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Investigation of Greenhouse Gas Emissions from the Soil Amended with Rice Straw Biochar

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Abstract

Biochar, which is a byproduct from pyrolysis of any kinds of biomass, has received attention recently for its potential to mitigate climate change if we are to apply it to agricultural soil. However, the effects of biochar application on greenhouse gas emissions are difficult to be generalized because we do not fully understand the mechanisms how biochar influences soil functions. In this study, Korean rice paddy soil was incubated for 30 d amended with biochars made from Chinese/Korean rice straw at low (300-400°C) and high (600°C) pyrolysis temperatures. The controls were prepared by amendment with the straw materials and nothing. Biochar addition significantly decreased the CO_2 and CH_4 evolution compared to the straw amendment. However, the FDA activity, microbial biomass, the abundance of methane related microorganisms were not changed by biochar addition. We observed an increase in the soil N_2O emissions with the biochar. We attributed it to the increased microbial nitrification followed by pH increase by biochar addition. Overall data suggests that care should be taken when we apply biochar to the rice paddy soils that are acidic and heavily fertilized, because it might stimulate N_2O emission through nitrification, although CO_2 and CH_4 are not changed or reduced.

Keywords: *biochar, climate change, greenhouse gas emission, rice paddy soil, soil improvement*

1. Introduction

Rice paddy soil is a major source of greenhouse gas emissions in the agricultural sector. Global methane emissions from flooded rice paddy soils are estimated to be 40-53 Tg per year, which account for 6-10% of the total methane emissions (IPCC, 2001; Aselmann and Crutzen, 1989; Cao *et al*., 1996; Wassmann *et al.*, 1993). Recent studies have reported that nitrous oxide emissions from paddy fields are also significant, accounting for approximately 20% of the total emissions from croplands (Xing *et al*., 2009; Zou *et al*., 2005). Therefore, many strategies have already been suggested for the reduction of greenhouse gas emissions from rice paddy fields (Shen, 2014; Wang *et al*., 2012; Wassmann *et al*., 1993). Biochar application was suggested as one of the promising options to mitigate climate change by increasing soil carbon (C) sequestration and reducing greenhouse gas emissions (Zhang *et al*., 2010). However, the effects of biochar application on soil cannot be consistently predicted, because the feedstocks for biochar include a broad range of products. Therefore, we need to determine which biochars are "effective." in terms of climate change mitigation and soil quality improvement.

One way to evaluate the effectiveness of biochar in mitigating climate change is to monitor the soil sequestration of C and additional greenhouse gas emissions after applying biochar to soils. The effects of biochar application on greenhouse gas emissions vary, depending on the kinds of raw materials of the biochar and the types of soils to be amended (Lu *et al*., 2014; Yoo *et al*., 2014; Wang *et al*., 2012; Yoo and Kang, 2012). In order to predict these inconsistent effects, we must understand the mechanisms by which biochar influences soil ecosystems. The emission of greenhouse gases, primarily CO_2, CH_4, and N_2O, is closely related to the soil C and N dynamics, which are primarily mediated by soil microbiological activities that can be influenced directly and indirectly by biochar addition. The direct effects of biochar addition on soil microbes are related to the materials on the biochar surface. Although pyrolyzed materials are believed to be chemically inert, it was reported that some labile matter still exists on the surface (Bruun *et al*., 2012; Pereira *et al*., 2011; Cross and Sohi, 2011; Zimmerman *et al.,*

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2011). The residual bio-oils and recondensation products adsorbed onto the surfaces of some types of biochar might have toxic effects on soil microorganisms (Brown *et al.*, 2006; McClellan *et al*., 2007). On the other hand, many researchers have reported that the microbial activity was enhanced when biochar was added, because the biochar surfaces can serve as favorable sites for microorganisms due to the greater concentrations of adsorbed nutrients (Baldock and Smernik, 2002; Hamer *et al*., 2004; Pietikäinen *et al*., 2000).

Microbial activity can also be influenced indirectly via changes in the soil's chemical and physical environment. It has been widely reported that biochar addition increases the soil's pH (Van Zwieten *et al*., 2010) and Cation Exchange Capacity (CEC) (Liang *et al.*, 2006), which are important factors for microbial activity. An increase in the CEC would lead to better nutrient retention and reduced leaching (Liang *et al.*, 2006; Major et al., 2010; Major, *et al.*, 2012; Yao *et al*., 2012). Many studies have observed that the total porosity and aeration in soil increased after biochar amendment, and those changes in the soil's physical conditions influenced the microbial activity by providing microhabitats, and changing the oxygen and water status (Lehmann *et al.*, 2011; Mukherjee and Zimmerman, 2013). In summary, soil microbial activity is an important factor that influences greenhouse gas emissions and the investigation of the changes in the soil microbial activity caused by biochar addition could provide us with the theoretical background needed to select an effective type of biochar.

In this study, we set out to explain the effects of biochar on the emissions of CO_2, CH_4, and N_2O. We compared the effects of biochar amendment to soil with those of its raw material, rice straw. Rice straw is the most common agricultural by-product in China and Korea, and amendment with rice straw has been widely accepted as a way to improve the soil fertility for rice production in both countries (Zou *et al*., 2005). However, since rice straw incorporation increases soil methane release, an alternative to rice straw incorporation could be the amendment of biochar made from rice straw to soils, because it could reduce the methane emissions from the rice paddy fields. In Korea, as the demand for rice straw is high for other purposes, such as forage of domestic animals, if we decided to use biochar incorporation to agricultural soils, there might be the possibility for the future utilization of Chinese rice straw as a raw material for biochar production. Considering these situations, we set up the objectives of this study as follows: 1) to compare the effects of the addition of biochars made from Chinese and Korean rice straw on the greenhouse gas emissions from Korean rice paddy soil and 2) to relate the patterns of the gas emissions with changes in the soil chemical and microbiological factors.

