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Effect of biochar application on soil and plant

(バイオ炭施用が土壌および植物生育に及ぼす効果)

**Hokkaido University
Graduate School of Agriculture
Division of Bio-systems Sustainability
Doctor Course**

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CHAPTER 1

General Introduction

Food problem and climate change

World population will increase to 8.3 billion by 2030 (Eickhout *et al.*, 2006). It will be necessary to increase crop production to support this population increase. Fertilizer plays a key role of this process. Agriculture depending on the natural fertility of the soil has now been shifted to that depending on fertilizer, particularly chemical fertilizer. However, excess use of chemical fertilizer increases the probability of environmental pollution. Organic fertilizer can be alternatively used as fertilizer as well as soil amendment to improve soil properties. However, organic fertilizer is less effective than chemical fertilizer because N and P mainly occur in organic form in organic fertilizer.

There are also environmental issues which needs to be addressed such as the climate change. Climate change caused by an increase in atmospheric concentrations of greenhouse gases (GHGs) is predicted to cause catastrophic impacts on our planet (IPCC, 2006). Since pre-industrial times, the atmospheric concentrations of CO₂, CH₄ and N₂O in 2013 have increased by 42%, 153%, and 21%, respectively (World Meteorological Organization, 2014). Agricultural lands occupy about 40-50% of the Earth's land surface and agriculture is responsible for 10-12% of total global anthropogenic emissions of GHG in 2005 (IPCC, 2007). On the other hand, rice is one of the most important staple foods for more than 50% of the world's population, and it is cultivated on almost 155 million ha in the world (Kogel-Knabner *et al.*, 2010). Global warming potential (GWP) of

GHG emissions from rice systems was about four times higher than either wheat or maize (Linguist *et al.*, 2012). Concerning about global food security and reducing greenhouse gas emission, there is an urgent need to establish effective agricultural management practice that increase food production with mitigating GHG emission.

Biochar

Biochar is a product of thermal decomposition of organic material under oxygen-limited condition and at relatively low temperature ($<700^{\circ}\text{C}$), and used as soil amendment (Sohi *et al.*, 2009; Lehmann and Joseph, 2009). It has a high carbon (C) content and varying C to nutrient ratio depending on feedstock used. Biochar can be produced from a wide range of biomass sources such as shrubs, crop residues, green waste and livestock manures. The chemical and physical properties of biochar depend on its feedstock type and pyrolysis condition such as temperature, time duration, and air supply (Sohi *et al.*, 2009). For example: biochar produced at high temperature pyrolysis is more resistant to mineralization and contains lower amounts of volatile matter on its surface (Spokas, 2010). These characteristics suggest that biochar can be used to effectively sequester CO_2 from the atmosphere over long time scales (Woolf *et al.*, 2010). In addition, biochar has a large surface area and high porosity (Downie *et al.*, 2009), and can develop both positive and negative surface charges, indicating that biochar can absorb either positively and negatively charged compounds, thus decreasing

nutrients leaching. Porous structures of biochar are also provide an appropriate habitat for several kinds of soil microbe (Ogawa, 1994).

Effect of biochar on soil properties and plant growth

Biochar research emanated from the discovery of fertile black soils in Amazonia (Terra Preta do Indio) (Schimmelpfennig and Glaser, 2011). Soils of the Terra Preta or Amazonian dark earth were affected by human management over many years ago and characterized by a sustainable enhanced fertility due to high levels of soil organic matter and nutrients than adjacent soils (Glaser *et al.*, 2001; Glaser, 2007). The charred organic material, known as biochar, identified as the key component of Terra Preta (Glaser *et al.*, 2001). Biochar has been proposed as a possible mean to improve soil fertility and sequester C to mitigate climate change (Sohi *et al.*, 2010). The beneficial effects on soil chemical properties are mainly due to pH increase (Topoliantz *et al.*, 2007; Masulili *et al.*, 2010; Yuan *et al.*, 2011), improved nutrient availability (Chan *et al.*, 2008; Haefele *et al.*, 2008), nutrient retention (Glaser *et al.*, 2002; Lehmann *et al.*, 2003), and cation-exchange capacity (Glaser *et al.*, 2002; Topoliantz *et al.*, 2002; Masulili *et al.*, 2010; Yuan *et al.*, 2011) of soil. Biochar has also positive influences on improving soil physical properties: increasing the water-holding capacity and available soil water (Glaser *et al.*, 2002; Masulili *et al.*, 2010), decreasing the bulk density and soil strength (Masulili *et al.*, 2010). Moreover, biochar could change soil biological community structure and abundance (Pietikainen *et al.*, 2000). Thereby, indirectly affected the growth and yield in

various crop species, including cowpea and rice (Lehmann *et al.*, 2003), radish (Chan *et al.*, 2008), soybean (Tagoe *et al.*, 2007), and maize (Yamato *et al.*, 2006). A meta-analysis found that biochar application to soil overall increased crop yields by ~10% (Jeffery *et al.*, 2011)

Biochar and greenhouse gas emissions

Biochar could act as a long term carbon sink in soil (Lehmann *et al.*, 2006) due to the recalcitrance of its microbial decomposition (Seiler and Crutzen, 1980). By slow decay of biochar in soils, only a small amount of CO₂ returns to the atmosphere (Woolf *et al.*, 2010). As a result, biochar application can mitigate and even reduce the global warming (Lehmann *et al.*, 2007). Previous studies indicated that biochar amendment reduced CO₂ (Liu *et al.*, 2011; Yoo and Kang, 2012), CH₄ (Liu *et al.*, 2011; Feng *et al.*, 2011; Yoo and Kang, 2012), NO (Nellisen *et al.*, 2014), and N₂O (Zhang *et al.*, 2010; Wang *et al.*, 2012; Zhang *et al.*, 2012; Singla and Inubushi, 2014) emissions, which could contribute to mitigating global warming. However, there is limited understanding of the mechanism through biochar impact on GHG emissions. A number of mechanisms have been proposed to explain these effects. Biochar amendment reduced the activity of C-mineralizing enzyme, therefore reducing soil CO₂ emission (Jin, 2010). In addition, on biochar surface which have high pH and abundant alkaline metals, CO₂ precipitates as carbonate, explains the decrease in CO₂ emission (Joseph *et al.*, 2010; Lehmann *et al.*, 2011). The improvement of soil aeration and porosity caused by biochar amendment may also increase

methanotrophic activity and thereby decrease CH₄ emission (Troy *et al.*, 2013). The mechanisms of N₂O reduction in biochar-amended soils could be attributed to reduced N availability due to biochar's adsorption of substrates such as ammonium and nitrate (Bruun *et al.*, 2011; Case *et al.*, 2012), changes in microbial community structure (Bruun *et al.*, 2011), a decrease in soil redox potential (Case *et al.*, 2012) or microbial inhibition by volatile organic compounds contained in biochar (Spokas *et al.*, 2010).

Meanwhile, many studies reported the different effects of biochar amendment on soil GHG emissions. For instance, increase of CO₂ emission by biochar amendment was estimated (Wang *et al.*, 2012). No significant effect (Knoblauch *et al.*, 2008) and increasing effect (Zhang *et al.*, 2010) in CH₄ emissions were observed. Wheat-derived biochar did not notably reduce NO emission from paddy field (Xiang *et al.*, 2015). Moreover, enhancing N₂O emissions by biochar was demonstrated by Yoo and Kang (2012). These different effects of biochar are presumably due to the differences in biochar types and properties, types of the soils, the microbiological circumstances, or water and fertilizer managements.

Objective of this study

As described above, biochar can affect the crop production and GHG emission. However, its effects vary widely. Then, two different experiments were conducted in this study to understand these biochar effects more in detail. Firstly, I examined the effect of biochar on microbial community structure and mineral

availability in soils growing different crop species under different organic manure treatments to understand the mechanisms of growth enhancement by biochar in soils with organic manure (Chapter 2). Meanwhile, in the second experiment, we investigated the potential effect of different types of biochar application on GHG emissions and soil properties under different soil moisture conditions using soil from rice paddy field (Chapter 3).

CHAPTER 2

Effect of biochar application on mineral and microbial properties of soils growing different plant species

2.1. Introduction

Driven by population growth, increased human pressure on land has forced the conversion of natural landscapes into agricultural fields while simultaneously depleting the land under agricultural use (Lal, 2009). Therefore, there is an urgent need to establish effective agricultural management practices that not only increase food production but also prevent the negative environmental impacts of intensive agriculture. There are various fertilizers and soil amendments that are able to improve soil fertility and crop productivity. Fertilizers are necessary to increase crop production, and are supplied mainly in the form of chemical amendments. However, the continuous and excessive use of chemical fertilizers may result in environmental pollution. In addition, many countries face challenges related to high costs and shortages of chemical fertilizers. Organic fertilizers may be alternatively used as chemical fertilizers; such organic fertilizers can also act as soil amendments which improve the physical, chemical, and biological properties of soil. However, organic fertilizers are less effective than chemical fertilizers because nitrogen (N) and phosphorus (P) mainly occur in organic forms in organic fertilizer.

Biochar is a product of the thermal degradation of organic material under oxygen-limited conditions. With respect to appearance, it is similar to charcoal produced by natural burning; however, it is distinguished by its use as a soil

amendment (Sohi et al., 2009; Lehmann and Joseph, 2009). Many studies have shown the beneficial effects of biochar on soil chemical properties such as pH (Topoliantz et al., 2007; Masulili et al., 2010; Yuan et al., 2011), nutrient availability (Chan et al., 2008; Haefele et al., 2008), nutrient retention (Glaser et al., 2002; Lehmann et al., 2003), and cation-exchange capacity (Glaser et al., 2002; Topoliantz et al., 2002; Masulili et al., 2010; Yuan et al., 2011). Improvements in the growth and yield of plants following biochar application have also been reported in various crop species, including cowpea and rice (Lehmann et al., 2003), radish (Chan et al., 2008), soybean (Tagoe et al., 2007), and maize (Yamato et al., 2006).

Recently, the use of organic fertilizers in soil to increase crop productivity has received considerable attention. The incorporation of organic fertilizers is a useful approach for maintaining organic matter content in soil and thereby enhance soil biological activity and increase nutrient content, which, in turn, contributes to increasing crop productivity (Dikinya and Mufwanzala, 2010; Diacono and Montemurro, 2010). However, in order to supply available nutrients to plants, organic fertilizers need to be mineralized by soil microorganisms. Biochars have been shown to have a positive effect on soil fertility and plant growth (as described above); however, little information is available on their effects when combined with manure, particularly in terms of the mineral and microbial properties of soil. Therefore, this study assessed the effects of biochar on the microbial community structure and mineral availability in soils growing different crop species under different organic manure treatments.

2.2. Materials and methods

Experimental setup

A pot experiment was conducted using soybean and sorghum under four soil treatment combinations (cattle farmyard manure with/without biochar and rapeseed cake with/without biochar) to examine the effects of wood biochar on the microbial community structure and mineral availability in soils (Table 2.1). Soils (Gleyic Fluvisol) were collected from the 0–25 cm layer at the experimental farm of Hokkaido University. The soil was air-dried and passed through a 2.0 mm mesh screen. Then, 0.8 L of soil and 0.8 L of perlite were mixed and placed in a plastic pot (1.6 L). The biochar used in this experiment was purchased from Shimokawa City Forest Organization Carbon Industry and was produced from broad-leaved trees at 400 °C; the biochar had a Carbon (C) content of 71.8%. The amount of fine biochar (<0.25 mm) applied to each pot was 28 g (equivalent to a field application rate of 35 t ha⁻¹). Two different types of organic fertilizer were used: cattle farmyard manure (0.8% N, 1.7% P₂O₅, and 1.8% K₂O) and rapeseed cake (5.3% N, 2.0% P₂O₅, and 1.0% K₂O); 31.75 g of cattle farmyard manure (providing 0.254 g N, 0.540 g P₂O₅, and 0.572 g K₂O) or 4.793 g of rapeseed cake (providing 0.254 g N, 0.096 g P₂O₅, and 0.048 g K₂O) was applied to each pot. In treatments with rapeseed cake, 0.878 g of calcium superphosphate and 0.381 g of K₂SO₄ were added to make the application rates of each of N, P₂O₅, and K₂O equal to 100 kg ha⁻¹. Chemical properties of biochar and each organic manure used in this study were shown in the Supplementary material, Table 2.S1. After mixing of the soil, perlite, fertilizer, and biochar, the pots were incubated

for 4 weeks in a greenhouse under moderately moist conditions (40–60% of field capacity depending on the soil condition).

