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Variable effects of biochar application to soils on nitrification-mediated N_2O emissions

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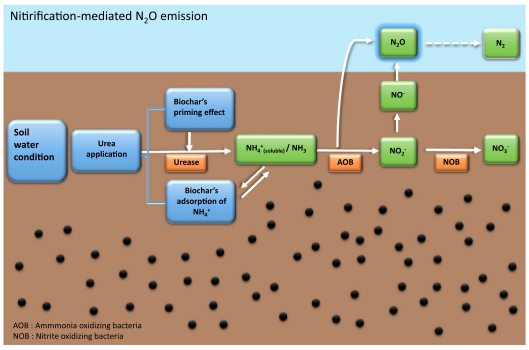
HIGHLIGHTS

- N_2O emissions were increased or not changed when both biochar and urea were applied to dry soils with low C content.
- Soil water status right after urea and biochar application was the primary determinant to predict the effect of biochar on N_2O emissions, together with soil C status and biochar's adsorptive capacity.
- Our study is unique in that we obtained the results from multiple field experiments covering the whole cropping periods.

GRAPHICAL ABSTRACT



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ABSTRACT

Although a meta-analysis on biochar's effects on N_2O emission reported an overall reduction in N_2O emission by adding biochar to the soils, there are still variations in the changes in N_2O emission, especially from field results. The objectives of this study are 1) to compare the effects of biochar addition on N_2O emission between three agricultural upland field experiments, where soil water status was dry favoring nitrification and 2) to identify main factors explaining biochar's variable effects on N_2O emission. Three field experiments were conducted: Exp A in the cultivated grassland treated with rice husk biochar at 2 ton ha^{-1} + urea (CHAR) and with urea only (CON); Exp B in the cabbage field with CHAR and CON treatments; and Exp C in the pepper field with CHAR, CON, and CHAR + DCD (dicyandiamide, nitrification inhibitor) treatments. In Exp A and C, cumulative N_2O emissions significantly increased by 82.5% and 55.8% in the CHAR than CON treatments, respectively, while in Exp B, there was no difference in cumulative N_2O emission between the CHAR and CON. Based on results from using nitrification inhibitor and soil % water filled pore space (WFPS), we assumed that the main N_2O production mechanism was nitrification. Our results suggest that soil water status right after urea application is the primary determinant of different effects of biochar on N_2O emission in addition to soil C status and biochar's adsorption. Principal component analysis using the 25 compiled data also supported our results. This study identified the specific field conditions under which biochar could have stimulating effects on N_2O emission. Mitigation potential of biochar application should be reconsidered if biochar and urea were amended to dry soils with low C contents.

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1. Introduction

Approximately 70% of anthropogenic N_2O emission originates from the agricultural sector (Bouwman et al., 2010; Smith et al., 2008), primarily from widespread use of synthetic nitrogen (N) fertilization. As N_2O is a potent greenhouse gas, this means that a management strategy is needed to cut N_2O emission, especially with regard to agricultural soils. Biochar amendment to soils has been suggested as a potential strategy for reducing N_2O emission, but the mitigation effects of biochar application have not yet been fully verified.

Cayuela et al. (2014) compiled 30 peer-reviewed literatures and collected 261 experimental treatments to investigate the impact of biochar on N_2O emission and to test whether the often proposed reductions in N_2O emissions occurred across multiple studies. They found that biochar decreased soil N_2O emissions by 54% on average but that the effects of biochar on N_2O emission varied depending on soil pH, texture, soil water status, biochar C/N ratio, and application rates. In addition, there are still many studies which reported no difference or even an increase in soil N_2O emissions after biochar amendment (Clough et al., 2010; Suddick and Six, 2013).

Inconsistent effects of biochar addition on N_2O emission might be related to different mechanisms of N_2O production in soils under various conditions. Singh et al. (2010) reported positive and negative effects of biochar addition on N_2O emission and suggested a possible mechanism of N_2O emission increase via enhanced nitrification. Sánchez-García et al. (2014) studied two soils with contrast responses to biochar amendment and reported that in the soil with reduced N_2O emission by biochar addition, the main pathway leading to N_2O was denitrification, while in the soil with increased N_2O emission, the main process of N_2O production was nitrification. Li et al. (2015) reported a slight increase in N_2O emission with the addition of wheat straw biochar and confirmed that the production of N_2O occurred via nitrification through use of a nitrification inhibitor. Lin et al. (2017) reported that the application of wheat straw biochar to rice paddy soil significantly increased N_2O emission and attributed this increase to the enhanced abundance of ammonia-oxidizing bacteria (AOB) *amoA* genes, which is the first step in the nitrification process. The abovementioned findings led us to hypothesize that biochar addition would have a stimulating effect when the main N_2O production process is nitrification. However, in prior studies, enhanced nitrification related to biochar did not always lead to increased N_2O emission. Xu et al. (2014) reported that biochar amendment stimulated both nitrification and denitrification processes, while reducing N_2O emission overall. Verhoeven et al. (2017) also reported that biochar addition reduced N_2O emission in upland systems to the same degree as rice paddy systems where denitrification is dominant. Thus, a better understanding of the main mechanism of N_2O production and influential factors is needed in order to effectively predict the effects of biochar on N_2O emission.