2. Materials and Methods

2.1 Soil and Biochar Preparation and Characterization

The soil for the incubation studies was collected from the surface (0-10 cm depth) of a rice paddy Hwasung si, Gyeonggi-do, Korea on Mar. 2013. The permission for the sampling was issued from the Gyeonggido Agricultural Research & Extension Services. Three soil cores were randomly collected, composited, and used for bulk density determination. In the laboratory, the soil samples were passed through a 2-mm sieve and air dried for 2 wks. The soil texture was determined using the hydrometer method.

We prepared rice straws from the domestic farms in China and Korea and used them as our amendments of organic matter and biochar. The owners of the land gave permission to conduct the study using the materials produced from their farms. We used the same rice species from China and Korea, which is *Oryza sativa L*., to reduce the variability of feedstock itself. The straws from China and Korea were air dried and chopped into 1 cm length and stored at a cool and dry place before application. We prepared four different biochars from Chinese and Korean rice straws at low (300-400ºC) and high (600ºC) temperatures. From Chinese rice straw, C-Char400 and C-Char600 were produced and from Korean rice straw, K-Char300 and K-Char600 were produced. In Korea, rice straw material is already widely used for multiple purposes, so it might not be economically efficient to use it as a feedstock for biochar production. Therefore, we could not exclude the possibility of importing Chinese rice straw as biochar feedstock if we wanted to apply biochar to soils as a new management practice. This possible scenario is the reason to compare the effects of biochar made from Chinese and Korean rice straw.

The pH of the soil and biochar was determined with a glass electrode using a 1:1 (w/v) and 1:5 (w/v) soil- and biochar-to-deionized-water ratio, respectively. The bulk densities of the amendments were measured by measuring the dry weight of the materials of the known volume using mass cylinder. The total C and N contents were determined by combustion analysis using a Carlo Erba NS 1500 C/N analyzer (Carlo Erba, Milan, Italy). Hot water extractable C (HWC) was measured following the method by (Haynes and Francis, 1993). The NO_3^- and NH_4^+ concentrations were determined using the salicylate microplate method (Sims *et al*., 1995).

2.2 Incubation

The soil microcosms were constructed using 0.30 L glass jars with a septum. Each jar contained 50 g of oven-dry-weight-equivalent soil. The treatments consisted of amendment with Chinese biochar produced at 400ºC (C-Char400), Chinese biochar produced at 600ºC (C-Char600), Korean biochar produced at 300ºC (K-Char300), and Korean biochar produced at 600ºC (K-Char600). The treatment samples were compared with working controls amended with Korean rice straw (K-straw), Chinese rice straw (C-straw), or no addition (No-Add). The application rate of biochar was 4% by weight, which was in the middle level of the additions reported by Kolb *et al.* (2009) and Yanai *et al.* (2007). After the biochar was added, the soil was adjusted to 200% Water Filled Pore Space (WFPS) in order to maintain the waterlogged conditions and then it was incubated in the dark at

25ºC for 30 days. We applied mineral N as $(NH_3)_2CO_3$ at a 200 kg N ha^{-1} rate to all of the treatments including the controls. Our incubation experiment was performed with six replications. After 15 days, half of the samples were used to analyze the soil's chemical and biological parameters, and the results were labeled as 15D samples. When the incubation was over after 30 days, the rest of the samples were analyzed and labeled as 30D samples. During the incubation, the glass containers were sealed except when the lids of the containers were opened every 3d in order to aerate the microcosms. The headspace was recirculated with ambient air.

2.3 Measurements

Gas samples from the headspace were collected at 0, 0.5, 1, 3, 6, 15, 21, and 30 d after the initiation of the incubation. The concentrations of CO_2, CH_4, and N_2O were measured using gas chromatography (Agilent 7890A, USA) with two detectors. The CO_2 and CH_4 were detected using a hydrogen Flame Ionization Detector (FID), and the N_2O was detected using an Electron Capture Detector (ECD). The gas fluxes were calculated from the changes in the headspace concentration over the measured period using the following equation (Troy *et al*., 2013).

$$Flux = \frac{dGas}{dt} \times \frac{V}{A} \times \frac{p \times 100 \times MW}{R} \times \frac{273}{273 + T} \quad (1)$$

where *dGas/dt* is the change in the gas concentration over time; V is the volume of the incubation container; *p* is the atmospheric pressure; MW is the molecular weight of the gas; *R i*s a gas constant, 8314 $Jmol^{-1}K^{-1}$; A is the area of the container; and T is the temperature in Celsius.

After 15 d and 30 d of incubation, destructive soil sampling was conducted and the samples were labeled 15D and 30D, respectively. In order to measure the soil C sequestration after 30 d of incubation, the total C was measured by combustion analysis using a Carlo Erba NS 1500 C/N analyzer (Carlo Erba, Milan, Italy). In order to determine the labile C content, the hot water extractable C (HWC) was measured following the method by Haynes and Francis (1993). In order to investigate the N availability, the NH_4^+–N and NO_3^- –N concentrations were determined using 2M KCl extraction and colorimetric methods (Sims *et al*., 1995).