Table 2.1. Treatments in this study.

Treatment	Description
B0M1	no biochar + cattle farmyard manure
B1M1	biochar + cattle farmyard manure
B0M2	no biochar + rapeseed cake
B1M2	biochar + rapeseed cake

Seeds of soybean (*Glycine max* (L.) Merr. cv. Toyoharuka) and sorghum (*Sorghum bicolor* (L.) Moench cv. Hybrid Sorgo) were sterilized with 10% (v/v) NaClO solution for 1 min and then rinsed in deionized water. The seeds were sown and germinated in vermiculite. After the first two leaves appeared (post 10–12 days), two of each species were transplanted to each pot. Depending on the soil condition during the experiment, all pots were then watered with deionized water to 40–60% of their field capacity. The pot experiment was performed for 30 days (November 15–December 12, 2015) for soybean and 40 days (November 15–December 23, 2015) for sorghum. The experiment was conducted in a greenhouse at an almost constant average temperature of 25°C.

Soil sampling and analysis

Soil samples were collected at the time of plant sampling (30 and 40 days after sowing for soybean and sorghum, respectively). After removing the plants (as described later), the soil in each pot was mixed and remaining roots were

removed. Fresh soil was taken for determining the microbial community structure and activity analyses using EcoPlate™ (Biolog Inc., CA, USA). The remaining soil was air-dried and sieved for chemical analysis.

EcoPlate contains three replicate wells of 31 of the most useful carbon sources and water (no substrate; tetrazolium dye only as a blank). For assessing microbial carbon utilization patterns, a 1 g soil sample was thoroughly shaken by hand with 10 ml of sterile saline solution (0.85% NaCl) and diluted 1000 times with the same saline solution. A subsample of 150 µL was inoculated directly into each well of the EcoPlate. Three replicate suspensions were prepared for each soil sample. The EcoPlates were then placed in an incubator at 25 °C; purple color was formed when the microbes utilized the carbon source and began to respire. The color development was measured every 24 h for 5–6 days using a microplate reader (Sunrise Remote, TECAN A-5082, Austria) at 595 nm. Changes in the pattern were compared and analyzed using principle components analysis (PCA). The average well color development (AWCD) in each plate, which indicates microbial activity, was calculated as follows:

$$AWCD = [\Sigma(R_i - C)]/31,$$

where R_i and C are the optical density (OD) values at 595 nm of the response wells (containing sole carbon sources) and the control well (water), respectively.

Soil pH (H₂O) was determined at a soil/water ratio of 1:2.5 using a pH meter (Mettler Toledo, MP220, 2005). For determination of inorganic N (NH₄-N and NO₃-N) concentration in the soils, 4 g samples were extracted with 40 mL of 2 M KCl by shaking for 1 h. The soil extracts were passed through filter paper (No. 6,

Advantec, Tokyo, Japan). The $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations were determined by colorimetric methods. Available P was extracted with Truog's solution and measured by spectrophotometry (U-5100, HITACHI, Japan) at 710 nm. Excluding N, the concentrations of mineral elements in the soil were determined by extracting 2 g of soil with 40 mL of 1 M ammonium acetate, shaking for 30 min, and passing through filter paper (No. 5C Advantec, Tokyo, Japan). Thereafter, 5 mL of the filtrate was digested with 2 mL of 61% HNO_3 . The concentrations of P, aluminum (Al), arsenic (As), boron (B), calcium (Ca), cadmium (Cd), cobalt (Co), cesium (Cs), copper (Cu), iron (Fe), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), rubidium (Rb), selenium (Se), strontium (Sr), vanadium (V), and zinc (Zn) in the digested solution were measured using inductively coupled plasma mass spectrometry (ICP-MS) (ELAN, DRC-e, Perkin Elmer, MA, USA).

Plant sampling and analysis

Plants were harvested at the end of the vegetative growth. Roots of the plants were washed clean with tap water. The plants were then separated, washed with de-ionized water, and dried in an oven at 70°C for 7 days, before being weighed and ground for mineral analysis. The concentrations of mineral elements in the plant samples were determined as described above.

Data analysis

All experimental data were statistically analyzed using Minitab 16 (Minitab, Inc, United States). Analysis of variance (ANOVA) followed by Tukey's test were used to detect significant differences among treatments. To compare the results of the treatments with and without biochar, paired Student's t-tests were applied. PCA was used to profile the microbial communities and minerals in the soils.

2.3. Results

Growth and mineral accumulation of plants

The total dry weight of both the plant species grown with rapeseed cake significantly increased because of the biochar application, particularly for sorghum (1.21 and 1.48 times higher than that without biochar for soybean and sorghum, respectively) (Fig. 2.1). A similar trend was also found for cattle farmyard manure; however, this was not statistically significant (Fig. 2.1). The concentrations of some metal elements in leaf material are shown in Table 2.2. Overall, irrespective of the plant species and manure type, the biochar application decreased or did not affect the concentration of these elements.

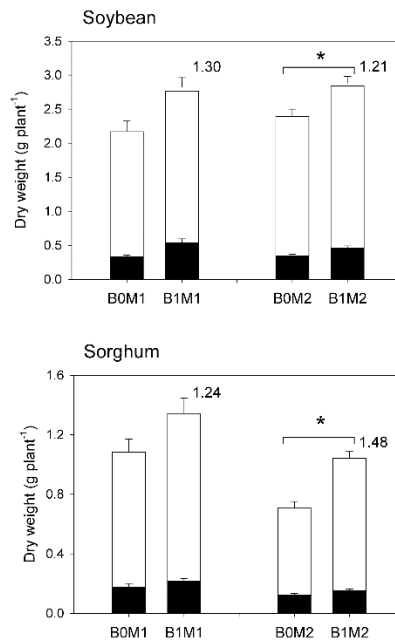


Figure 2.1. Dry weight of soybean and sorghum. ■ and □ indicate root and shoot, respectively. The error bars represent the standard error of the mean (n = 4). * indicates a significant difference between treatments with and without biochar in each organic manure treatment (M1 or M2) ($P < 0.05$, Student's *t*-test). Relative value of the B1 treatment to the B0 treatment is indicated on the bar of B1 in each manure treatment. B0: without biochar; B1: with biochar; M1: cattle farmyard manure; M2: rapeseed cake.

General chemical properties of soil

Soil pH was higher in soil receiving cattle farmyard manure (Table 2.3). The biochar application significantly increased soil pH under sorghum with each type of organic manure, but not in soybean. The concentration of inorganic N ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) did not differ significantly between the treatments (Table 2.3). Available P concentration was higher in soils receiving cattle farmyard manure; however, no biochar effect was observed for either plant grown soil (Table 2.3).

Table 2.2 Concentration (mg kg^{-1} dry weight) of some metal elements in plant leaf.

		Al			Ba			Cd			Co			Sr			Zn		
Soybean	B0M1	55.33	± 6.48	c	79.4	± 5.9	a	0.261	± 0.018	ab	0.175	± 0.006	b	91.2	± 5.5	a	154.0	± 8.4	a
	B1M1	70.71	± 3.55	bc	48.7	± 5.0	b	0.164	± 0.013	c	0.181	± 0.006	b	71.2	± 2.6	b	124.7	± 8.3	b
	B0M2	94.16	± 4.29	a	13.3	± 0.3	c	0.303	± 0.007	a	0.223	± 0.011	a	79.4	± 1.7	ab	173.9	± 3.1	ab
	B1M2	85.61	± 4.97	ab	20.2	± 0.6	c	0.244	± 0.004	b	0.204	± 0.007	ab	70.5	± 0.7	b	148.9	± 9.5	c
Sorghum	B0M1	65.38	± 7.90	b	14.8	± 0.9	a	2.11	± 0.22	bc	0.080	± 0.006	b	24.7	± 1.1	ab	108.6	± 3.9	a
	B1M1	40.71	± 2.42	b	14.5	± 0.8	a	1.71	± 0.09	c	0.055	± 0.003	b	22.0	± 1.3	b	79.4	± 3.9	b
	B0M2	360.54	± 41.32	a	8.4	± 0.8	b	2.81	± 0.13	a	0.308	± 0.028	a	28.3	± 0.5	a	114.3	± 3.2	a
	B1M2	61.92	± 4.77	b	7.7	± 0.4	b	2.61	± 0.07	ab	0.068	± 0.004	b	24.9	± 0.7	ab	93.9	± 1.9	c

Different letters indicate significant difference at $P < 0.05$ in each species (Tukey's multiple range test).

Table 2.3 pH and concentration (mg kg⁻¹ dry soil) of NH₄-N, NO₃-N, Truog-P, and ammonium acetate-extractable S in soil

		pH			NH ₄ -N			NO ₃ -N			P			S		
Soybean	B0M1	5.26	±0.04	a	105.3	±20.1	a	14.9	±0.9	a	546	±17	a	91	±5	c
	B1M1	5.41	±0.06	a	85.6	±20.8	a	9.0	±2.0	b	568	±22	a	98	±2	c
	B0M2	4.88	±0.02	b	38.7	±10.3	a	13.3	±1.1	ab	287	±12	b	362	±13	a
	B1M2	4.99	±0.02	b	47.6	±8.2	a	10.4	±1.3	ab	267	±5	b	303	±6	b
Sorghum	B0M1	5.23	±0.03	b	38.1	±5.2	a	21.0	±1.3	ab	595	±24	a	92	±2	b
	B1M1	5.47	±0.03	a	33.1	±1.7	a	12.2	±0.7	b	601	±20	a	109	±7	b
	B0M2	4.81	±0.02	d	22.8	±3.1	a	28.8	±3.9	a	293	±23	b	1506	±418	b
	B1M2	4.98	±0.01	c	34.9	±5.0	a	21.8	±2.4	ab	278	±9	b	9245	±2390	a

Different letters indicate significant difference at $P < 0.05$ in each species (Tukey's multiple range test).

The concentration of ammonium-acetate-extractable S in soil increased under sorghum because of biochar application with rapeseed cake (Table 2.3).

Microbial activity and community structure of soil

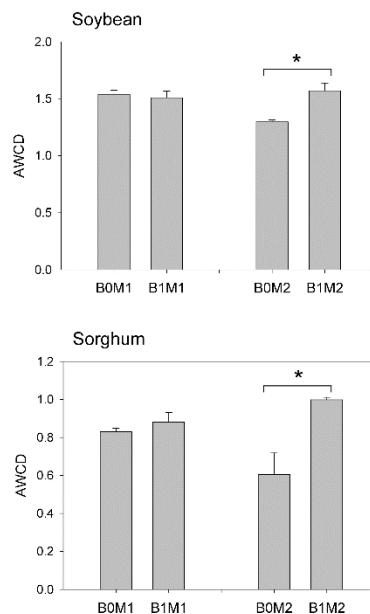


Figure 2.2 AWCD values of soybean and sorghum grown soils. Error bars represent the standard error of the mean ($n = 4$). * indicates a significant difference between treatments with and without biochar in each organic manure treatment (M1 or M2) ($P < 0.05$, Student's t -test). B0: without biochar; B1: with biochar; M1: cattle farmyard manure; M2: rapeseed cake. Data recorded 144 and 120 h after the incubation started were used for soybean and sorghum, respectively.