Meta-analyses on the effects of biochar addition on N_2O emission were recently conducted by Cayuela et al. (2014) and Verhoeven et al. (2017). These analyses were based on data collected from a wide range of water conditions from flooded to very dry soils and variable mechanisms of N_2O production. Considering that the Intergovernmental Panel on Climate Change (IPCC)'s greenhouse gas inventory includes soil N_2O emission only from agricultural upland systems, it is more practical for us to focus on the effects of biochar on N_2O emission from upland field soils (Liu et al., 2016). Under drier soil conditions where the soil % water filled pore space (WFPS) is 35–60%, N_2O was primarily produced from the microbial nitrification process (Bateman and Baggs, 2005). In the nitrification process, N_2O is formed from NH_2OH, which is oxidized from NH_4^+/NH_3 by ammonia oxidizing bacteria (AOB). Nitrite is further oxidized into nitrate by nitrite oxidizing bacteria (NOB). Considering the higher sensitivity of NOB to NH_3 toxicity compared to AOB, NO_2^- tends to be accumulated when the soil contains high levels of NH_3, resulting in high possibility of N_2O emission (He et al., 2016; Venterea et al., 2015). To understand the effects of biochar addition on nitrification-mediated N_2O emission, we need to investigate the conditions and factors for nitrification influenced by biochar.

In this study, we conducted three independent field experiments of agricultural upland systems where the soils were mostly dry. We assumed that the main N_2O production mechanism was nitrification because of the dry soil water status (Bateman et al., 2004). We considered many of the factors involved in the nitrification process, including soil pH, soil water content, soil inorganic N availability, and microbial activity (Che et al., 2015; He et al., 2016; Nelissen et al., 2012), all of which could potentially be influenced by biochar amendment. To have more generalized knowledge on the variable effects of biochar on nitrification-mediated N_2O emission, we collected data from published papers on N_2O emission and biochar addition with nitrification as the main N_2O production process. By conducting principal component analysis, we identified the main factors determining the direction and magnitude of changes in N_2O emission via biochar amendment.

The objectives of this study were 1) to compare the effects of biochar addition on N_2O emission between three agricultural field studies where soil water status of each experiment was dry favoring nitrification and 2) to identify the main factors influencing biochar's effects on N_2O emission using statistical analysis.

2. Materials and methods

2.1. Site description and biochar preparation

The field experiments were conducted in three different soils, all located in the central area of S. Korea. Annual mean temperatures ranged from 11.8 to 12.5 °C and precipitations ranged from 1227 to 1312 mm (Table 1, Korea Meterological Administration, http://www.kma.go.kr/weather/observation). Experiment A (Exp A) was set up on October 25, 2011 in a cultivated grassland planted with tall fescue (*Festuca arundiancea*), orchard grass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), and white clover (*Trifolium repens*). Experiment B (Exp B) was set up on September 1, 2014 and planted with cabbage (*Brassica rapa* var. glabra) and Experiment C (Exp C) was set up on May 10, 2015 and planted with pepper (*Capsicum annuum*).

Biochar used in this study was rice husk biochar, which was a commercial product sold by the Farmers' Association in Gangjin-gun, Korea. It was produced by pyrolyzing rice husks at 350 °C in a pyrolysis reactor (DCH-400) from Daewon GSI Co., Gyeongsangbuk-Do, Korea. The detailed procedures for char production are provided at www.daewonces.co.kr.

2.2. Field experiment

In Exp A, the CHAR treatment applied rice husk biochar at 2 ton ha^{-1} on October 25, 2011 together with 140 kg N ha^{-1} of urea fertilizer. The control (CON) was only applied with urea fertilizer at the same rate. The experiment lasted for 350 d because cultivated grassland was planted with perennial grass species. In Exp B, the CHAR (2 ton ha^{-1} rice husk biochar and urea application) and CON (urea application only) treatments were employed with a urea application rate of 190 kg N ha^{-1}. The duration of this experiment was 42 d (the growing period of cabbage). In Exp C, in addition to the CHAR (2 ton ha^{-1} rice husk biochar and 225 kg N ha^{-1} urea application) and CON (225 kg N ha^{-1} urea application only) treatments, a third treatment was also applied, CHAR + DCD, where we added rice husk biochar, urea and dicyandiamide (DCD). DCD is a representative nitrification inhibitor (Di and Cameron, 2002) that was applied at 10% of the urea applied by weight. The duration of this experiment was 120 d. All experiments used a completely randomized block design and had three replicates.

The biochar applied had a particle size of <3 mm and was incorporated into the soil profile to a depth of 10 cm using shovels. In Exp C, the DCD was applied in solution by dissolving 100 g of DCD powder in 1000 ml of the deionized (D.I.) water. The non-biochar plots were

Table 1
Site description and physico-chemical properties of soils from the Exp A, Exp B, and Exp C.

			Exp A	ExpB	ExpC
	Location		Chunan-Si, Chung-cheongnam-Do (36°N, 127°E)	Hwaseong-Si, Gyeonggi-do (37°N, 127°E)	
	Annual temperature		11.8 °C	12.0 °C	
	Annual precipitation		1227 mm	1312 mm	
Soil	Soil texture		Sandy loam	Sandy loam	Sandy loam
	pH		7.20	6.90	5.90
	Total C	%(w/w)	0.42	0.61	0.89
	Total N		0.06	0.08	0.11
	C:N		7.00	7.63	8.45
	NH_4^+	mg kg^{-1}	26.00	4.00	–
	NO_3^-		4.00	16.00	35.00

also mixed with shovels and in Exp C, the same amount of D.I. water was added to the CHAR and CON soils as that in the CHAR + DCD treatment.

2.3. Gas sampling and analysis

Gas samples were taken every month during the growing season in Exp A and every week in the other experiments using a chamber method (Wang et al., 2011). Two chambers (20 cm in diameter, 25 cm in height) were inserted 5 cm deep into the soil for each plot. On sampling dates, the chambers were closed with airtight lids for 40 min and gas samples were withdrawn from the headspace of the closed chamber using a 10 ml 3 way syringe (BD Luer-LokTip).