The microbial biomass C was measured using the $CHCl_3$ fumigation extraction method (Vance *et al*., 1987). The 0.5 M K_2SO_4 solution was used in order to extract the fumigated and unfumigated samples, and the C contents in the extracts were analyzed using a TOC analyzer (TOC-V, Shimadzu, Japan). The overall soil microbial enzymatic activity was evaluated using the Fluorescein Diacetate (FDA) hydrolysis method Adam and Duncan (2001). The fumigation–extraction method was used to measure the microbial C (Solaiman, 2007; Vance *et al.*, 1987), with 0.45 as the extraction factor. The soil enzymatic activity involved in the decomposition of specific compounds was measured using methylumbelliferyl compounds as model substrates (Kang and Freeman, 1999). The enzymes under analysis were b-glucosidase, cellobiohydrolase, and N-acetylglucosaminidase. These enzymes play a key role in the decomposition of cellulose, hemicelluloses, and chitin, respectively. In order to estimate the abundance of methane-related microorganisms, real time quantitative PCR was performed using an I-Cycler TM (Version 3.0a, Bio-Rad, Hercules, CA, USA) and SYBR Green (Bio-Rad) as the detection system in a reaction mixture of 30 ml with a specific primer for each group. The detailed method can be found in the study by Seo *et al.* (2014).

2.4 Statistical Analysis

Analysis of variance was performed using the MIXED procedure of SAS 9.1 (SAS Institute, 2001) on the soil CO_2, CH_4, and N_2O emission rates; HWC; microbial biomass C; FDA activity; exo-enzyme activities (b-glucosidase, cellobiohydrolase, N-acetylglucosaminidase); abundance of methanogens and methanotrophs; NH_4^+ and NO_3^- –N contents; and pH. The treatments and dates were the fixed variables for the soil gas analysis. The least squares means were used to test for significant differences among the treatments at the 5% probability level. For the CH_4 and N_2O emission rates, comparisons were made at two levels: among the straw-added treatments (C-Straw and K-Straw) and the No-Add control, and among the biochar amendments (C-Char400, C-Char600, K-Char300, and K-Char600) and the No-Add control due to a huge difference between the values from the straw and biochar treatments.

3. Results and Discussion

3.1 Characteristics of the Soil and Biochar

The physicochemical properties and elemental analysis of the soil, feedstock, and biochar are shown in Table 1. The biochar and straw were both alkaline, with the highest pH in the K-Char600 and the lowest in the K-Straw. The elemental analysis of the rice straw and biochar revealed that the atomic H/C ratio of the rice straw was 1.50 on average and it was reduced to 0.76 for the biochar produced at a low temperature (C-Char300 and K-Char400) and 0.31 for the biochar produced at a high temperature (C-Char600 and K-Char600). This result indicates that the higher the pyrolysis temperature, the higher the aromaticity of the biochar. The high aromaticity of the biochar is also consistent with the amount of labile C contained in the biochar as suggested by Krull *et al.* (2011). The HWC content was higher in the C-Char400 and K-Char300, than in the C-Char600 and K-Char600. The C/N ratios of the rice straw were higher than those of the biochars. The Chinese rice straw and biochars had lower C/N ratios than the Korean ones, which indicated more extensive N fertilization in China than in Korea with the exception of the C-Char600, which had a similar C/N ratio to the K-Char600. We will further discuss the relationship between the C/N ratios of the amended materials and the greenhouse gas emissions later in this study. The bulk densities of the rice straw and the biochar were similar with an average of

Table 1. Physico-chemical Characteristics of Soil and aMended Materials

pH	Bulk Density	Total C	Total N	C/N ratio	NH_4^+	NO_3^-	P_2O_5	SiO_2	K	Ca	Na	Mg
	g cm^{-1}	g kg^{-1} soil			mg kg^{-1} soil							
5.4 (1:5)	0.87	14.07	1.18	11.92	8.18	4.21	44	68	0.23	6.7	0.33	1.47

		pH	Bulk Density	C	N	H	C/N ratio	H/C ratio	HWC	NH_4^+	NO_3^-
			g cm^{-1}	%					mg kg^{-1} soil		
Amend-ment	C-Straw	8.31	0.12	38.80	0.49	4.89	79.18	1.51	31346.0	14.13	7.14
	K-Straw	7.19	0.12	40.18	0.31	4.99	129.61	1.49	31425.2	41.15	2.76
	C-Char400	7.84	0.10	61.71	1.27	3.41	48.59	0.66	3189.0	2.77	5.49
	C-Char600	9.86	0.14	72.83	1.06	1.79	68.71	0.30	204.0	0.61	0.56
	K-Char300	6.75	0.16	50.50	0.89	3.62	56.74	0.86	9414.8	3.20	1.51
	K-Char600	10.54	0.13	50.31	0.79	1.35	63.68	0.32	1772.0	0.74	2.46

0.13 g cm^{-3}.