The microbial activity in soil was estimated by AWCD. A high value of AWCD reflects higher microbial activity. Figure 2.2 presents the AWCD values obtained from the EcoPlate. For both soybean and sorghum grown soils with rapeseed cake, biochar application significantly increased AWCD compared to the case without biochar. When PCA was conducted to assess the utilization patterns of

the different carbon sources, the total variance explained by the first two components was 39% and 44% for soybean and sorghum, respectively. In the soybean grown soil, the score plot of PCA showed a separation between the B0M2 treatment and the other treatments in PC1 (Fig. 2.3). Meanwhile, in the score plot of the sorghum grown soil, the soil with biochar application shifted negatively along PC1 in both the cattle farmyard manure and rapeseed cake treatments (Fig. 2.3).

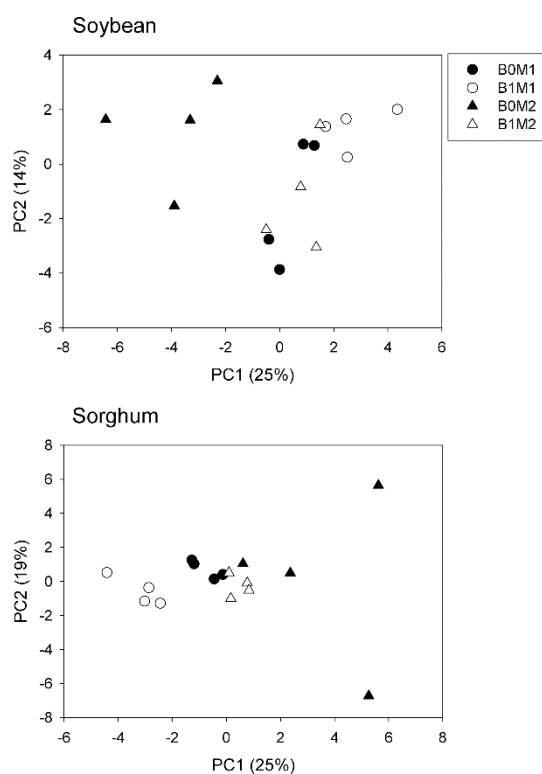


Figure 2.3 Principal component analysis of carbon source utilization activity (EcoPlate) in soybean and sorghum grown soils. B0: without biochar; B1: with biochar; M1: cattle farmyard manure; M2: rapeseed cake. Data recorded at 144 and 120 h after the incubation started were used for soybean and sorghum, respectively.

Mineral profile of soil

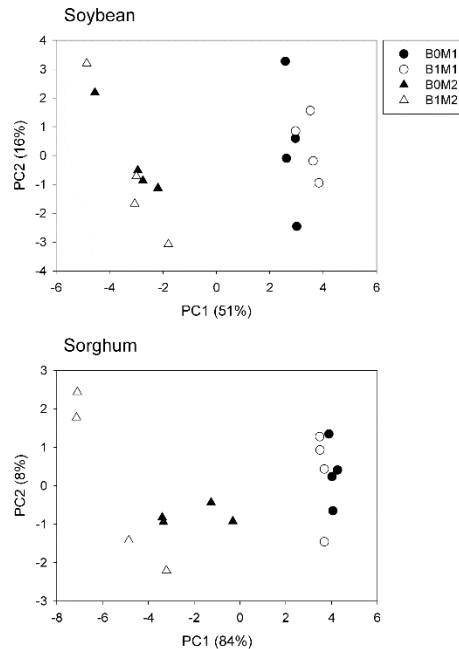


Figure 2.4 Principal component analysis of ammonium acetate-extractable mineral elements in soybean and sorghum grown soils. B0: without biochar; B1: with biochar; M1: cattle farmyard manure; M2: rapeseed cake.

PCA was also used to examine the treatment effects on the mineral profile of the soils. Figure 2.4 shows the score plot of the first two components from the ammonium-acetate-extractable concentrations of each mineral element in soils growing soybean or sorghum. The first two components accounted for 67% and 92% of the total variance for the soybean and sorghum soils, respectively. In both species, a clear separation in the score plot was observed between the different types of organic fertilizer applied to the soil (Fig 2.4). Moreover, the biochar application altered the profile of the extractable mineral elements in the sorghum grown soil with rapeseed cake (Fig. 2.4), whereas it did not affect that in the soybean grown soil (Fig.2.4).

2.4. Discussion

It has been reported that the positive effect of biochar on plant growth may be related to the nutrient-retention capacity of biochar (Glaser et al., 2002) and its sorption capacity for toxic metals and some phytotoxic compounds (Hille and den Ouden, 2005; Lair et al., 2006). In the present study, the biochar application enhanced the growth of both the plant species grown in soils with both types of manure application (Fig. 2.1), indicating that the combined application of organic manure with biochar is effective at increasing crop yield. This growth promotion effect was more remarkable in the soil with rapeseed cake, particularly for sorghum. Therefore, different plant species as well as different types of organic manure may affect soil-biochar interactions differently. This raises the question regarding the factors causing these differences.

In both soybean and sorghum grown soils, AWCD-estimated microbial activity was increased by biochar application with rapeseed cake (Fig. 2.2). It has been reported that biochar provides a suitable habitat for microorganisms (Pietikainen et al., 2000) and produces substances that stimulate the growth of microbes (Kasozi et al., 2010). Rapeseed cake may have suitable characteristics for the exertion of these positive effects of biochar on microorganisms. The enhanced microbial activity can be expected to enhance the mineralization of rapeseed cake applied to soils. Although significant differences were not found for both plant species in inorganic N and available P concentrations between soils with and without biochar in the rapeseed cake treatment, a significant increase was found in extractable S concentrations because of biochar application under sorghum in

the rapeseed cake treatment (Table 2.3). Moreover, significant positive correlation was found between extractable S concentration in soil and utilization (absorbance) for phenylethyl-amine in the EcoPlate in sorghum with rapeseed cake application ($r = 0.94$, Supplementary material, Figure 2.S1). These results imply that the biochar enhanced the microbial decomposition of organic matter, containing organic S in this soil, resulting in superior growth of the sorghum. In fact, for the rapeseed cake treatments, S concentration in the leaves of sorghum significantly increased by biochar application (data not shown, Student's *t*-test, $P < 0.05$).

It has also been suggested that biochar may change the soil microbial community structure (Lehmann et al., 2011). In the present study, PCA of the EcoPlate data demonstrated that biochar application clearly changed the microbial community structure, particularly in sorghum grown soils (Fig. 2.3). Correlation analysis was conducted to determine the factor(s) responsible for biochar-induced changes in the microbial community structure. In the PCA, the PC1 scores using the EcoPlate showed a negative correlation with soil pH for sorghum but not for soybean (which showed weak positive correlation) (Fig. 2.5). In fact, biochar application did not significantly affect soil pH under soybean but increased it under sorghum (Table 2.2). Together, some interactions between the sorghum rhizosphere and biochar may affect soil pH; this may be the primary factor altering the microbial community structure in soils.

Under soybean, biochar application had little effect on the profile of ammonium-acetate-extractable mineral elements of the soil for both types of manure

application (Fig. 2.4). For sorghum, however, biochar application altered the profile of the extractable elements in the soil applied with rapeseed cake (Fig. 2.4). This alteration was mainly due to the increase in the extractable concentrations of certain metals in soils due to biochar application (Table 2.4). The biochar application increased soil pH in sorghum grown soil applied with rapeseed cake (Table 2.3), which cannot explain the results of the extractable metals in this study because increasing the pH normally decreases the availability of certain metal cations such as Al and Cd (von Uexküll and Mutert, 1995; Xian and In Shokohifard, 1989).

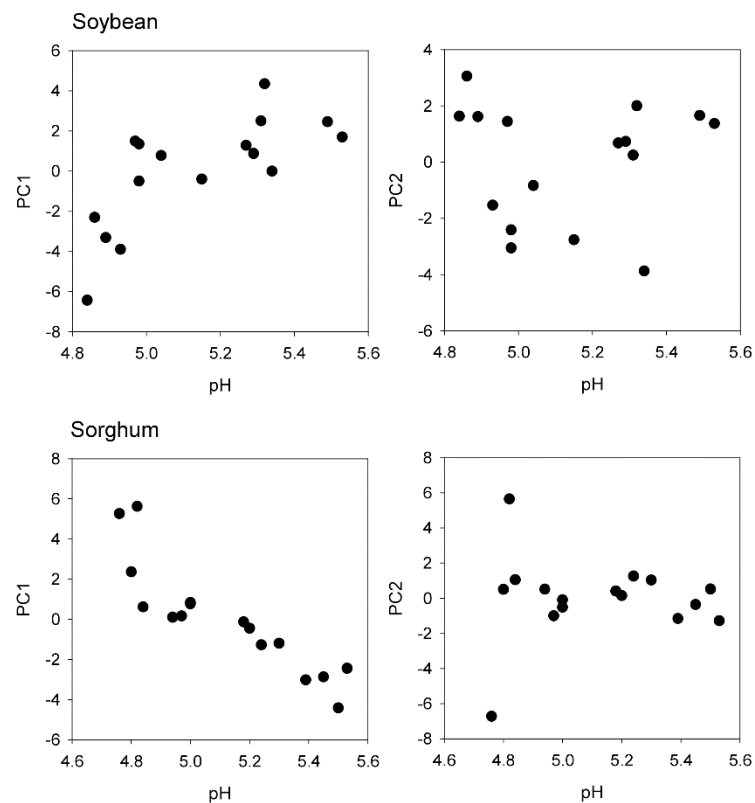


Figure 2.5 Correlation of soil pH with PC1 or PC2 of PCA in the EcoPlate for soybean and sorghum grown soils.

In contrast to the effects of biochar on soil, concentrations of these metals in the leaves of sorghum grown in the soil with rapeseed cake did not change, or they tended to show a decrease due to the biochar application (Table 2.2). Because biochar application increased microbial activity in the soils applied with rapeseed cake (Fig. 2.2), it possibly enhanced organic matter decomposition in this soil, producing chelating organic compounds that solubilized some metals but also made those metals less available to sorghum roots. In fact, when analyzing the correlation between extractable concentration of each of these metal elements and utilization (absorbance) for each carbon source in the EcoPlate, highly significant correlation ($P < 0.01$) was found in several carbon sources (4-hydroxy benzoic acid and Al/Ba; phenylethyl-amine and Zn; α -D-lactose and Zn) only in sorghum grown soil with rapeseed cake application (Supplementary material, Figure 2.S1). These carbon utilization characteristics of microbial community in sorghum grown soil with rapeseed cake might be related to the production of chelating compounds from soil organic matter to solubilize certain metals in soil.

In conclusion, biochar application can be an important agricultural practice for increasing the efficiency of organic manure for crop cultivation. However, its effects differ depending on the plant species and organic manure type. These differences may be attributed to the complicated interactions between the plant rhizosphere, biochar, organic manure, and soil microorganisms. In order to elucidate these interactions, detailed analysis of the dynamics of microorganisms and organic/inorganic substances in the rhizosphere of soils applied with different types of organic manure is needed.