Gas samples were analyzed using a gas chromatograph (Agilent 7890A, USA) equipped with an electron capture detector. Gas fluxes were calculated based on the changes in headspace concentration over the measured period using the following equation (Troy et al., 2013):

$$N_2O\ \text{flux} = \frac{dN_2O}{dt} \times \frac{V}{A} \times \frac{P * 100 * MW}{R} \times \frac{273}{273 + T} \quad (1)$$

where, dN_2O/dt is the difference in N_2O concentrations between the initial and end time points, V is the volume of the chamber, A is the surface area that the chamber covers, P is the atmospheric pressure, MW is the molecular weight of N_2O, R is a gas constant, 8314 J mol^{-1} K^{-1}, and T is the absolute temperature.

2.4. Soil sampling and analysis

Soil samples were collected from each plot from a depth of 0–15 cm using a soil core sampler (4.9 cm i.d., Forest supplier, USA). Samples were sealed in plastic bags and taken to the laboratory after sampling. To measure soil gravimetric water content, approximately 20 g of soil was taken from the plastic bag and dried in an oven at 105 °C for 24 h. Soil bulk density was determined from the soil cores taken by weighing the dry weight of the soil in a known volume. The water filled pore space (WFPS) was determined using the total pore volume and gravimetric water content (Li et al., 2015).

After air-drying for 2 weeks, soil samples were passed through a 2 mm sieve and analyzed for physico-chemical characteristics. The soil texture was determined using a hydrometer method; pH was determined using a glass electrode 1:1 (w/v) in deionized water; and NH_4^+ and NO_3^- concentrations were determined using the salicylate microplate method (Sims et al., 1995). Total C and N contents were measured via combustion analysis using a Carlo Erba NS 1500 C/N analyzer (Carlo Erba, Milan, Italy). Hot water extractable C (HWC) was determined following the method of Haynes and Francis (1993). Soil microbial activity was evaluated via the fluorescein diacetate (FDA) hydrolysis method (Adam and Duncan, 2001). The phyico-chemical characteristics of biochars were also analyzed using the same method for soil analysis except for pH determination. We used a glass electrode 1:5 (w/v) in D.I water to determiner biochar pH, instead of 1:1.

2.5. Statistical analysis

Statistical analysis using the general linear model (GLM) procedure of SAS 9.4 (SAS Institute, 2013) was conducted on the N_2O emission rate, cumulative N_2O emission, soil NH_4^+ and NO_3^- contents, soil pH, HWC content, and microbial FDA activity. The least square means were used to test for significant differences among treatments at the 5% probability levels.

To obtain in-depth insight into nitrification-mediated N_2O emission, we compiled 25 experimental treatments from the eight peer-reviewed articles published between 2010 and 2017. Data were obtained from both laboratory and field experiments (Clough et al., 2010; He et al., 2016; Li et al., 2015; Lin et al., 2017; Liu et al., 2014; Pereira et al., 2015; Singh et al., 2010; Xu et al., 2014). A literature search was conducted using the Google Scholar data base using the keywords "biochar" OR "nitrous oxide" OR "N_2O" OR "nitrification". Among the search results, we carefully selected articles that discussed nitrification as a possible N_2O production mechanism. In the first place, we compiled factors influencing nitrification, which were soil % WFPS, soil C and N contents, soil pH, soil NH_4^+ and NO_3^- contents, soil cation exchange capacity (CEC), biochar C and N contents, biochar pH, biochar application rate, biochar CEC, and pyrolysis temperature. These were used as input variables for principle component analysis (PCA). We carried out PCA analysis via "prcomp" in GNU R (ver 3.3.2) for the tests. After identifying important PCs of which the eigenvalues were >1, we ran the stepwise multiple regression between the PCs and % N_2O emission change observed from the compiled experiments to identify the relationships of the soil and biochar variables and N_2O emission from different researches. We tested whether the direction and magnitude of N_2O change could be successfully accounted for with key variables which we identified from the PCA.

3. Results

3.1. Soil and biochar characterization

The soil texture of Exp A, B, and C was sandy loam (Table 1). Soil pH in Exp A and B were close to neutral (6.90–7.20), while the pH was 5.90 in Exp C. In terms of soil carbon (C) status, all the experiments were relatively low in total C (<1%).

The characteristics of biochar are summarized in Table 2. The pH was 10.3 and the C/N ratio was relatively low compared to biochars used in other studies (Krull et al. (2009). The HWC content in the rice husk was 2.9 g kg^{-1} soil. Compared to the woody biochar used for Sánchez-García et al. (2014)'s study, our biochars contained 22 fold greater labile C. The reasons for the low C/N ratio included the low pyrolysis temperature and high ash content (46%).

3.2. Effects of biochar addition on N_2O emission

In Exp A, the temporal pattern in N_2O emission rates was influenced both by seasonal temperature and soil water content (Fig. 1A). In Exp B, a very high rate of N_2O emission was observed in the initial period of the experiment and then decreased sharply (Fig. 1B). This pattern was consistent with the change in soil water content and less influenced by air temperature due to short growing period. In Exp C, the temporal pattern in N_2O emission was both related to the temperature change and the % WFPS (Fig. 1C).

Effects of biochar addition on N_2O emission rate varied with time and experiments. In Exp A, N_2O emission rates were higher on days 35, 58, 149, and 213 in the CHAR treatment than those in the CON when the soil %WFPS levels were <30% (Fig. 1A). In Exp B, there were no significant differences in N_2O emission rates between the CHAR

Table 2
Characteristics of biochar.

Feedstock	Pyrolysis condition	pH	Total C	Total N	C:N	CEC[a]	HWC[b]
		1:5(water)	%(w/w)			meq 100 g^{-1}	g kg^{-1}
Rice husk	350 °C	10.30	47.02	0.70	67.17	33.54 ± 0.87	2.9

[a] CEC, cation exchange capacity.
[b] HWC, hot water extractable Carbon.

