**	0.16	0.85**	0.5*	-0.75**	0.3	-0.51*
	P value	(0.002)	(0.3)	(0.06)	(0.003)	(0.5)	(0.000)	(0.04)	(0.000)	(0.22)	(0.032)
EC	Correlation	0.50*	-0.24*	0.51*	-0.89**	0.17	0.86**	0.53*	-0.64**	0.29	-0.44*
	P value	(0.000)	(0.04)	(0.03)	(0.000)	(0.5)	(0.000)	(0.03)	(0. 004)	(0.24)	(0.07)

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

ISSN: 2413-5267

References

Bushnaf K.M. (2015). Effect of biochar or activated carbon on soil nutrients and its availability. *Proceedings of the Second Conference of Environmental sciences, 15-17 December,* Zliten, Libya.

Castro M.S., Peterjohn W.T., Melillo J.M., Steudler P.A., Gholz H.L., and Lewis D. (1994). Effects of Nitrogen-Fertilization on the Fluxes of N_2O, CH_4, and CO_2 From Soils in A Florida Slash Pine Plantation. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, 24(1): 9-13.

Clough T.J., Bertram J.E., Ray J.L., Condron L.M., O'Callaghan M., Sherlock R.R., and Wells N.S. (2010). Unweathered Wood Biochar Impact on Nitrous Oxide Emissions from a Bovine-Urine-Amended Pasture Soil. *Soil Sci. Soc. Am. J.*, 74(3): 852-860.

Dalal R.C., Wang W.J., Robertson G.P., and Parton W.J. (2003). Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. *Australian Journal of Soil Research*, 41(2): 165-195.

Feng Y., Xu Y., Yu Y., Xie Z., and Lin X. (2012). Mechanisms of biochar decreasing methane emission from Chinese paddy soils. *Soil Biology and Biochemistry*, 46: 80-88.

Hütsch B.W., Webster C.P., and Powlson D.S. (1994). Methane oxidation in soil as affected by land use, soil pH and *N* fertilization. *Soil Biology and Biochemistry*, 26(12): 1613-1622.

Karhu K., Mattila T., Bergström I., and Regina K. (2011). Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity–Results from a short-term pilot field study. *Agriculture, Ecosystems & Environment*, 140(1–2): 309-313.

Knoblauch C., Maarifat A.A., Pfeiffer E.M., and Haefele S.M. (2011). Degradability of black carbon and its impact on trace gas fluxes and carbon turnover in paddy soils. *Soil Biology and Biochemistry*, 43(9): 1768-1778.

Lehmann J., Pereira da Silva J., Steiner C., Nehls T., Zech W., and Glaser B. (2003). Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil*, 249(2): 343-357.

Mochizuki Y., Koba K., and Yoh M. (2012). Strong inhibitory effect of nitrate on atmospheric methane oxidation in forest soils. *Soil Biology and Biochemistry*, 50: 164-166.

Nesbit S.P., and Breitenbeck G.A. (1992). A laboratory study of factors influencing methane uptake by soils. *Agriculture, Ecosystems & Environment*, 41(1): 39-54.

Ortega-Calvo J.J., and Saiz-Jimenez C. (1998). Effect of humic fractions and clay on biodegradation of phenanthrene by a Pseudomonas fluorescens strain isolated from soil. *Applied and Environmental Microbiology*, 64(8): 3123-3126.

Saito M., and Marumoto T. (2002). Inoculation with arbuscular mycorrhizal fungi: the status quo in Japan and the future prospects. *Plant and Soil*, 244(1-2): 273-279.

ISSN: 2413-5267

Sitaula B.K., Bakken L.R., and Abrahamsen G. (1995). *CH_4* uptake by temperate forest soil: Effect of *N* input and soil acidification. *Soil Biology and Biochemistry*, 27(7): 871-880.

Smith M.S., and Tiedje J.M. (1979). Phases of Deniterfication Following Oxygen Depletion in Soil. *Soil Biology & Biochemistry*, 11(3): 261-267.

Spokas K.A., and Reicosky D.C. (2009). Impacts of Sixteen Different Biochars on Soil Greenhouse Gas Production. *Annals of Environmental Science*, 3: 179-193.

Steiner C., Teixeira W.G., Lehmann J., Nehls T., de Macedo J.L.V., Blum W.E.H., and Zech W. (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil*, 291(1-2): 275-290.

Steudler P.A., Bowden R.D., Melillo J.M., and Aber J.D. (1989). Influence of nitrogen fertilization on methane uptake in temperate forest soils. *Nature*, 341(6240): 314-316.

Taghizadeh-Toosi A., Clough T.J., Condron L.M., Sherlock R.R., Anderson C.R., and Craigie R.A. (2011). Biochar Incorporation into Pasture Soil Suppresses in situ Nitrous Oxide Emissions from Ruminant Urine Patches. *J. Environ. Qual.*, 40(2): 468-476.

Thies J.E., and Rillig M.C. (2009). Characteristics of biochar: biochar properties. In Lehmann J., and Joseph S. (Eds.) *Biochar for Environmental Management–Science and Technology*. Earthscan.

VanZwieten L., Singh B., Joseph S., Kimber S., Cowie A., and Chan K.Y. (2009). Biochar and emissions of non-*CO_2* greenhouse gases from soil. In Lehmann J., and Joseph S. (Eds.) *Biochar for Environmental Management – Science and Technology*. Earthscan.

Warnock D.D., Lehmann J., Kuyper T.W., and Rillig M.C. (2007). Mycorrhizal responses to biochar in soil - concepts and mechanisms. *Plant and Soil*, 300(1-2): 9-20.

Yanai Y., Toyota K., and Okazaki M. (2007). Effects of charcoal addition on *N_2O* emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. *Soil Science and Plant Nutrition*, 53(2): 181-188.

Zhang A., Bian R., Pan G., Cui L., Hussain Q., Li L., Zheng J., Zheng J., Zhang X., Han X., and Yu X. (2012a). Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of consecutive rice growing cycles. *Field Crops Research*, 127(0): 153-160.

Zhang A., Liu Y., Pan G., Hussain Q., Li L., Zheng J., and Zhang X. (2012b). Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. *Plant and Soil*, 351(1-2): 263-275.

Zhang A., Liu Y., Pan G., Hussain Q., Li L., Zheng J., and Zhang X. (2012c). Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. *Plant and Soil*, 351(1-2): 263-275.