CHAPTER 3

Potential effect of wood and bone biochar on greenhouse gas emission of paddy soil under waterlogged and upland condition

3.1. Introduction

The atmospheric concentrations of CO₂, CH₄ and N₂O in 2013 have been increased 142%, 253% and 121%, respectively since pre-industrial times (World Meteorological Organization, 2014). Agricultural lands occupy about 40-50% of the Earth's land surface and agriculture accounted for 10-12% of total global anthropogenic emissions of GHG emission in 2005 (IPCC, 2007). Waterlogged paddy fields are considered one of the most important sources of methane production (Yagi *et al.* 1997; Wassman *et al.* 2000; Yan *et al.* 2003) and also emitted N₂O (Yan *et al.* 2000; Zou *et al.* 2007). Result of meta-analysis by Linnquist *et al.* (2012) reported that the global warming potential (GWP) of GHG emissions from rice systems was about four times higher than either wheat or maize. On the other hand, rice is the most important food for more than 50% of the world's population, and it is grown on almost 155 million ha of the world's surface (Kogel-Knabner *et al.* 2010). With an expanding world population, the demand for crops production of the largest source of human calories (rice, wheat, and maize) must increase by 1.29% annually to 2025 to meet growing demand (Cassman *et al.* 2003). There is an urgent need to establish technologies that mitigate GHG gas emission while increase crop production.

The crop rotation of rice paddy fields and upland crops is widely conducted, and various upland crops are cultivated in drained paddy fields in Japan (Ministry of

Agriculture, Forestry and Fisheries of Japan (2003) in Nishimura *et al.* (2005)). At converted paddy fields where water is drained in accordance to the requirement of upland crops cultivation may cause changes in soil properties (Takahashi *et al.* 2003; Kyuma, 2004; Chu *et al.* 2009; Tago *et al.* 2011). These possible changes may also influence the dynamic of GHG emission from drained paddy fields. So that the inventory of GHG from converted fields takes an important position. However, there was a limited information available in published literature.

Biochar is a product of thermal degradation of organic material under oxygen-limited condition, similar in its appearance to charcoal produced by natural burning but distinguish by its use as soil amendment (Sohi *et al.* 2009; Lehmann and Joseph, 2009). Some studies have indicated that biochar may play a significant role in reducing GHG emissions directly by sequestering carbon and or indirectly by improving soil fertility (Lehmann and Joseph, 2009; Sohi *et al.* 2010; Lehmann *et al.* 2011).

The effect of biochars on GHG emissions on paddy fields were inconsistent. A calculation revealed a reduction (Liu *et al.* 2011; Yoo and Kang, 2012) and increased of CO₂ emissions (Wang *et al.* 2012) by biochar amendment. In some studies (Liu *et al.* 2011; Feng *et al.* 2011; Yoo and Kang, 2012), CH₄ emissions were reduced after biochar application compared without biochar. However, there was no significant (Knoblauch *et al.* 2008) and increased (Zhang *et al.* 2010) effect on CH₄ emissions. Previous study with wheat-derived biochar did not notably reduce NO emission from paddy field (Xiang *et al.* 2015). On the

other hand, Nellisen *et al.* (2014), reported a reduction. Moreover, N₂O emissions were significantly suppressed by biochar amended paddy soils (Zhang *et al.* 2010; Wang *et al.* 2012; Zhang *et al.* 2012; Singla and Inubushi, 2014). Conversely, enhanced N₂O emissions by biochar was demonstrated by Yoo and Kang (2012).

Application of biochar for mitigating GHG emission has been studied and reviewed extensively. However, there was a little information reported on the effect of biochar from converted paddy fields. Moreover, to our knowledge, there is almost no papers reported the effect of bone charcoal on GHG emissions. Up to now, biochar which usually used in of GHG emissions studies from paddy soil was made from feedstock such as crops (Liu *et al.* 2011; Xiang *et al.* 2015), agricultural waste (Knoblauch *et al.* 2008; Zhang *et al.* 2010; Liu *et al.* 2010; Wang *et al.* 2012; Feng *et al.* 2012) and manure/animal waste (Yoo and Kang, 2012). Different psychochemical properties of biochar due to diverse sources with different charring methods could induced vary greatly effects on soil processes and GHG emissions (Spokas and Reicosky, 2009; Zimmermann *et al.* 2011).

Bone charcoal is charcoal made from animal bones. It is mostly composed of calcium phosphate and a small amount of carbon. Structurally, the calcium phosphate in bone charcoal is in the hydroxyapatite form (Hassan *et al.* 2008; Choy *et al.* 2004). In most studies, bone charcoal was used as a treatment for decontaminating polluted water. In particular, its potential to adsorb metal

species from contaminated water supplies (Larsen *et al.* 1994; Wilson *et al.* 2000; Wilson *et al.* 2003; Choy and McKay, 2005; Rugayah *et al.* 2014)

Considering those points above, this incubation experiment was set up in order to investigate the potential effect of wood and bone biochar application on CO₂, CH₄, NO, and N₂O emissions and soil properties under different soil moisture conditions (waterlogged and 60 % FWC for simulating paddy and upland conditions) using paddy soil.

3.2. Material and methods

Soil and biochar

Soil samples were collected from the 0-25 cm layer of paddy field at experimental farm of Hokkaido University (43°04'29".6N 141°20'16.3"E). The soil was air-dried and sieved pass through a 2.0 mm mess screen.

Biochar manufactured by Shimokawa City Forest Organization Carbon Industry and Kodama health Trading Co., Ltd. were used in this incubation study. These two different biochars were produced from fine and broad leaf tress at 400°C with C content of 71.8% and animal bone at 800°C with C content of 11.0% (hereinafter wood and bone biochar, respectively).

Incubation study

Before being placed into 1.8 L of Mason jars, 20 g air-dried soil was mixed with 0.35 g of biochar (equivalent to a field application rate of 35 t ha⁻¹) and urea as 0.06 g (equivalent to a field application rate of 90 kg Nha⁻¹) then by adding

deionized water adjusted to 60% FWC or waterlogged condition in a plastic cup. The different soil moistures were chosen according to the paddy field condition (waterlogged treatment) and the upland field condition (60% FWC treatment).

Six treatments have been set up:

1. Soil 20 g + 0 g biochar + 0.06 g urea + waterlogged
2. Soil 20 g + 0 g biochar + 0.06 g urea + 60% FWC
3. Soil 20 g + 0.35 g wood biochar + 0.06 g urea + waterlogged
4. Soil 20 g + 0.35 g wood biochar + 0.06 g urea + 60% FWC
5. Soil 20 g + 0.35 g bone biochar + 0.06 g urea + waterlogged
6. Soil 20 g + 0.35 g bone biochar + 0.06 g urea + 60% FWC

The jars were sealed tightly. A jar without a soil sample was also prepared and labeled as a blank. Ambient wet air was passed through a vinyl tube connected to the jar at a rate of 0.2 ml min^{-1} for 30 minutes to replace the gas in the jar completely. The jars were incubated at 20°C in incubator throughout the 40 days experiment.

Carbon dioxide, CH_4 , NO and N_2O emissions were measured at 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, 32 and 40 days after the initiation of incubation. About 250 ml of headspace air was taken from the jar into a Tedlar bag by using a 50 ml syringe for NO analysis. On the same time, 20 ml was also taken from jar head space by using a 25 ml syringe and injected into a 15 ml pre-evacuated vial for CO_2 , CH_4 and N_2O analysis. After sampling, the jars were opened for air exchange and were immediately closed tightly and then placed in incubator until

next sampling during incubation. Four replications were conducted in the experiment.

CO₂, CH₄, NO and N₂O sampling and measurement

Carbon dioxide analyzed using an infrared CO₂ analyzer (ZFP9FC11, Fuji Electric System, Tokyo, Japan). CH₄ was analyzed by gas chromatography with FID (GC-8A, Model, Shimadzu, Kyoto, Japan). NO gas determined using a chemiluminescence nitrogen oxide analyzer (265P Model, Kimoto Electric, Osaka-Japan) and N₂O by gas chromatography with ECD (GC 14 and GC-2014 Model, Shimadzu, Kyoto, Japan).

Gas fluxes are the change of gas concentration in glass jar during incubation. Positive flux indicates the gas emission from soil surface into the atmosphere, while negative flux indicates the gas uptake from the atmosphere. It is calculated using the equation: $F = \rho \times V/W \times \Delta c/\Delta t \times 273/T$. Where, F is the gas flux (mg kg⁻¹soil day⁻¹); ρ is the density of gas at the standard conditions (CO₂ = 1.977kg m⁻³, CH₄ = 0.717 kg m⁻³, NO = 1.340 kg m⁻³, and N₂O = 1.978kg m⁻³); V (m³) and W (kg) are the volume of glass jar and the air-dried soil weight; $\Delta c/\Delta t$ (mgkg⁻¹day⁻¹) is the change in gas concentration in the glass jar; T is the absolute temperature (°K).

Soil and biochar analysis

For soil chemical analysis, soil samples were extracted with distilled water (ratio 1:5) for measurement of electric conductivity (EC), pH, water extractable organic

carbon (WEOC), and NO_3^- -N, and with 2M KCl (ratio 1:10) for measurement of NH_4^+ -N before and after the incubation. An electric conductivity meter (CM-30V TOA, Japan) and pH meter (F-52 Horiba, Japan) were used for these measuring. Concentrations of WEOC and NO_3^- -N and were analyzed using a total organic carbon (TOC) analyzer (Model TOC-5000A, Shimadzu, Kyoto, Japan) and ion chromatography (QIC analyzer, Dionex Japan, Osaka, Japan) respectively. The NH_4^+ -N concentration was measured using Spectrophotometer (UV-Vis Mini Spectrophotometer 1240, Shimadzu, Kyoto, Japan) from soil-KCl extracted with indophenol-blue addition. The microbial biomass carbon (MBC) was determined by the fumigation-extraction method with TOC analyzer (Model TOC-5000A, Shimadzu, Kyoto, Japan). The value of biochar pH (ratio 1:5) was measured with pH meter (Mettler Toledo MP 220).

Data analysis

The statistical analyses were carried out using IBM SPSS statistics software version 20. A repeated-measures ANOVA were used to determine whether the dynamic of CO_2 , CH_4 , NO, and N_2O emissions were affected by biochar (B), soil moisture (W), sampling day or their interaction during incubation. A one-way ANOVA was also applied to determine cumulative of emissions and soil properties affected by biochar at different soil moisture. Comparisons of cumulative emissions and soil properties among the different treatments were made using Tukey's honestly significant difference (HSD) tests. Tukey's HSD tests was analyzed by using Minitab 16 (Minitab, Inc, United States).

3.3. Results

Characteristic of soil and biochar

Paddy soil used for incubation experiment had initial pH of 6.41, an EC of 7.04 mSm^{-1} , a WEOC content of 237 mgkg^{-1} , a NO_3^- -N content of 0.43 mgkg^{-1} and a NH_4^+ -N content of 54.36 mgkg^{-1} . The biochars produced from wood and animal bone were both alkaline with pH of 7.65 and 7.34 (1:5, H_2O), respectively.