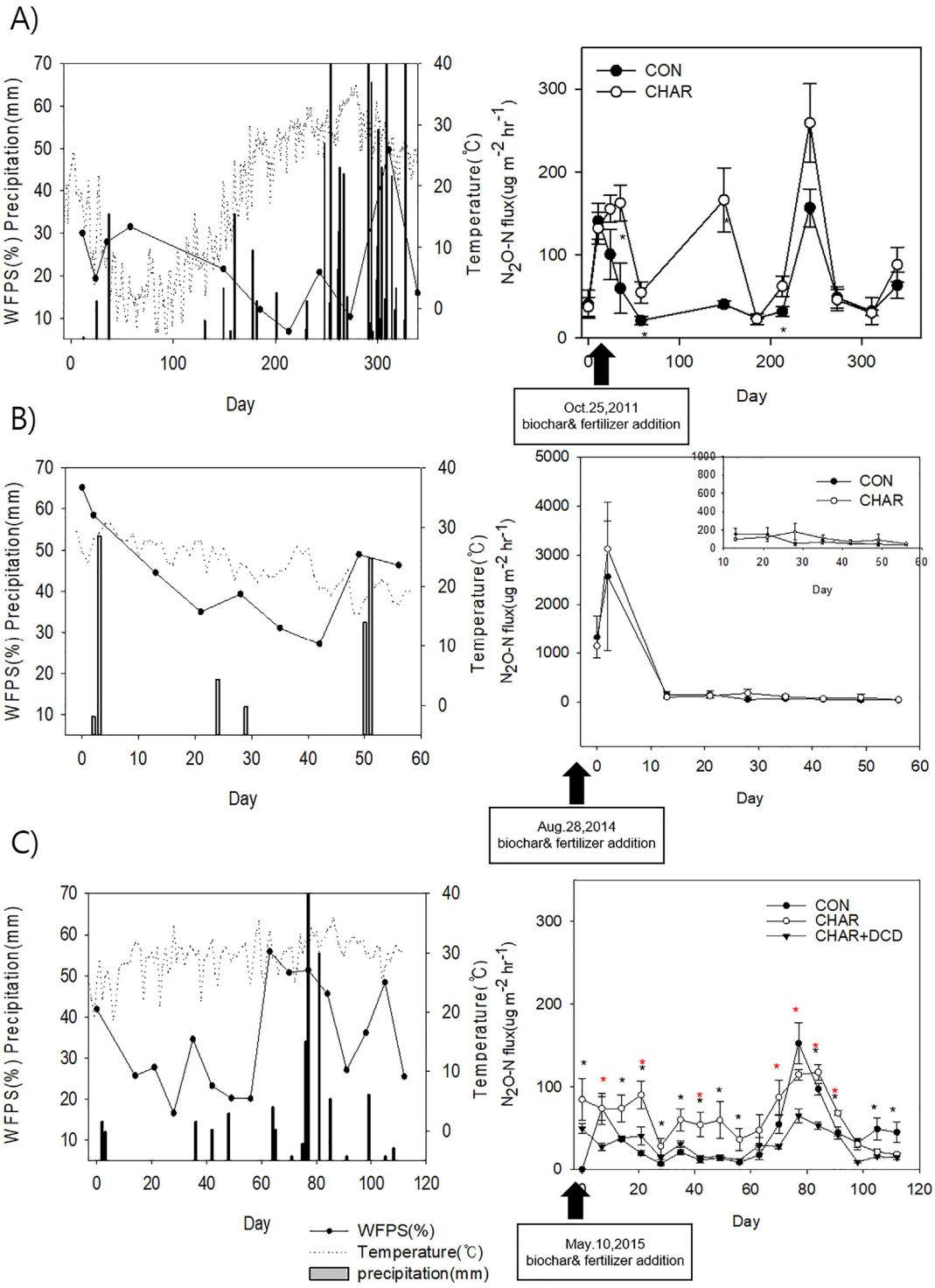

Fig. 1. Temporal changes in soil % water filled pore space (WFPS), air temperature, precipitation, and the N_2O emission rate in Exp A (A), Exp B (B), and Exp C (C). CHAR treatment involved application of rice husk biochar at 2 ton ha^{-1} in Exp A,B, and C. A black asterisk (*) indicates a significant difference in N_2O emission rates between the CHAR and CON treatments. A red asterisk (*) indicates a significant difference between the CHAR and CHAR + DCD treatment ($P < .05$).

and CON treatments on specific days (Fig. 1B). In Exp C, the CHAR treatment significantly increased N_2O emission from day 1 to day 56 compared to the CON and CHAR + DCD treatments and after day 84, biochar's stimulating effect was either nonsignificant or reversed (Fig. 1C).

Cumulative N_2O emissions from the CHAR treatment were significantly higher than the CON by 82.5 and 55.8% in Exp A and C, respectively (Fig. 2). In Exp C, cumulative N_2O emission was lower in the CHAR + DCD treatment compared to CHAR treatment, which was similar to that in the CON treatment. On the other hand, in Exp B, the cumulative N_2O emissions were not affected by CHAR treatment.

3.3. Effects of biochar on soil properties

Soil pH was not changed by CHAR treatment in Exp A, while it was increased in Exp B with limited significance and even increased in Exp C, respectively (Table 3). Soil HWC content and microbial FDA activity were increased by CHAR treatment in Exp A and C, although the significance level was low in Exp C. In Exp B, however, there was no change in HWC content and microbial FDA activity between the CHAR and CON.