3.2 Soil CO_2 and CH_4 Emissions and Related Microbial Activities

The accumulated amount of soil CO_2 emissions was significantly influenced by the treatments (Fig. 1(a)). The addition of C-straw and K-straw significantly increased the soil CO_2 emission rates in comparison to the No-Add control. The soils amended with all of the kinds of biochar emitted significantly less CO_2 than the straw-amended soils. The C-Char400 and K-Char300 treatments showed slightly higher CO_2 emissions than the C-Char600 and K-Char600 treatments during the first 15 days (0-15 days). For the accumulated CO_2 during days 16-30, the pattern of lower CO_2 emission rates in the biochar-treated soil compared to those of the straw-added soils was maintained, but the CO_2 emissions were higher in all of the biochar treatments than in the No-Add control. The higher CO_2 emission rates from the C-Char400 and K-Char300 compared to those from the C-Char600 and K-Char600 were related to the higher HWC contents in the C-Char400 and K-Char300 biochars (Table 1), and this result was consistent with the results of the study by Bruun *et al.* (2012). They reported that the biochar produced from fast pyrolysis at low temperatures might still contain bio-available C for the microbial population. Jones *et al.* (2011) and Zimmerman *et al.* (2011) observed an increase in the CO_2 emissions from the soils with biochar amendments compared to the non-amended soils, especially during the initial stage after application. However, J ones *et al.* (2011) argued that the initial C loss from the increased CO_2 evolution was negligible compared to the amount of C stored within the biochar itself. The results from the longer incubation and field experiments tended to show lower emission rates of CO_2 from biochar-treated soils, which indicated the resistant characteristics of biochar (Liu *et al*., 2011; Lu *et al.*, 2014). The HWC content that remained in the treated soils after 15 and 30 days of incubation revealed that the HWC content remained higher in the straw-added soils than in the biochar-added soils (Fig. 2(a)). The HWC contents in the biochar-added soils were similar to or lower than those in the No-Add control. The data on CO_2 emission and HWC implied that additional HWC contents derived from biochar might have been decomposed during the 30 d of incubation although we did not consider the priming effect of biochar on the existing soil C. Contrary to our expectations, the lower C/N ratio of the Chinese straw and biochar did not significantly influence the CO_2 emission patterns. Rather, the effects of the pyrolysis temperature were more apparent in the results of the CO_2 evolution pattern.

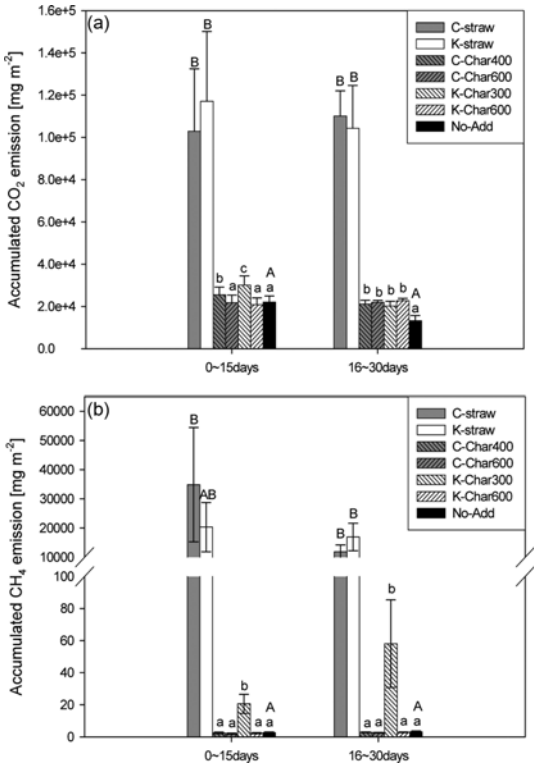

Fig. 1. Changes in Emission Rates on Accumulated Evolution Influenced by Treatments of: (a) CO_2, (b) CH_4 During 0-15 days and 16-30 days (Multiple comparisons were made among C-Straw, K-Straw, and No-Add represented as capital letters and among C-Char400, C-Char600, K-Char300, K-Char600, and No-Add represented as lower case letters. Different letters stand for significant difference at a 5% probability level)