Greenhouse gas emissions

Carbon dioxide

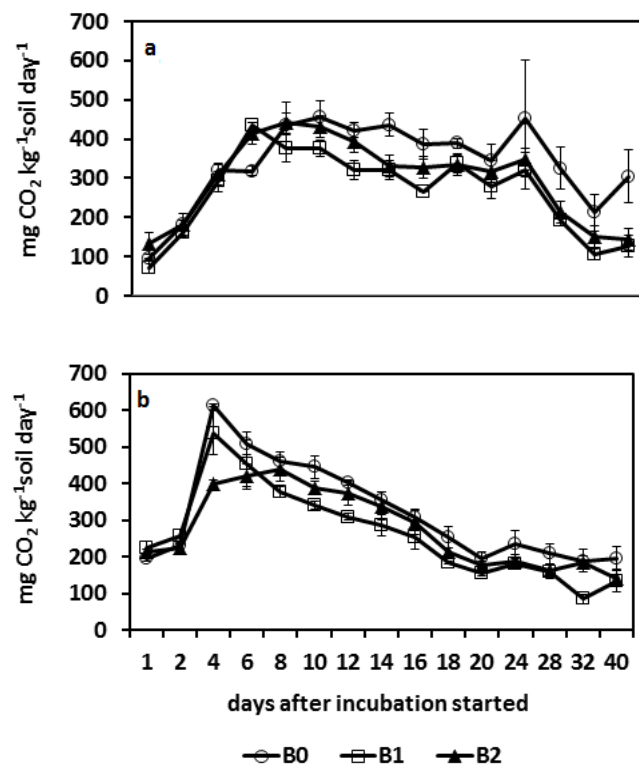


Fig. 3.1 Dynamic of CO₂ emissions influenced by biochar addition at waterlogged (a) and 60% FWC (b) during 40 days incubation. B0, B1 and B2 refer to without, wood and bone biochar. Emissions were expressed as average from four replicates. The vertical bars indicated the standard errors of means (\pm SE) from four replicates for each treatment.

The dynamics of CO₂ emission associated with each treatment during 40 days incubation is shown in Fig. 3.1. The average CO₂ emission varied from 71.39 to 613.06 mgkg⁻¹soil day⁻¹. From repeated-measures ANOVA indicated that CO₂ emission varied significantly with biochar, sampling day and interaction sampling day with biochar and soil moisture during incubation (Table 3.S1). At waterlogged, the average CO₂ emissions were higher with than without biochar at the early incubation (by 6 days incubation). Biochar significantly reduced CO₂ emissions at the later incubation. Average CO₂ emission for all treatments was steady between 300 and 450 mg kg⁻¹soil day⁻¹ until 24 days after incubation. Afterwards, CO₂ emissions gradually declined until the end incubation (Fig. 3.1a). CO₂ emissions at 60% FWC showed similar pattern and tendency for all biochar treatments, peaked at after 4 days incubation and then gradually declined (Fig. 3.1b). The reduction of CO₂ emissions by biochar was also observed at 60% FWC during incubation. In terms of cumulative emissions during 40 days incubation, CO₂ emissions under waterlogged were 5079, 3976 and 4470 mg kg⁻¹soil for B0, B1, and B2 treatments, respectively (Fig. 3.2a). Biochar significantly reduced cumulative CO₂ emissions by 21.7% and 12.0% for the treatment B1 and B2, respectively compared without biochar. At 60% FWC, with B1 or B2, the total amount of CO₂ reduced by 17.9% (3938 mg kg⁻¹soil) and 13.2% (4163 mg kg⁻¹soil), respectively than soil without biochar (4794 mg kg⁻¹soil) (Fig. 3.2b). Wood biochar addition was much more effective in reducing the cumulative CO₂ emissions at both soil moistures than in bone biochar (Fig. 3.2). Biochar addition

at different soil moistures exhibited almost similar total amount of CO₂ emissions (Fig. 3.2a and 3.2b).

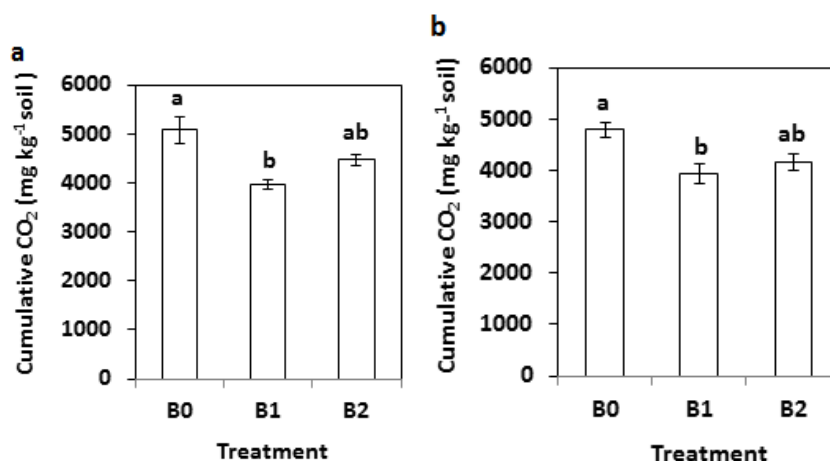


Fig. 3.2 Cumulative CO₂ emissions without and with biochar at waterlogged (a) and 60% FWC (b) during incubation. The vertical bars indicated the standard errors of means (\pm SE) from four replicates for each treatment. Significant differences between the means are indicated by different letters (Tukey's HSD test). Treatment codes described as: *B0* without biochar, *B1* wood biochar, *B2* bone biochar.

Methane

In this experiment, the temporal dynamic of CH₄ emission during incubation followed similar trends in all treatments and the average CH₄ emission ranged from -0.01 to 0.05 mg kg⁻¹soil day⁻¹ (Fig. 3.3). The greatest CH₄ emissions occurred within 6 days after the start of the incubation and then rapidly declined and were steadily low throughout the 40-days incubation for all treatments. Statistical analysis indicated that CH₄ emissions were significantly enhanced due to biochar addition at both soil moistures (Supplemental Table 3.1, Fig. 3.3). The effect of biochar addition on soil CH₄ emissions was different at the different soil moisture and their significant interaction also occurred on CH₄ emission. As

shown in Fig. 3.4, cumulative CH₄ emission for 40 days incubation ranging from 0.02 to 0.10 mg kg⁻¹soil.

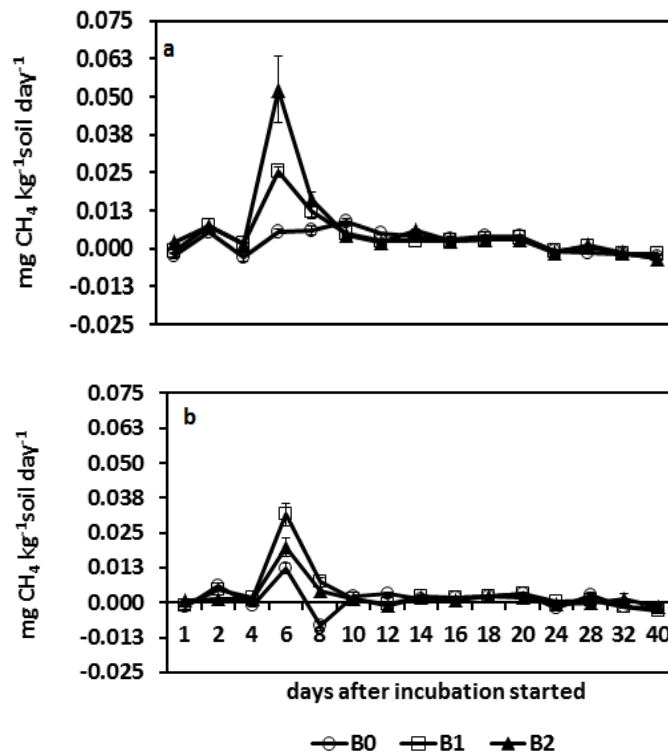


Fig. 3.3 Dynamic of CH₄ emissions influenced by biochar addition at waterlogged (a) and 60% FWC (b) during 40 days incubation. B0, B1 and B2 refer to without, wood and bone biochar. Emissions were expressed as average from four replicates. The vertical bars indicated the standard errors of means (±SE) from four replicates for each treatment.

At waterlogged, compared with no biochar (0.03 mg kg⁻¹soil), the cumulative of CH₄ emissions enhanced by 2.0 times (0.06 mg kg⁻¹soil) and 3.3 times (0.10 mg kg⁻¹soil) higher in the treatment B1 and B2, respectively (Fig. 3.4a). Similarly, a stimulator effect of biochar addition on CH₄ emissions was also observed at 60% FWC. Biochar resulted in 2.5-fold (0.05 mg kg⁻¹soil) and 1.5-fold (0.03 mg kg⁻¹soil) higher in the treatment B1 and B2, respectively (Fig. 3.4b).

¹soil) higher CH₄ emissions in B1 and B2 treatments than that of the B0 treatment (0.02 mg kg⁻¹soil) (Fig. 3.4b). At waterlogged, bone biochar had much more stimulating effect than wood biochar on total amount of CH₄ emissions throughout incubation. However, wood biochar emitted greater total amount of CH₄ emission than bone biochar at 60% FWC. The cumulative amount of CH₄ emission from waterlogged was higher than in 60% FWC for corresponding biochar treatment. Here the waterlogged treatment had a 1.2-2.8 times greater CH₄ emissions than the 60% FWC treatment.

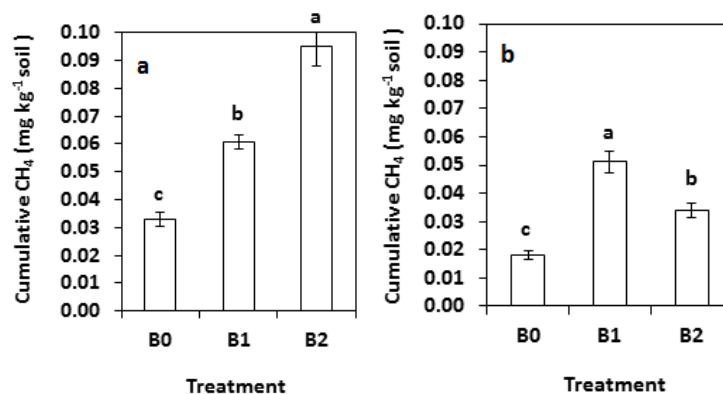


Fig. 3.4 Cumulative CH₄ emissions without and with biochar at waterlogged (a) and 60% FWC (b) during incubation. The vertical bars indicated the standard errors of means (\pm SE) from four replicates for each treatment. Significant differences between the means are indicated by different letters (Tukey's HSD test). Treatment codes described as: *B0* without biochar, *B1* wood biochar, *B2* bone biochar.

Nitric oxide

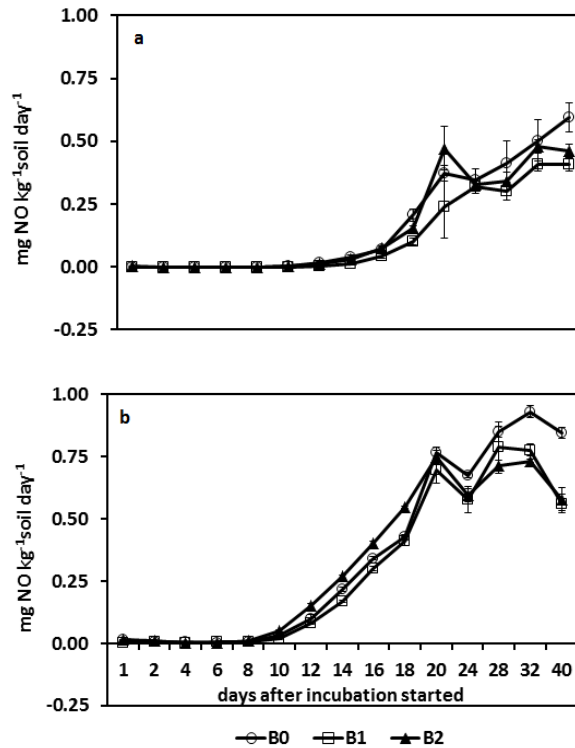


Fig. 3.5 Dynamic of NO emissions influenced by biochar addition at waterlogged (a) and 60% FWC (b) during 40 days incubation. B0, B1 and B2 refer to without, wood and bone biochar. Emissions were expressed as average from four replicates. The vertical bars indicated the standard errors of means (\pm SE) from four replicates for each treatment.