Soil NH_4^+ and NO_3^- contents after the growing periods varied significantly among Exp A, B, and C (Fig. 3). The overall NH_4^+ and NO_3^- content was higher in Exp B than in other experiments because of the short growing season of cabbage (42 d). In Exp A, the soil inorganic N level was lower than in other experiments probably because of the long duration of the experiment. As Exp A involves a year-round cultivated grassland system, the long duration time (350 d) could have decreased the remaining amount of fertilized N. In Exp C, the remaining level of soil inorganic N was in the moderate range between that in Exp A and B, reflecting the medium length of the cropping period (120d). The NH_4^+ plus NO_3^- content was not changed by CHAR treatment both in Exp A and C, while in Exp B, that was significantly increased in the CHAR treatment compared to CON. In addition, in Exp A and C, NO_3^- content was not significantly increased compared to NH_4^+ content, except for the CON in Exp A. However, in Exp B, soil NO_3^- content was significantly higher than NH_4^+ content in the CHAR treatment, while in the CON treatment, the difference was not significant.

3.4. Principal component analysis

We identified four principal components (PCs) with eigenvalues >1. PC1, PC2, PC3 and PC4 explained 30.0%, 23.2%, 11.7% and 9.5% of the total variance, respectively, all of which captured 74.5% of the variance in our data (Table 4). PC1 was strongly negatively correlated (i.e. |Loading| ≥ 0.4) with soil C and N contents, while PC2 was strongly positively correlated with biochar C content and negatively correlated with biochar CEC. PC3 had a strong positive correlation with biochar application rate and a negative correlation with soil NO_3^-. PC4 was strongly negatively correlated with soil %WFPS.

The Pearson correlation coefficient between % N_2O emission change by biochar addition and PC1 was 0.255 ($p = .12$) and that between % N_2O emission change and PC4 was 0.721 ($p < .05$). The correlations between N_2O emission pattern and PC2 and PC3 were very low and nonsignificant. Hence, we plotted the score plots between PC1 and PC4 and between PC3 and PC4 (Fig. 4). In the score plot between PC1 and PC4, data of % N_2O emission change by biochar addition were presented as dots (Fig. 4A). When PC4 (negatively correlated with soil water content) was positive, all of the points were from data showing a positive % N_2O emission change with biochar addition, while when PC4 was negative, more points indicated negative % N_2O emission change with biochar addition. In the score plot between PC3 and PC4, data from the laboratory/greenhouse and from the field studies were grouped separately. Data points far from the horizontal axis of 0, either positive or negative directions, were from the laboratory results (circle symbol), while data points near the horizontal axis of 0 were from the field results (square symbol).

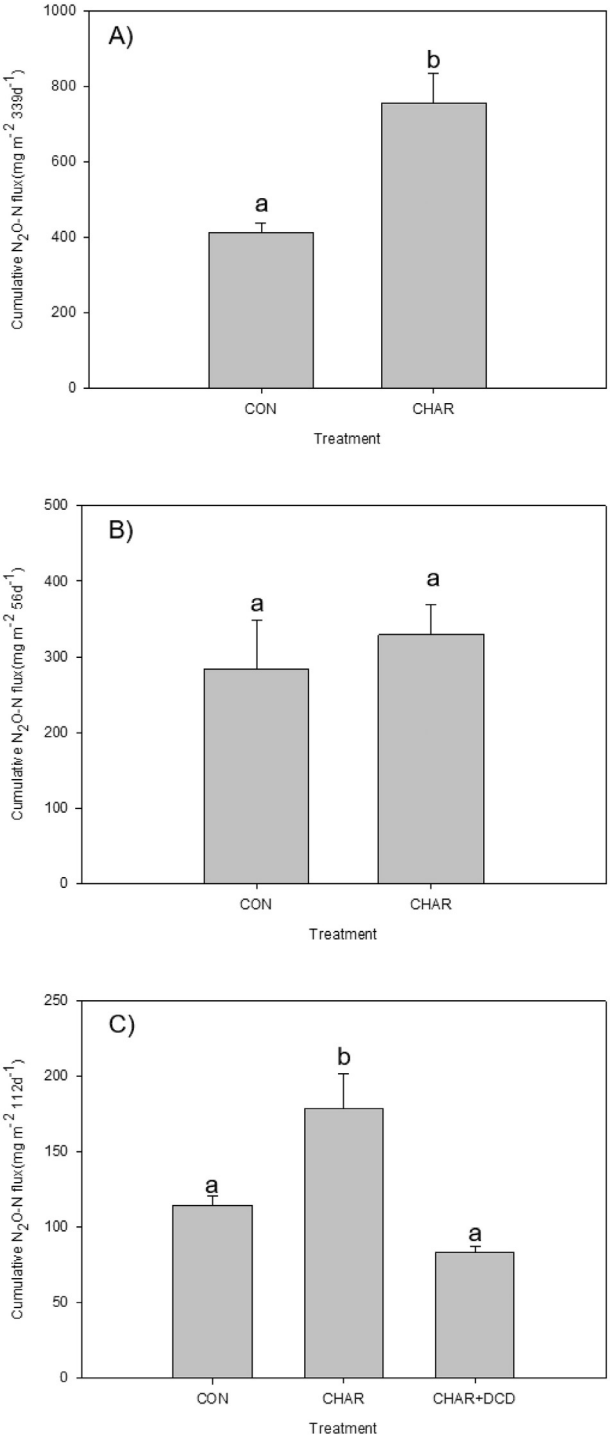

Fig. 2. Cumulative N_2O emission in Exp A(A), Exp B(B), and Exp C(C). The CHAR treatment involved application of rice husk biochar at 2 ton ha^{-1} in Exps A,B, and C. Bars with different letters indicate a significant difference among treatments ($P < .05$).