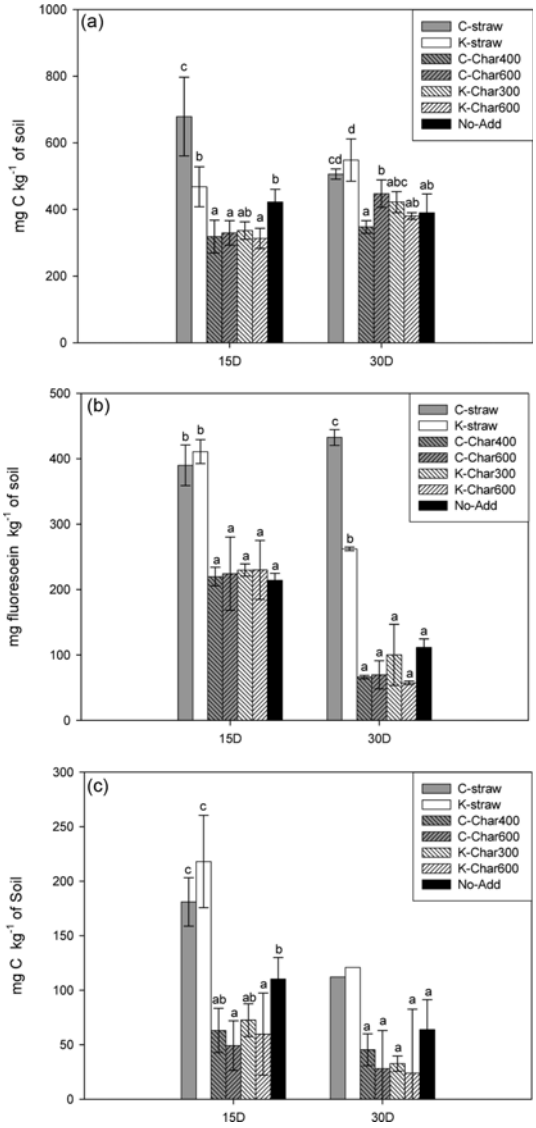

Fig. 2. Changes in: (a) Hot Water Extractable C (HWC), (b) FDA Activity, (c) Microbial Biomass C Influenced by Treatments for the 15D and 30D Samples (Multiple comparisons were made among C-Straw, K-Straw, and No-Add represented as capital letters and among C-Char400, C-Char600, K-Char300, K-Char600, and No-Add represented as lower case letters. Different letters stand for significant difference at 5% probability level)

The FDA activity was significantly increased in the straw-amended soils, but there was no treatment effect in the biochar-added soils (Fig. 2(b)). The microbial biomass C was also increased in the straw-amended soils compared with the No-Add soil (Fig. 2(c)). However, in the C-Char400 and K-Char300 soils, the microbial biomass C was not changed, while in the C-Char600 and K-Char600 soils, a slight decrease was observed. The microbial biomass C contents had high correlation coefficients with the CO_2 emission rates, FDA activities, and HWC contents, while the HWC contents and FDA activities had a relatively lower correlation with the CO_2 emission rates (Table 2). Sánchez-

Table 2. Pearson Correlation Coefficients between CO_2 Emission Rate, Hot Water Extractable C (HWC), Fluoresecein Dehydrogenase Activity (FDA), and Microbial Biomass C (MBC)

	CO_2 emission rate	HWC	FDA	MBC
CO_2 emission rate	1	0.4750	0.4797*	0.6961***
HWC		1	0.4130	0.7286***
FDA			1	0.7289***
MBC				1

*, **, *** indicates the significance level at 10, 5, and 1% probability levels, respectively.

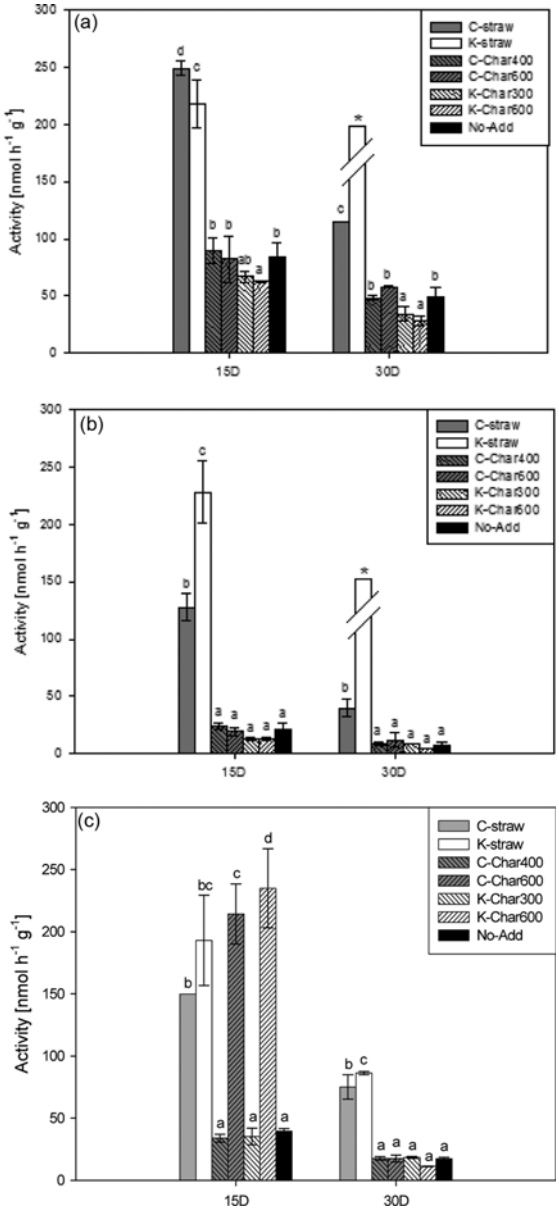

Fig. 3. Changes in Activities of: (a) b-glucosaminidase, (b) Cello-biohydrolase, (c) N-acetylglucosaminidase Influenced by treatments (*indicates the extremely high values which is beyond the detection range. Bars with different letters are significantly different at 5% probability level)

Table 3. Soil C Contents after 30d Incubation

	C-Straw	K-Straw	C-Char400	C-Char600	K-Char300	K-Char600	No-add
	gC kg^{-1} soil						
Soil C content	20.46b*	22.13b	32.20d	32.40d	27.23c	32.35d	14.83a

*Different letters indicate significant difference at a 5% probability level.

Monedero *et al.* (2008) also reported a good correlation between the microbial biomass C and the FDA activity. As a sufficient substrate for the microbial hydrolysis was added when measuring FDA activities, FDA activities were the proxy for potential microbial activities, which could be highly related to the microbial biomass. The relatively lower correlations between the CO_2 emissions with the HWC contents and the FDA activities indicated that there are other factors influencing CO_2 emission in the soil with biochar amendment. The HWC might be adsorbed to the biochar surface and could not be easily utilized by microbes (Chen, accepted) .