As seen in Fig. 3.5, in all treatments, similar trend of the dynamic NO emission were observed during incubation, varying between -0.002 and 0.931 mg kg⁻¹ soil day⁻¹. Variation of NO emissions was significantly affected by biochar, soil moisture and their interaction with sampling day (Table 3.S1). Up to the first 10 days after incubation, NO emissions were almost not detected (<0.05 mg kg⁻¹ soil day⁻¹), gradually increased and peaked at 20 and 32 days after incubation then declined for all treatments, except in treatment without biochar at waterlogged

condition. In the case of cumulative emissions throughout incubation period, total amount of NO emissions at waterlogged were 2.56, 1.82 and 2.35 mg kg⁻¹soil in B0, B1, and B2 treatments, respectively (Fig. 3.6a). B1 and B2 treatment suppressed NO emissions by 28.9% and by 8.2% compared B0 treatment. A similar to waterlogged, cumulative NO emissions at 60% FWC were also reduced by addition B1, 4.40 mg kg⁻¹soil (15.7%) and B2, 4.83 mg kg⁻¹soil (7.5%) than that B0 (5.22 mg kg⁻¹soil) (Fig. 3.6b). Wood biochar emitted smaller amount of NO emissions than bone biochar addition at both soil moistures. Average cumulative NO emission from waterlogged was 2.0-2.4 times smaller than that from 60% FWC for all corresponding biochar treatments (Fig. 3.6a and 3.6b).

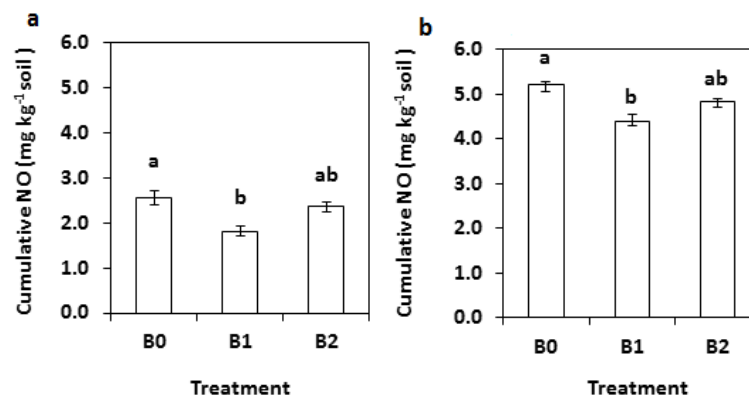


Fig. 3.6 Cumulative NO emissions without and with biochar at waterlogged (a) and 60% FWC (b) during incubation. The vertical bars indicated the standard errors of means (\pm SE) from four replicates for each treatment. Significant differences between the means are indicated by different letters (Tukey's HSD test). Treatment codes described as: *B0* without biochar, *B1* wood biochar, *B2* bone biochar.

Nitrous oxide

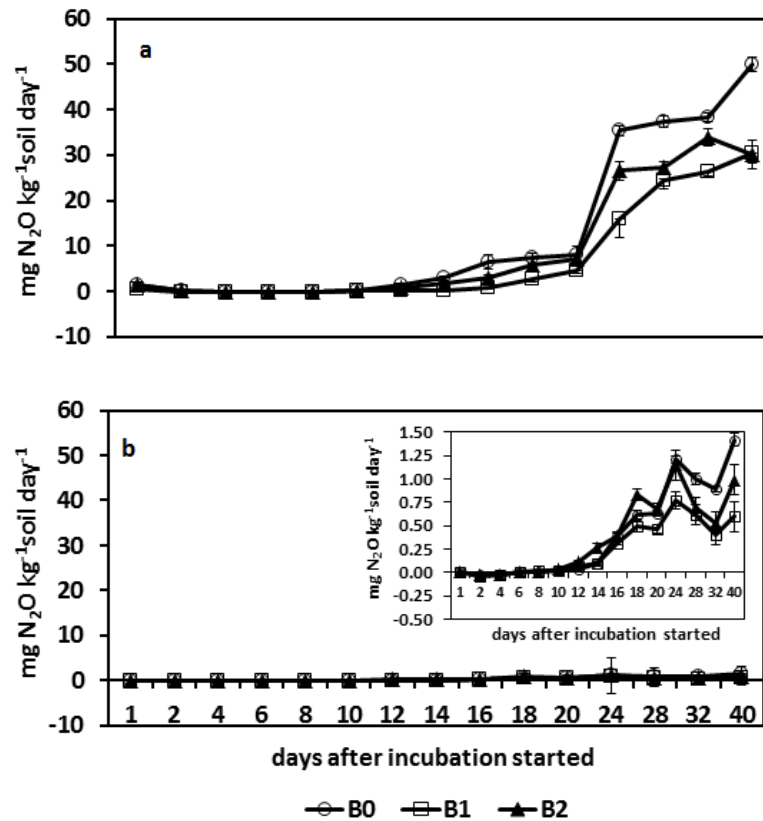


Fig. 3.7 Dynamic of N₂O emissions influenced by biochar addition at waterlogged (a) and 60% FWC (b) during 40 days incubation. B0, B1 and B2 refer to without, wood and bone biochar. Emissions were expressed as average from four replicates. For the clarity, a panel describing N₂O emission for 60% FWC during 40 days incubation was inserted. The vertical bars indicated the standard errors of means (\pm SE) from four replicates for each treatment.

The treatment and the dynamic of N₂O emission during incubation were presented in Fig. 3.7. Nitrous oxide emission throughout incubation ranged from -0.04 to 49.89 mg kg⁻¹soil day⁻¹. Soil N₂O emissions were affected significantly by biochar, soil moisture, sampling days and interaction among them (Supplemental Table 3.1). At waterlogged, in no biochar treatment, N₂O emission

were prolonged increase until the end incubation, while in the biochar-added soil, all N₂O emissions occurred until one week before the end incubation (Fig. 3.7a). Until the end incubation, N₂O emissions were remained small (-0.04 to 1.15 mg kg⁻¹soil day⁻¹) at 60% FWC (Fig. 3.7b). Nitrous oxide emissions at waterlogged (Fig. 3.7a) were greater than those from corresponding treatment at 60% FWC (Fig. 7b). The cumulative N₂O emissions at waterlogged were 189, 106, and 138 mg kg⁻¹soil for B0, B1 and B2 treatments respectively. As compared with no biochar, N₂O emission significantly reduced by 43.9% and 27.0% by B1 and B2 (Fig. 3.8a). Although a relatively lower average of cumulative N₂O emissions (< 6.3 mg kg⁻¹soil) at 60% FWC, there was a reduction by 39.9% and 10.4% for B1 and B2, respectively (Fig. 8b). Addition of wood biochar was much more effective in reducing N₂O emissions at both soil moistures. The average cumulative N₂O emission from waterlogged was 25-30 times greater than those from corresponding 60% FWC (Fig. 3.8a and 3.8b).

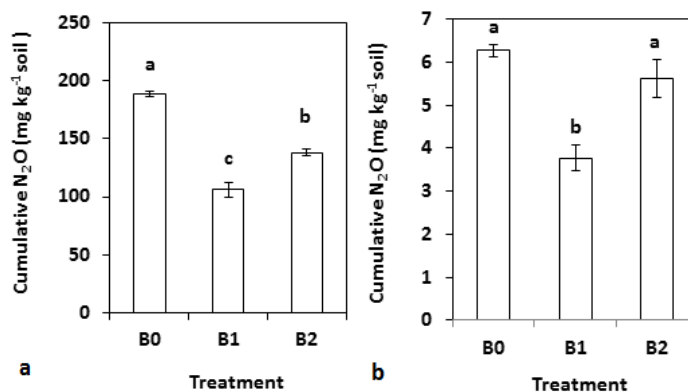


Fig. 3.8 Cumulative N₂O emissions without and with biochar at waterlogged (a) and 60% FWC (b) during incubation. The vertical bars indicated the standard errors of means (\pm SE) from four replicates for each treatment. Significant differences between the means are indicated by different letters (Tukey's HSD test). Treatment codes described as: *B0* without biochar, *B1* wood biochar, *B2* bone biochar.

Soil properties

Soil properties in response to biochar addition at each soil moisture after 40 days incubation are presented in Table 3.1. Biochar had no effect on soil EC at both soil moistures, which was an average of 101.98 mSm^{-1} to 129.43 mSm^{-1} across all treatments. Soil pH was significantly affected by biochar. At waterlogged, biochar addition significantly increased soil pH up 2.0 units, while no significant difference with B2 and a reduction of 0.4 unit with B1 at 60% FWC. The mean MBC and NH_4^+ -N content for all treatments fluctuated ranged 206.19 mgkg^{-1} to 268.67 and 953.50 mgkg^{-1} to 1092.78 mgkg^{-1} , respectively. No overall treatment effects were observed at each soil moisture. Biochar addition resulted in significant reduction in soil WEOC. At waterlogged, soil added B1 and B2 biochar decreased WEOC by 33.36% and 29.91%, respectively as compared without biochar. Meanwhile, at 60% FWC B1 and B2 treatments decreased WEOC with decreases of 39.58% and 25.37%, respectively than B0. NO_3^- -N content were significantly lower because of biochar addition at waterlogged, conversely it resulted in significant increase in NO_3^- -N content at 60% FWC compared without biochar treatment.

Table 3.1 Soil EC, pH, MBC, WEOC, NO₃⁻-N and NH₄⁺-N (mean±standard error, n = 4) following biochar addition at each soil moisture after 40 days incubation

Treatment		EC (mSm ⁻¹)			pH			MBC (mgkg ⁻¹)			WEOC (mgkg ⁻¹)			NO ₃ ⁻ -N (mgkg ⁻¹)			NH ₄ ⁺ -N (mgkg ⁻¹)								
W1	B0	129.10	±	6.33	a	5.65	±	0.12	b	230.22	±	27.09	a	573.78	±	10.91	a	87.34	±	14.56	a	1038.79	±	56.86	a
	B1	121.93	±	7.69	a	6.29	±	0.03	a	249.71	±	20.10	a	382.37	±	8.48	b	34.71	±	8.38	b	1010.08	±	58.20	a
	B2	129.43	±	11.59	a	6.45	±	0.06	a	206.19	±	32.40	a	402.19	±	8.60	b	33.25	±	11.33	b	1092.78	±	27.28	a
W2	B0	105.53	±	3.38	a	6.87	±	0.01	a	239.99	±	16.45	a	473.13	±	5.81	a	15.14	±	1.25	c	957.65	±	43.63	a
	B1	108.73	±	5.20	a	6.52	±	0.08	b	268.67	±	24.27	a	285.88	±	28.78	b	52.03	±	4.74	a	998.19	±	52.70	a
	B2	101.97	±	4.68	a	6.90	±	0.05	a	259.44	±	14.56	a	353.10	±	36.88	b	31.48	±	2.11	b	953.50	±	58.82	a

Different letter within a column indicate significant difference between the treatments at P < 0.05

3.4. Discussion

Carbon dioxide

A stimulating effect of biochar addition on CO₂ emission (Wang *et al.* 2011; Wang *et al.* 2012; Lin *et al.* 2015) was also observed by our study at early incubation at waterlogged condition (Fig. 3.1a). The initial increase in CO₂ emission on biochar addition than in no biochar could be attributed by short-term mineralization of biochar (Smith *et al.* 2010) which may stimulate microbial activity (Zimmermann *et al.* 2011; Jones *et al.* 2011). This effect diminished by day 6 of the incubation which is in agreement with previous result (Smith *et al.* 2010). Probably due to slow decomposition of biochar under waterlogged condition because of the low O₂ availability, CO₂ emissions were almost steady constant until 24 days after incubation. Afterwards, since the labile organic C contained in biochar and soil decreased and no other sources were available, CO₂ emission at both soil moistures decreased rapidly at later until the end incubation (Fig. 3.1).