4. Discussion

It has been widely reported that soil water status is a very important variable explaining changes in N_2O emission (Chintala et al., 2014; Maag and Vinther, 1996; Schindlbacher, 2004; Yanai et al., 2007). Hence, we related the effect of biochar addition on temporal change in N_2O emission rates with soil % WFPS. In Exp A and C, biochar amendment had significant stimulating effects on N_2O emission throughout experiments and the soil % WFPS was maintained at a level lower than approximately 40% (Fig. 1A & C). On the other hand, in Exp B, a

Table 3
Changes in soil pH, hot water extractable carbon (HWC), and fluorescein diacetate hydrolysis activity (FDA) with CHAR treatment. The CHAR treatment involved application of rice husk biochar at 2 ton ha^{-1} in Exps A,B, and C. Numbers in parentheses indicate standard error and an * indicates a significant difference between the CON and CHAR treatments ($P < .05$).

		pH	HWC[a]	FDA
		1:5	gC kg^{-1}soil	mg fluorescein g^{-1} soil
ExpA	CON	7.2 (±0.20)	0.18 (±0.02)	0.22 (±0.02)
	CHAR	7.2 (±0.20)	0.24* (±0.03)	0.31* (±0.02)
ExpB	CON	6.90 (±0.31)	0.62 (±0.04)	1.12 (±0.27)
	CHAR	7.27 (±0.22)	0.67 (±0.03)	1.09 (±0.05)
ExpC	CON	5.60 (±0.12)	1.41 (±0.06)	0.16 (±0.01)
	CHAR	4.86* (±0.04)	1.47 (±0.05)	0.18 (±0.07)
	CHAR + DCD	5.07* (±0.08)	1.55 (±0.05)	0.16 (±0.04)

[a] HWC, hot water extractable carbon.

positive effect of biochar addition on N_2O emission was not observed when the soil % WFPS level was either higher or lower than 40% (Fig. 1B). This indicates that dry soil condition is not the sole variable explaining stimulating effects of biochar on N_2O emission. This further implies that understanding the complex interactions between soil water status and other conditions influencing nitrification should be considered to explain the variable effects of biochar on N_2O emission (Cayuela et al., 2014; Chen et al., 2015; Verhoeven et al., 2017; Yoo and Kang, 2012).

Considering the results by Bateman and Baggs (2005) who reported that the main process of soil N_2O emission was nitrification when the soil %WPFS was 35–60%, we assumed that the main mechanism of N_2O emission was nitrification in Exp A and C because the % WFPS was maintained lower than 40% throughout the experiments. In addition, in Exp C, we verified that the main mechanism of N_2O production in this soil was nitrification because cumulative N_2O emission was significantly lower in the CHAR + DCD than CHAR treatments (Fig. 3). On the other hand, in Exp B, as the soil % WFPS from day 1 to day 12 was higher than 50%, we should consider denitrification as well as nitrification as the possible mechanism of N_2O emission during this period. However, after day 12, the soil %WFPS was maintained below 40% and we also assumed that nitrification was the main mechanism of N_2O emission.

The pipe model of nitrification was very helpful to understand the complex interactions among soil NH_4^+, NO_2^-, NO_3^-, and N_2O emission (Firestone and Davidson, 1989). Urea fertilization could provide the soil with NH_4^+ and NH_3 via hydrolysis of urea and/or NH_3 volatilization (Clay et al., 1990; Rawluk et al., 2001). Soil NH_4^+ and NH_3 could serve as a substrate for AOB and ammonia oxidizing archea (AOA) and they are transformed into NO_2^- which would be further oxidized into NO_3^- by NOB (Norman and Barrett, 2014). Venterea et al. (2015) reported that urea application to soil can cause elevated soil NO_2^- levels, which in turn promote elevated N_2O production. Their study suggested the mechanism of elevated N_2O emission via urea application as follows: both AOB and NOB are sensitive to NH_3 toxicity, but, in general, NOB are more sensitive than AOB. Thus, soil NO_2^- accumulates in the presence of high NH_3 levels because NOB activity is inhibited and this could increase N_2O emission. As we fertilized soil with urea in all the experiments, urea would be hydrolyzed and/or volatilized into NH_4^+/NH_3 and these processes depend on soil temperature, rainfall, and soil water conditions, etc. (Clay et al., 1990; Rawluk et al., 2001). In Exp A and C, right after urea application, moderate precipitation occurred

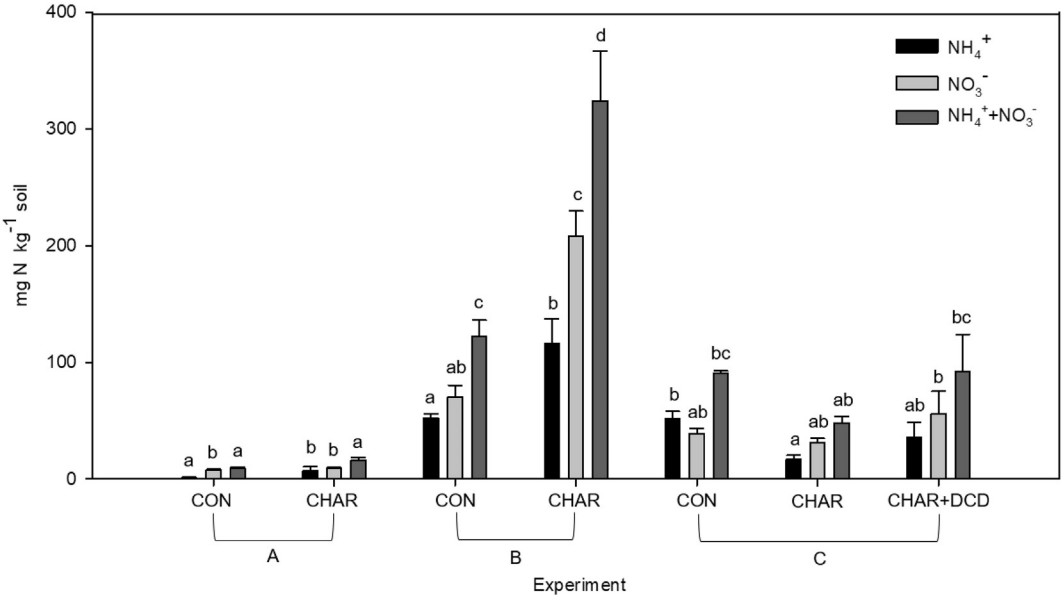

Fig. 3. Treatment effects on soil NH_4^+, NO_3^- and $NH_4^+ + NO_3^-$ content on the last day of the experiment. Comparisons were conducted within each experiment. Bars with different letters indicate significant differences among treatments ($P < .05$).