The activity of the exo-enzymes (β-glucosidase, cellobiohydrolase, and N-acetylglucosaminidase) was influenced by both the straw and biochar treatments (Fig. 3). Compared to the No-Add treatment, all of the enzyme activities increased with the C-Straw and K-Straw treatments, whereas the effects of the biochar addition varied by the kinds of enzyme. Considering that there was no biochar effect on overall microbial activity (FDA activity), our results on specific enzymes seem to be contradictory. However, Bailey *et al.* (2011) reported that biochar addition could increase enzyme activities without an increase in overall microbial activity because biochar could provide chemical modification to enzymes or absorption sites for the longer term stability of enzymes. For β-glucosidase, there was no difference in the activity between the Chinese biochar treatments (C-Char400 and C-Char600) and the No-Add control, while for the K-Char300 and K-Char600 soils, the activity was slightly reduced. The data on the β-glucosidase showed the different responses in the Chinese and Korean biochars, indicating that the difference in the β-glucosidase activities might be related to the different C status for two biochars. In our study, the lower C/N ratios of the Chinese biochar might have stimulated the β-glucosidase activity. For cellobiohydrolase, all of the biochars did not change the activity of this enzyme in comparison to that of the control. The N-acetylglucosaminidase activity was enhanced for the C-Char600 and K-Char600 in the 15D samples, but no other significant changes were seen. The initial increase in this enzyme during the early stage of incubation implied that the enzyme involved in the soil N dynamics could be influenced by the different mineral N contents of the amended materials. The NH_4^+ contents of the C-Char600 and K-Char600 were lower than those of the C-Char400 and K-Char400 (Table 1). The lower ammonium contents might have stimulated the activity of the N-acetylglucosaminidase, which involves decomposition of chitin (Chung *et al*., 2007). Jin *et al*. (2013) argued that the changes in the activity of the various exo-enzymes together with the microbial biomass and FDA activity could represent changes in the microbial community structure. If we related the reduction in the microbial biomass in the C-Char600 and K-Char600 treatments with the maintained and/or stimulated activities of the exo-enzymes in these treatments, we could suggest that the addition of biochar, especially produced at higher temperatures, might have increased the metabolizing efficiency of the soil microbes. This result was consistent with those of Dempster *et al.* (2010), who reported a decrease in the microbial biomass and community by the biochar addition at high application rates, while the activities of the exo-enzymes were maintained in the biochar treatment.

The soil C contents increased in all of the treated soils (Table 3). In comparison to the No-Add control, the straw amendment increased the C contents by 40% and the biochar amendment enhanced the C storage by 84-119%. The smallest increase in the C content observed in the K-Char300 treatment was probably due to the higher labile C content of this biochar.

The average rate of CH_4 emissions was also significantly affected by the straw treatments (Fig. 1(b)). In the C-straw and K-straw treatments, the CH_4 emission rate was approximately 600 times greater than that from any of the other treatments. Considering that the CO_2 emission rate in the straw amendment treatment was three times higher than that of the other treatments, the huge increase in the CH_4 emissions indicated that the decomposition of straw occurred primarily under anaerobic conditions, resulting in methanogenesis (Le Mer and Roger, 2001). In contrast to the straw amendment treatments, the biochar treatments did not significantly change the soil CH_4 emission rates except for the K-Char300 treatment, which showed a 2-16 times greater rate than that from the No-Add control. We attributed the increase in the CH_4 emissions from the K-Char300 treatment to its extremely higher labile C content (Table 1). The HWC content in the K-Char300 was 25 times higher than those in any of the other biochars on average. The very high amount of labile C in the K-Char300 treatment indicates that this material contains enough available C to boost the methanogenesis in the soil in a similar way that organic matter can. The other biochar treatments did not significantly change methane emission probably from the two reasons. The first one was that the labile C contents existent on biochar were not sufficient enough to stimulate methane emission. Although the C-Char400 contains higher HWC content than C-Char600 and K-Char600, the amount is only one third of that in K-Char300 (Table 1) and as a result of higher CO_2 emission from the C-Char400 and K-Char300, the remaining soil HWC contents were almost identical except for the slightly high level in C-Char600 (Fig. 2(a)). The second reason could be that the addition of biochar can increase aeration status in the soils. Case

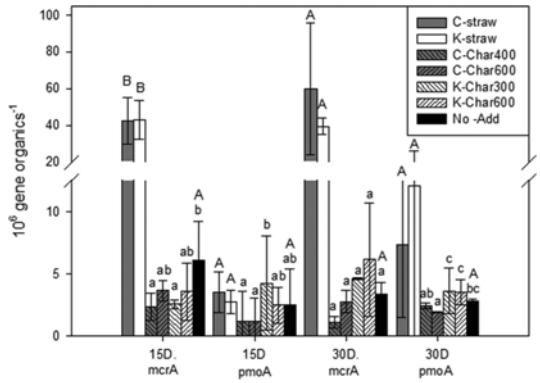

Fig. 4. Changes in Abundances of Methanogens (mcrA) and Methanotrophs (pmoA) Influenced by Treatments for the 15D and 30D Samples (Multiple comparisons were made among C-Straw, K-Straw, and No-Add represented as capital letters and among C-Char400, C-Char600, K-Char300, K-Char600, and No-Add represented as lower case letters. Different letters stand for significant difference at 5% probability level)

et al. (2012) reported that 10% biochar amendment decreased soil bulk density and enhanced soil aeration. It was also suggested that increased soil aeration could decrease methane production and/or increase methane oxidation in soil (Van Zwieten *et al*., 2009).