By contrast, at both soil moisture condition, biochar addition (at rate 35 t ha⁻¹) decreased cumulative CO₂ emission (Fig. 3.2), which is in a good agreement with Wang *et al.* (2011) who found that rice husk biochar at rate of 26.67 g kg⁻¹ soil (equivalent to a field application rate of 50 t ha⁻¹) had a reduced effect on CO₂ emission of paddy soil during the second 30-day incubation period. Liu *et al.* (2011) also found a calculation of CO₂ emissions revealed a 6.3 fold reduction if rice residues were transferred to biochar before being applied to paddy soils. Reduced cumulative CO₂ emissions from rice paddy soil amended with swine

manure biochar have reported by other study (Yoo and Kang, 2012). Biochar can influence CO₂ emission from paddy soil through its effects on C cycling in the soil–water–gas system. In the incubation condition, the equilibrium of CO₂ concentration was established between the air and water phases. Under more alkaline conditions, more CO₂ was dissolved in the water phase (Liu *et al.* 2011). The increased soil pH due to biochar addition would be responsible for the reduction CO₂ emissions. Therefore, soil added with wood biochar (pH 7.65) had smaller cumulative CO₂ emissions than in bone biochar (pH 7.34) (Fig. 3.2). In addition, on biochar surfaces which have high pH and abundant alkaline metals, CO₂ precipitates as carbonates, explains the decrease in CO₂ (Joseph *et al.* 2010; Lehmann *et al.* 2011). In this case, biochar addition may also have suppressed the microbial respiration of the soil microbial community (Yoo and Kang, 2012). Changes in the microbial community composition or in enzyme activities are responsible for lower mineralization of soil C observed with biochar addition. The activity of two carbohydrate-mineralizing enzymes decreased after biochar addition therefore reducing CO₂ emissions (Jin, 2010). Moreover, slow decay of biochar in soils due to recalcitrant form of carbon in biochar returns a small amount of CO₂ to the atmosphere (Woolf *et al.* 2010). Water extractable organic C (WEOC) is a measure of the readily available resource for microbial growth and biological decomposition (Liang *et al.* 1996; Jensen *et al.* 1997) and had strong relationship with CO₂-C mineralization (Paul and Beauchamp, 1989). Our study resulted lower WEOC because of biochar addition (Table 3.1) at each soil moisture, it may help to explain the smaller amount of CO₂ emissions emitted

during incubation. Biochar addition at different soil moistures exhibited almost similar total amount of CO₂ emissions (Fig. 3.2a and 3.2b), which was consistent with what was already reported (Bruun *et al.* 2011).

Methane

Soil pH increased by biochar addition (Table 3.1) could affect the methanogen activity in soils. This may explain the enhancing CH₄ emission at the early incubation (first 6 days after incubation) at both soil moistures (Fig. 3.3). The activity of methanogens is usually optimum around neutrality or under slightly alkaline conditions and is very sensitive to variations in soil pH (Le Mer and Roger, 2001 and references therein). Emission of CH₄ formed during biochar production from the pore and or surface biochar (Spokas and Reicosky, 2009, Yu *et al.* 2013) may be another reason for the initial increase in CH₄ emission at both soil moistures. A great provision of substrate (CO₂) at initial incubation (Fig. 3.1a and 3.1b) which promoted methanogenic activity, subsequently enhanced CH₄ emission at the first week incubation (Fig. 3.3a and 3.3b), which also supports our result.

The addition of biochar in soil had been proposed to reduce CH₄ emission. Reduction in CH₄ emission from paddy soil treated with biochar has been reported (Karhu *et al.* 2011; Liu *et al.* 2011; Feng *et al.* 2012; Yoo and Kang, 2012; Dong *et al.* 2013). However, in this study, biochar promoted CH₄ emissions over the incubation at both soil moistures. This result is agreed to some previous studies from paddy soil (Knoblauch *et al.* 2008; Zhang *et al.* 2010;

Knoblauch *et al.* 2011; Wang *et al.* 2012; Yu *et al.* 2013). Amount of CH₄ emissions will vary and depend on soil type (Spokas and Reicosky, 2009; Zheng-Qin *et al.* 2007), feedstock material, pyrolysis and properties of biochar used (Spokas and Reicosky, 2009; Van Zwieten *et al.* 2010) water management (Yu *et al.* 2013) and different land uses (Wang *et al.* 2012)

Emission of CH₄ is the net effect of two processes, CH₄ production by methanogens and CH₄ uptake or consumption by methanotrophs (Knowles, 1993). The net emission will be positive or negative depending on the relative magnitudes of these processes. The labile organic C contained in biochar which provided a source of methanogenic substrate and created locally anaerobic microsite in soil favoring CH₄ production (Van Zwieten *et al.* 2009) may be responsible for enhancing cumulative CH₄ emission in biochar treatment at both different soil moistures (Fig. 3.4). Soils with more labile C tend to have a higher microbial biomass. However in our result, no significant change in microbial biomass carbon was detected between without and with biochar at each soil moisture condition after 40 days incubation (Table 3.1). An inhibitory effect of chemical in the biochar on the activity of methanotrophs as found by Spokas *et al.* (2010) was also may partly be a reason for the increased CH₄ emissions.

Biochar increased the soil porous by its high porosity and large surface (Downie *et al.* 2009; Spokas and Reicosky, 2009). But, under high moisture content, the soil porous spaces were filled with water and thus restricted CH₄ and O₂ diffusion and thereafter limiting in CH₄ consumption (Gulledge and Schimel, 1998). These may probably contribute the higher cumulative CH₄ emissions at waterlogged

condition (Fig. 3.4a) than that in 60% FWC (Fig. 3.4b) on corresponding biochar treatment. Methanotrophs require oxygen for CH₄ oxidation (Dalal *et al.* 2008) and their activity is highest at around 60% WFPS and decrease at above this moisture content (Castro *et al.* 1995; Karhu *et al.* 2011) may also explain our result. Yu *et al.* (2013) was also confirmed that under the high soil moisture, 85% and 100% water filled pore space (WFPS), biochar enhanced CH₄ emissions from forest and paddy soil, meanwhile biochar increased CH₄ uptake at 35% and 60% WFPS compared without biochar.

Nitric oxide

The dynamic of NO presented in figure 3.5 suggest that nitrification and denitrification process were involved for NO production in this present study. Other main process of NO forming, namely chemodenitrification (Pilegaard, 2013) may not be responsible for NO production, due to the soil pH at before (data was not shown) and after incubation (Table 3.1) was around neutral. Soil moisture is one of the factors thought to be important in the regulation of NO emission (Venterea *et al.* 2005; Pilegaard, 2013). High moisture content impeded aeration of the soil which reduces the microbial activity and or restricted gas diffusivity, as a result low NO emission found at waterlogged (Fig. 3.5a) than in at 60% FWC (Fig. 3.5b) on correspond treatment and sampling day. Findings of Venterea *et al.* (2005) that NO production was highly sensitive to WFPS and decreased substantially as WFPS increased from 21 to 63% support our result.

The production and consumption of NO in soil is a result of both microbial activity and chemical reaction. The main processes involved are microbial nitrification and denitrification, and chemodenitrification. Therefore, N availability, mainly in the form of NO_3^- and NH_4^+ , soil moisture and soil pH are controlling factor on forming of NO emission (Pilegaard, 2013). Content of NO_3^- -N presented in Table 3.1 suggest that nitrification would have been either enhanced at 60% FWC or decreased at waterlogged on biochar treatment. And NO emissions were concomitantly reduced at both soil moistures. Reduction of NO emission from biochar treatment suggests that two kind biochars may have certain compounds (Spokas *et al.* 2010) that suppressed the denitrifying activity which converted NO_3^- -N to NO at 60% FWC. Thereby, this case may also contribute the accumulation of NO_3^- -N in biochar treated soil at 60% FWC. On the one hand, a decrease in the availability of substrate for denitrification on biochar addition, subsequently decreasing NO emission at waterlogged condition. It is well documented that biochar application increased pH owing its alkalizing effect on soil. The pH-increasing effect in biochar-added soil prevents chemical decomposition of NO_2^- to NO (Islam *et al.* 2008; Braida *et al.* 2000). Thus, increased soil pH may also be responsible for reducing NO emission (Pilegaard, 2013; Obia *et al.* 2015; Cayuela *et al.* 2014).

In spite of functioning as a transport medium for NO_3^- and NH_4^+ , soil water content also has a strong impact to the rate of O_2 supply and thereby controls whether aerobic processes such as nitrification or anaerobic processes such as denitrification dominate within the soil (Wolf and Russow, 2000). In general,

nitrification dominates at values of WFPS below 60% and denitrification dominates at values above 60%. Due to limited substrate diffusion at very low water content and limited gas diffusion at high water content, maximum NO emission generally occurs at low to medium soil water content (Pilegaard, 2013). The maximum of NO emission in our study was found at 60% FWC and decreased towards waterlogged condition. On the other hand, because NO is highly reactive, it will more readily be consumed at high soil moisture (Pilegaard, 2013). Thus, cumulative NO emission was lower at waterlogged (Fig. 6a) than in at 60% FWC (Fig. 6b) in present study. Schindlbacher *et al.* (2004) found that optimal moisture for NO emission differed significantly between the soil, and ranged between 15% WFPS in sandy soil and 65% WFPS in loamy soil.

Nitrous oxide

In this study, biochar addition with rate approximately 1.8% (equivalent to a field application rate of 35 t ha⁻¹) significantly suppressed N₂O emissions at both soil moistures condition (Fig. 3.7) and thus, to cause a smaller cumulative emission compared with no biochar at each soil moisture during the entire incubation (Fig. 3.8). Probably due to a small concentration of biochar in soil (Bruun *et al.* 2011), the potential effect of biochar to reduce N₂O emissions was observed at 12 days after incubation at both soil moistures (Fig. 3.7). Reduction in N₂O emission was observed immediately after the addition of large amounts (\geq 8-60%) biochar (Yanai *et al.* 2007; Spokas and Reicosky, 2009). At waterlogged (Fig. 3.7a), N₂O emission were several times greater than those from corresponding treatment at

60% FWC (Fig. 3.1b) suggest that denitrification was the main process to produced N_2O emission.

Reduced N_2O emissions by up 44% in this present study is in line with previous studies that biochar addition decreased N_2O emission from paddy fields (Zhang *et al.* 2010; Wang *et al.* 2012; Zhang *et al.* 2012; Singla and Inubushi, 2014). For example, study of Zhang *et al.* (2010) found that biochar amendment of 10 tha^{-1} and 40 tha^{-1} decreased N_2O emissions by 40-51% and 21-28%, respectively with or without N fertilization. N_2O emissions from soil added with biochar vary depending on the feedstock used to produce biochar, soil properties, soil moisture condition and the addition of exogenous nitrogen (Van Zwieten *et al.* 2009; Zhang *et al.* 2010; Spokas and Reicosky, 2009).