Table 4
Statistics of principal components on soil and biochar characteristics. Values in the lower panel are the scores of variables for each principal component.

		PC1	PC2	PC3	PC4
Eigenvalue		2.00	1.74	1.23	1.11
Proportion of variance (%)		0.30	0.23	0.17	0.10
Cumulative proportion (%)		0.30	0.53	0.65	0.75
Loadings	Soil TC(%)	−0.46	0.10	−0.19	−0.21
	Soil TN(%)	−0.46	0.15	−0.12	−0.12
	WFPS(%)	−0.17	0.12	−0.07	−0.72
	Soil pH	0.22	−0.15	−0.39	−0.20
	NH_4^+ (mg kg^{-1})	−0.23	0.32	0.16	−0.11
	NO_3^- (mg kg^{-1})	−0.34	−0.14	−0.44	0.29
	CEC (cmol kg^{-1})	0.21	0.34	−0.33	0.17
	Biochar TC(%)	0.19	0.47	0.03	−0.03
	Biochar TN(%)	−0.40	−0.11	−0.16	0.38
	Biochar pH	−0.17	−0.38	0.30	−0.12
	Application rate (w/w%)	−0.20	0.09	0.53	0.24
	Biochar CEC (cmol kg^{-1})	0.12	−0.45	−0.18	−0.11
	Pyrolysis temperature (°C)	0.00	0.33	−0.18	0.16

and dry period was followed for a while (Fig. 1A and C), which is optimal for NH_3 volatilization (Jones et al., 2013). In addition, biochar amendment to soil could further increase NH_4^+/NH_3 contents due to the priming effect of biochar (Luo et al., 2011; Nelissen et al., 2012; Zimmerman et al., 2011). We observed that there was higher HWC content and microbial FDA activity in the CHAR than CON soil in Exp A and C (Table 3). High amount of labile portion of rice husk biochar might have stimulated urease activities in these experiments because the soil microorganisms were limited with very low C content in these sites. Due to higher urease activity in the CHAR soil, we expected higher NH_4^+ contents in the CHAR soil, which might accumulate NO_2^-, leading to higher N_2O emission. The lack of increase in final NO_3^- content compared to NH_4^+ in the CHAR treatment indirectly implied that further oxidation of NO_2^- to NO_3^- might have been hindered (Fig. 3).

On the other hand, in Exp B, where soil C content is also low and the same biochar was applied, there was no increase in N_2O emission by biochar addition. The most evident difference between Exp B and Exp A & C was the relatively high soil water content during the initial period. In this situation, applied urea is rapidly hydrolyzed and there would be high possibility of leaching of hydrolyzed NH_4^+. Biochar's adsorptive capacity of NH_4^+ to its surface was widely reported (Clough et al., 2010; He et al., 2016; Yang et al., 2015). In this experiment, the CHAR soil might have adsorbed more NH_4^+ and this was reflected by the higher final soil NH_4^+ contents in the CHAR treatment than the CON (Fig. 3). As the soil in Exp B is also limited with very low C content similar to that in Exp A and C, urease activity might also have been stimulated by char addition. However, in Exp B, presumed priming effect of biochar on urease activity might not lead to excess NH_4^+ because biochar could serve as slow releasing agent for excessive NH_4^+. Thus, accumulation of NO_2^- in the CHAR treatment might be prevented, resulting in no increase in N_2O emission by CHAR treatment compared to the CON soil.

Our results were not consistent with the discussion by Singh et al. (2010) and Sánchez-García et al. (2014) who attributed increased N_2O emission by biochar amendment to enhanced nitrification. If we use a simple equation to calculate nitrification rate using the changes in soil NH_4^+ and NO_3^- contents and plot the relationship between nitrification rate and N_2O emission, we found that the correlation was significantly negative in Exp B (Fig. 5A). Whereas, in Exp A and C, there was no trend between nitrification rate and N_2O emission. This indicates that we need to understand the effect of biochar on each step of nitrification including urea hydrolysis, NH_3 volatilization, NO_2^- accumulation, etc. for better prediction of the direction of change in N_2O emission. In Exp A and C, biochar stimulated overall microbial activity including urease activity and this provided the soil with excessive NH_4^+ and this would lead to NO_2^- accumulation and concomitant N_2O emission. In this situation, the widely reported liming effect of biochar was counterbalanced by the acidification due to enhanced oxidation by biochar addition (Boer and Kowalchuk, 2001; Wrage et al., 2001). In Exp A, soil pH was not changed by CHAR treatment and it was rather decreased in Exp C (Table 3). The decrease in soil pH related to biochar addition was also observed in Li et al. (2015). In Exp B, on the other hand, biochar's liming effect was evident because final soil pH was increased by the CHAR treatment. However, the priming effect of biochar was not clearly observed because soil HWC and microbial FDA activity were not increased by the CHAR treatment (Biederman and Stanley Harpole, 2012; Zhang et al., 2010). The main reason for different effects of biochar in the Exp A & C and Exp B could be initial soil water status right after urea application and the timing of biochar application.