The abundance of the methanogens was 5-20 times higher in the C-Straw and K-Straw treatments than in the No-Add control both in the 15D and 30D samples, while the abundance of the methanotrophs was not changed by the same treatments (Fig. 4). Contrast to the results from straw amendments, biochar amendments did not increase the abundance of methanogens, rather, it was slightly decreased in the C-Char400 and K-Char300 treatments for the 15D samples. The no change in the abundance of methanogens in the K-Char300 soil was un-expected because significantly higher CH_4 emission due to higher labile C content of this biochar was observed from this soil. In the case of K-Char300 treatment, the high content of HWC in this biochar increased methane emission without boosting up the abundance of methanogens. This result indirectly implies that the soil physical condition influenced by biochar addition might limit the growth of methanogens. However, further investigation is needed to solve this apparent contradictory result. Overall results indicated that the amendment of biochar increased microbial metabolic efficiency without the change in the abundance of methane-related microbes, resulting in no change in CH_4 emission.

Inconsistent with our results, many researchers have observed a reduction in the CH_4 emissions from the biochar-amended soils in comparison with the soils with no additions (Kammann *et al*., 2012; Liu *et al*., 2011; Rondon, 2006; Wang *et al*., 2012; Yoo and Kang, 2012). Karhu *et al.* (2011) reported a reduction in the CH_4 emissions and attributed it to an increase in the soil aeration caused by the biochar addition. Liu *et al*. (2011) also observed a reduction in the CH_4 emissions and no significant change in the community structure of the methanogens and methanotrophs. They attributed the reduction in the CH_4 emissions to the increased pH and the decreased microbial biomass resulting from the biochar addition. In the study by Liu *et al.* (2011) , the soil pH started at 5.9 and increased up to 8.5 in the biochar-treated soils. As has been reported, most methanogenic archaea grow at pH values near neutral with a range of 6.5-7.5 (Wang *et al*., 1993; Yang and Chang, 1998). Therefore, the biochar addition in Liu *et al.* (2011)'s study probably increased the soil pH beyond the optimal range for methanogenesis. However, in our study, although the biochar addition increased the soil pH from 5.4 to 6.8, this increase was not enough to suppress the CH_4 emissions from the biochar added soils.

3.3 Soil N_2O Emissions and Available N Contents

The soil N_2O emission rates decreased following the addition of C-Straw and K-Straw in comparison to the No-Add control and the values were very small and almost null during the 16-30 days. The reduction in the N_2O emissions by organic matter incorporation into soils has been widely reported (Pelster *et al*., 2013) and attributed to the subsequent immobilization of the mineral N. Very low N_2O emissions from the straw amended soils could be the conversion of N_2O to N_2. Since methanogenesis was prevalent in the C-Straw and K-Straw treatments, we could assume that the C-Straw and K-Straw soils were under severely oxygen-limiting conditions which were favorable for complete denitrification (Cayuela *et al*., 2014). Due to possible immobilization of available N and consumption of NO_3^- through denitrification, the amount of NH_4^+ and NO_3^- were decreased in the C-Straw and K-Straw treatments (Table 4).

The biochar amendment did not change the N_2O emissions in

Table 4. Changes in Soil NH_4^+ and NO_3^- Contents and pH Influenced by Treatments for the 15D and 30D Samples

			C-Straw	K-Straw	C-Char400	C-Char600	K-Char300	K-Char600	No-add
15D	NH_4^+	(mg kg^{-1} soil)	0.41a*	0.35a	0.68a	0.42a	0.69a	0.44a	35.04b
	NO_3^-		9.63a	13.41a	35.62b	35.90b	31.50b	31.88b	29.53b
	pH		6.32c	6.23c	5.83b	6.31c	5.83b	6.67d	5.60a
30D	NH_4^+	(mg kg^{-1} soil)	0.90a	0.65a	1.03a	0.60a	0.55a	0.28a	15.56b
	NO_3^-		12.85a	19.70b	32.80d	30.58cd	28.42cd	28.10c	28.52cd
	pH		6.17b	6.1b	5.94b	6.46c	5.96b	6.76d	5.32a

*Different letters stand for significant difference at a 5% probability level.

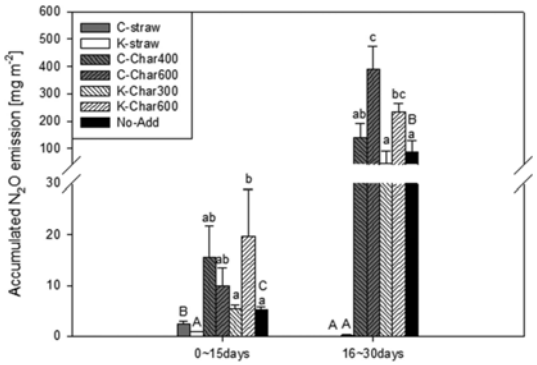

Fig. 5. Changes in Emission Rates on Accumulated Evolution influenced by Treatments of N_2O (Multiple comparisons were made among C-Straw, K-Straw, and No-Add represented as capital letters and among C-Char400, C-Char600, K-Char300, K-Char600, and No-Add represented as lower case letters. Different letters stand for significant difference at a 5% probability level)