The data presented in Fig. 3.7a and Table 3.1 suggest that biochar might reduce NO_3^- -N availability, thereby it would decrease the total N denitrified and it would favor the last step of denitrification to N_2 (Weier *et al.* 1993; Cayuela *et al.* 2013). Meanwhile, at 60% FWC biochar enhanced nitrification with higher of NO_3^- -N than in without biochar (Table 3.1), however concurrently it reduced N_2O emission. The reduction suggest that biochar may have contained the inhibitory compound (Spokas *et al.* 2010) that suppress denitrification process which convert NO_3^- -N to N_2O . In addition, Cayuela *et al.* (2013) demonstrated that biochar increase soil pH and subsequently promoted the reduction of N_2O to N_2 . Moreover, effect of adding high temperature biochar which caused the decreased of N_2O emission (Ameloot *et al.* 2013; Nelissen *et al.* 2014) was probably the

reason in explaining the decreased of N₂O emission especially for bone biochar produced at 800°C.

Most N₂O emitted from agricultural soil is produced through denitrification (Cayuela *et al.* 2013 and references therein). N₂O emissions increased with increasing soil moisture or decreasing water tension have been reported from previous studies (Schindlbacher *et al.* 2004; Yanai *et al.* 2007; Bruun *et al.* 2011; Saarnio *et al.* 2013). Our result demonstrated a similar trend, namely that total amount of N₂O was much more emitted at waterlogged condition than at 60% FWC. The averaged cumulative N₂O emissions at waterlogged were 25-30 times greater than those from corresponding treatments at 60% FWC (Fig. 3.8a and 3.8b). Moreover, N₂O emissions were almost not detected at 60% FWC. Under aerobic soil condition (low water content), production of N₂O is usually restricted and originates to a greater extent from the nitrification process (Batemann and Bags, 2005). The negligible N₂O emission at lower water content was also confirmed by Yanai *et al.* (2007) and Bruun *et al.* (2011).

3.5. Conclusions

The results of our incubation study showed that biochar addition into paddy soil caused a significant decrease in cumulative CO₂, NO and N₂O emissions compared without biochar. However, it stimulated the CH₄ production than in no biochar. At waterlogged represented paddy field condition, had a greater cumulative CH₄ and N₂O emission, but smaller NO emission than in at 60% FWC which represented upland condition for corresponding biochar treatment.

Total amount of CO₂ was almost similar at both conditions. Wood biochar had great potential effect in reducing CO₂, NO and N₂O emissions. Moreover, there is an interesting potency of bone biochar effect on CH₄ emission, that was promoting at waterlogged and concomitantly depressing at 60% FWC compared to effect of wood biochar. Thus, adding soils with biochar pyrolyzed from plant and animal bone residues could be used as a means to manage C sequestration and mitigate global warming from converted paddy fields due to crop rotation.

Summary

There is an urgency to establish effective agricultural management practice to increase food production while preventing the negative agricultural impacts on the environment. One such approach is the use of biochar. Biochar is a product of thermal degradation of organic material under oxygen-limited condition, similar in its appearance to charcoal produced by natural burning but distinguished by its use as soil amendment. Biochar has unique properties that expected to be a valuable soil amendment to sustainably increase soil health and productivity, as well as an appropriate tool for sequestering carbon in soils for the long term.

Biochar is widely used as a soil amendment to increase crop yields, but its effects on the dynamics of microbial communities and minerals in soils has not been fully elucidated, particularly in soils with organic manure application. On the other hand, application of biochar for mitigating greenhouse gas (GHG) emission from paddy and upland soils has been studied extensively. However, there was little information reported on the effect of biochar on GHG emission from soil in converted paddy fields which may have different characteristics of microbial community compared with general upland soil. Considering those points above, two experiments was conducted. Firstly, a pot experiment was carried out to examine the effect of biochar on microbial community structure and mineral availability in soils growing different crop species under different organic manure treatments. Secondly, an incubation experiment was set up in order to investigate the potential effect of different types of biochar application

on GHG emissions and soil properties under different soil moisture conditions using soil from rice paddy fields.

1. Effect of biochar application on mineral and microbial properties of soils growing different plant species

A pot experiment was conducted using soybean and sorghum under four combination soil treatments (cattle farmyard manure with or without biochar and rapeseed cake with or without biochar) to examine the effect of wood biochar on microbial community structure and mineral availability in soils. Growth of both species was improved by the biochar application, particularly in sorghum with rapeseed cake application. Principal component analysis using the data of Biolog EcoPlate™ indicated that biochar application changed the microbial community structure in soil, particularly in soil grown sorghum. Biochar application had little effect on the profile of ammonium acetate-extractable mineral elements in soil with both types of manure application in soybean. In sorghum, however, biochar application altered the profile of the extractable elements in soil with rapeseed cake. This alteration is mainly due to the increase of the extractable concentration of some metals in soils. By contrast, concentrations of these metals in leaves of sorghum grown in soil with rapeseed cake did not change or tended to be decreased by the biochar application. Because biochar application increased microbial activity in soils with rapeseed cake, estimated by the average well color development in Biolog EcoPlate, biochar application possibly enhanced organic matter decomposition in soil with rapeseed cake producing chelating organic

compounds that can solubilize some metals but make them less available for sorghum root. These species-specific changes in soil properties by biochar application may be related to the superior growth in sorghum with rapeseed cake.

2. Effect of wood and bone biochars on greenhouse gas emission of paddy soil under waterlogged and upland conditions

A soil incubation experiment was conducted with the following treatments: soil treated with and without manufactured biochar (wood and animal bone) at two soil moisture conditions (waterlogged for simulating paddy field condition and 60% of the field water capacity for simulating upland field condition converted from paddy field). During the incubation, production of CO₂, CH₄, NO, and N₂O emissions from soil in each treatment were determined. Average over two different soil moisture conditions, biochar addition significantly decreased the cumulative emission of CO₂, NO, and N₂O from the soils by 16%, 15%, and 30%, respectively, while enhanced CH₄ emission by 2.3 times as compared with those without biochar. Wood biochar was more effective than bone biochar in reducing CO₂, NO, and N₂O emissions. Different soil moisture conditions significantly influenced CH₄, N₂O and NO emissions, while did not CO₂ emission. A greater cumulative of CH₄ and N₂O and smaller NO emissions were observed in soil at waterlogged conditions than in soil at 60% FWC conditions for each biochar treatment. Interestingly, bone biochar application enhanced CH₄ emission more markedly under waterlogged conditions.

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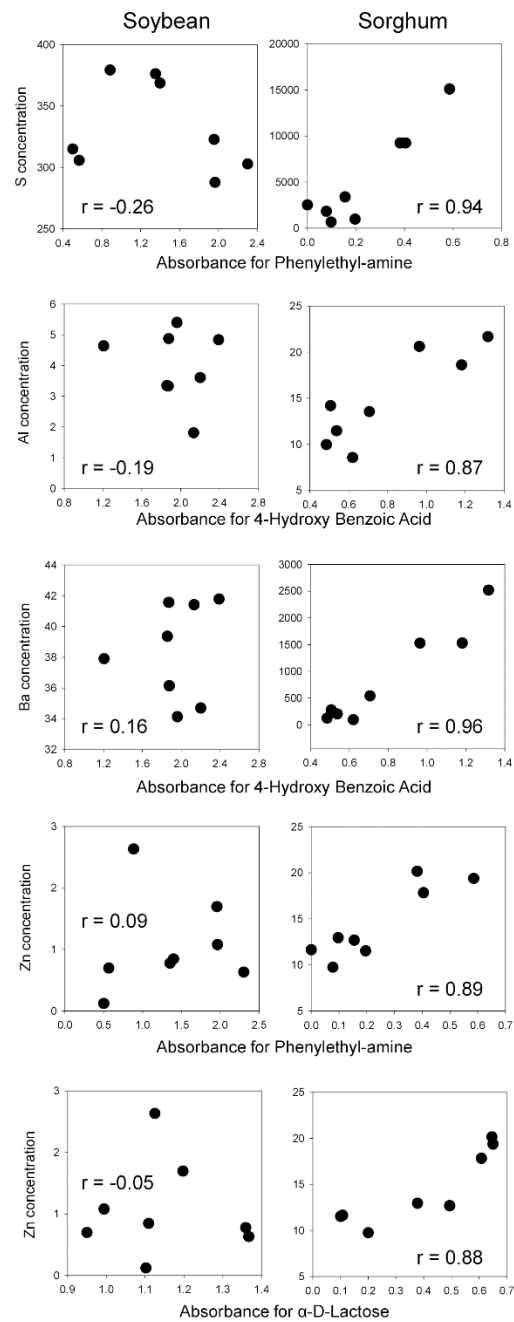
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Supplemental data

Supplemental Table 2.S1 Mineral concentration (mg kg⁻¹ dry weight) of biochar and organic manures used in this study

	P	K	Ca	Mg	Al	Fe	Mn	Na
Biochar	234 ±7	2158 ±45	3491 ±211	245 ±17	186 ±10	271 ±12	109 ±2	50 ±5
Cattle farmyard manure	17055 ±515	32465 ±370	11573 ±601	9552 ±270	1132 ±27	1827 ±193	411 ±14	12913 ±269
Rapeseed cake	12921 ±83	15555 ±366	14109 ±608	8060 ±75	28 ±1	121 ±2	85 ±6	134 ±15

Concentration of each element was determined as described in plant mineral analysis in Material and Methods section



Supplemental Figure 2.S1 Correlation between concentration of extractable element (mgkg^{-1} dry soil) in soil and utilization (absorbance in Biolog Ecoplate) of carbon source in microbial community of soil with rapeseed cake (including both with and without biochar). Only pairs with significant positive correlation ($P < 0.01$) were shown.

Supplemental Table 3.S1 F value from repeated-measures ANOVA for the effect of biochar (B), soil moisture (W), sampling day and their interaction on CO₂, CH₄, NO and N₂O emission during incubation

Source	df	CO ₂	CH ₄	NO	N ₂ O
Between subject					
B	2	8.513**	28.674***	10.626**	52.981***
W	1	1.143ns	41.401***	341.906***	1661.280***
B x W	2	0.193ns	13.579***	0.147ns	47.259***
Within subject					
Sampling day	14	78.786***	114.315***	507.340***	503.761***
Sampling day x B	28	2.168**	15.272***	4.690***	13.255***
Sampling day x W	14	19.824***	7.212***	48.263***	457.128***
Sampling day x B x W	28	1.672*	8.767***	1.713*	11.773***

*ns not significant, *P < 0.05, ** P < 0.01, *** P < 0.001*

Supplemental Table 3.S2 Summary from one-way ANOVA for the effect of biochar (B) in each soil moisture (W) on soil EC, pH (H₂O), MBC, WEOC, NO₃⁻-N and NH₄⁺-N

	Soil moisture	Degrees of freedom	Sum of squares	F value	P value
EC (mSm ⁻¹)	In waterlogged	2	143.782	0.231	0.798
	In 60% FWC	2	91.207	0.567	0.586
pH	In waterlogged	2	1.424	29.801	<0.001
	In 60% FWC	2	0.360	15.322	0.001
MBC (mgkg ⁻¹)	In waterlogged	2	3801.302	0.652	0.544
	In 60% FWC	2	1714.902	0.600	0.569
WEOC (mgkg ⁻¹)	In waterlogged	2	88633.073	125.453	<0.001
	In 60% FWC	2	71981.989	12.147	0.003
NO ₃ ⁻ - N (mgkg ⁻¹)	In waterlogged	2	7596.665	6.940	0.015
	In 60% FWC	2	2733.560	35.996	<0.001
NH ₄ ⁺ - N (mgkg ⁻¹)	In waterlogged	2	14104.464	0.718	0.514
	In 60% FWC	2	4875.752	0.225	0.803