The results from PCA identified the main factors explaining the variance of the data and they supported our explanation on the effects of biochar on nitrification-mediated N_2O emission. PC1 was related to soil C and N contents, PC2 was related to biochar characteristics, PC3 was related to biochar application rate, and PC4 was highly related to soil %WFPS. The highly significant and negative correlation coefficient between the PC4 and % N_2O change by biochar addition implies that when the soil water content is low, biochar amendment tends to have a stimulating effect on N_2O emission and when the soil water content is high, biochar amendment could reduce N_2O emission. This interpretation was demonstrated in the biplot between PC1 and PC4. Data points reporting the increase in N_2O emission by biochar addition were located in the positive PC4 area, while the ones reporting the decrease in N_2O

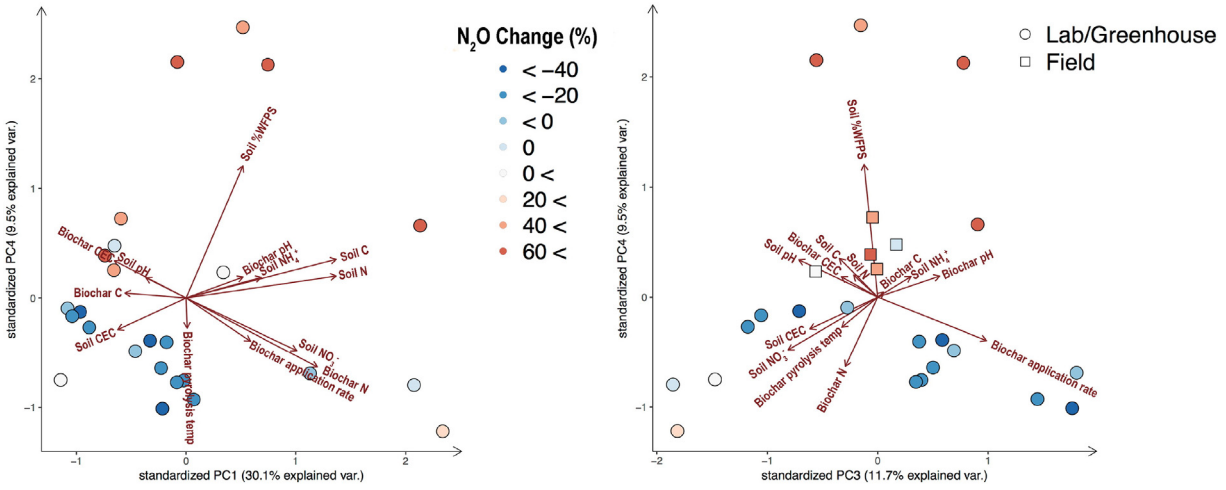

Fig. 4. Principal component plots using PC1 and PC4 (A) and PC3 and PC4 (B). Arrows represent input variables projected in the loading spaces. Different symbols indicate the changes in N_2O emission by biochar addition from the compiled data.

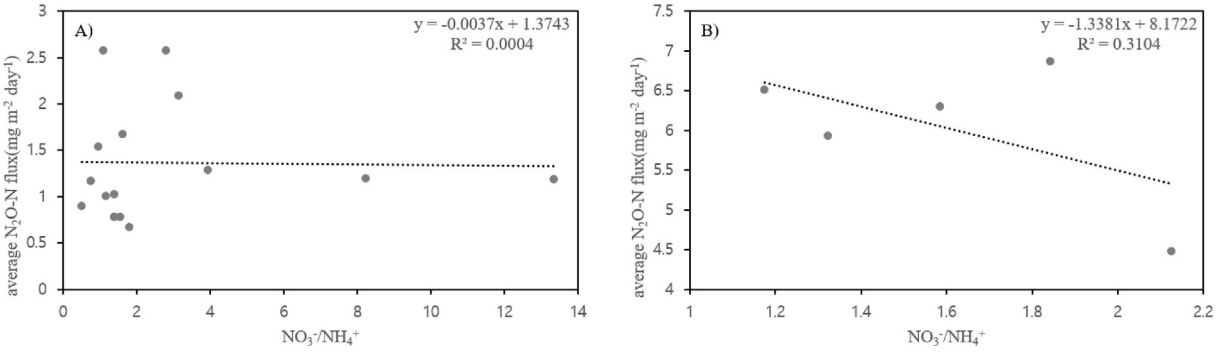

Fig. 5. The relationship between nitrification rate and N_2O emission rate in A) Exp A and C and B) Exp B.

emission by biochar addition were located in the negative PC4 area (Fig. 4A). In the score plot between the PC3 and PC4, we identified the difference between data from the lab/greenhouse and field experiments. Data collected from the laboratory/greenhouse was located in the area of higher absolute values (either positive or negative) along with y axis and data from the field was located near zero along close to the x axis, indicating that data from the lab might overestimate the effects of biochar on N_2O emission. This was consistent with the discussion by Cayuela et al. (2014) and Verhoeven et al. (2017). Our statistical analysis indicated that soil water status is the primary determinant predicting the direction of change (increase or decrease) in N_2O emission. As the secondary factors, soil C content could influence the role of biochar in soil N dynamics.

5. Conclusions

This study compared the effects of biochar addition on N_2O emission between three field experiments, where soil water status was dry favoring nitrification. Reduction in N_2O emission by biochar addition was not found in this study. Instead, N_2O emissions were increased or not changed when the biochar and urea were applied together to soils with low C content. Soil water status during the period right after application of biochar and urea was the main determinant of variable effects of the same biochar on N_2O emission.

Our discussion on the three field experiments was further supported and generalized by the PCA results. PC4, which showed significant negative correlation with soil %WFPS, was highly correlated with the direction and magnitude of change in N_2O emission by biochar addition. PC1, which was significantly correlated with soil C and N contents, was also correlated with the change in N_2O emission. Our study is unique in that we obtained the results from multiple field experiments covering the whole cropping periods. In addition, this study identified specific conditions where biochar addition would have stimulating effects on N_2O emission. Mechanism based investigation of nitrification-mediated N_2O emission improved our understanding of the variable effects of biochar. Further studies should focus on measuring soil NO_2^- and nitrification-related microbial organisms to confirm these findings.

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