comparison to the No-Add control during the 0-15 days except for the K-Char600 treatment (Fig. 5). During days 16-30, the N_2O emissions were not changed by the amendment with C-Char400 or K-Char300, but they increased in the C-Char600 and K-Char600 soils compared to those of the No-Add control. The increase in the N_2O emissions by the biochar amendment in the rice paddy soils was contrary to many reports that have observed a reduction in the N_2O emissions after adding biochar to soils in saturated conditions (Case *et al*., 2012; Kammann *et al*., 2012; Liu *et al*., 2011; Wang *et al*., 2012; Wang *et al*., 2011; Yanai *et al.*, 2007; Zhang *et al*., 2010). The explanations for the reduction were enhanced soil aeration (Rogovska *et al*., 2011), the increased pH (Rondon *et al*., 2006), and the microbial immobilization of the soil NO_3^-. On the other hand, when the soil was not under saturated conditions, the increase in the N_2O emissions by the biochar addition was reported by Yoo and Kang (2012) and Troy *et al*. (2013). The incubation conditions of these studies were 70% Water-Filled Pore Space (WFPS) and 26% gravimetric water content, respectively.

Sánchez-García *et al.* (2014) emphasized the importance of identifying the predominant N_2O formation pathways in order to understand the mechanisms by which the biochar addition changes the N_2O emissions. Bateman and Baggs (2005) found that nitrification was the primary process producing N_2O at 35-60% WFPS, whereas denitrification was the predominant process above 70% WFPS. However, the N_2O production originates from much more complicated pathways; therefore, the %WFPS could only provide a rough estimate for the primary process of N_2O production. In our study, although the soils were completely saturated, we observed a pattern of increased N_2O emissions with biochar addition. The soil pH was slightly increased from 5.4 to 5.9 in the C-Char400 and K-Char400 treatments, while a substantial increase was observed from 5.4 to 6.5~6.8 in the C-Char600 and K-Char600, respectively. This difference in the change in the soil pH might partly explain the different patterns of the N_2O emissions among the biochar treatments. Although the relationship between soil pH and N_2O emissions is particularly complicated, N_2O emissions tend to decrease in soil with a higher pH when denitrification prevails (ŠImek and Cooper, 2002) and increase in soil with a higher pH when nitrification prevails (Sánchez-García *et al.*, 2014). As we observed an increase in the N_2O emissions from the C-Char600 and K-Char600 soils together with the soil pH, we could assume that the prevalent process of N_2O emissions from these soils was from nitrification. The changes in the NH_4^+ and NO_3^- further supported this idea. In all of the biochar amended treatments, the NH_4^+ contents were dramatically lower, while the NO_3^- contents increased, indicating that the biochar amendment stimulated nitrification, which was also reported by Sánchez-García *et al.* (2014), Singh *et al.* (2010), Yoo and Kang (2012). We still needed to determine why in our soils, which were completely waterlogged during the incubation period, the N_2O emissions primarily originated from nitrification. According to Beccari *et al.* (1992), nitrification can occur when the dissolved concentration of oxygen is greater than 2 mg l^{-1}. During our incubation, we aerated the incubation jars every 3 d, which probably ensured partly aerated conditions in the waterlogged jars. The overall results indicated that the primary process of the N_2O production in our incubation might have been nitrification and the pH increase in the C-Char600 and K-Char600 soils might have stimulated N_2O emission. However, we still could not exclude the possibility of activated denitrification by biochar addition as mechanism for enhanced N2O flux because denitrification process often does not simultaneously change with nitrate or denitrifier abundances (Song *et al*., 2010; Song *et al.*, 2012).

Our observations of the changes in N_2O emissions following biochar addition were unique, because we found enhanced N_2O emissions from the biochar-added soils even when the soil water conditions were saturated. This result implied that care should be taken when applying biochar to rice paddy soils that are heavily fertilized with the mineral N as is common in China and Korea.

4. Conclusions

The results of this study showed that biochar amendment did not significantly increase the CO_2 or CH_4 emissions from the rice paddy soils. As a result, the C storage in the biochar added soils was increased by 84-119%. The results of the microbial biomass C and the exo-enzyme activity implied that the addition of biochar might have increased the metabolizing efficiency of soil microbes, especially in those treatments of biochar produced at high temperatures, because we observed a reduction in the microbial biomass C, and maintained or increased activities of the exo-enzymes. We observed a significant increase in the N_2O emissions from the treatments of biochar produced at higher temperatures. In the rice paddy soils in Korea, which are generally acidic and heavily fertilized with the mineral N, amendment with biochar could enhance nitrification, which could be further stimulated by an increase in the soil pH by the biochar addition.

In this case, the ability of the biochar to reduce the denitrification by increasing the aeration might be counterbalanced by the stimulation of the nitrification process. Our observations of the increased N_2O emissions in the waterlogged rice paddy soils following the biochar addition were unique and could be added to the existing database about the effects of biochar on N_2O emissions from agricultural soils. In soils with low organic C and heavy N fertilization, such as those in China and Korea, biochar addition could increase the N_2O emissions even from water logged rice paddy soils. Despite the increased N_2O emissions from the biochar-added soils, soil amendment with biochar made from straw had the capacity to decrease the Global Warming Potential (GWP) by approximately 1600 gC m^{-2} in comparison to the soils amended with rice straw.

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