Effect of Biochar Application on the Efficacy of the Nitrification Inhibitor Dicyandiamide in Soils

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A series of laboratory incubation experiments was conducted to evaluate the effect of biochar application on the efficacy of the nitrification inhibitor (NI) dicyandiamide (DCD) in Cambisol (pH 7.14) and Latosol (pH 4.83). The feedstocks (eucalyptus wood, coconut coir, and rice straw), pyrolysis temperatures (350, 500, and 650 ˚C), and application rates (0.5, 1.0, 2.5, and 5.0% of 200 g soil) were identified as influential factors. The results showed that biochar could significantly reduce the effectiveness of DCD on nitrification inhibition. Biochar produced from eucalyptus wood with a large surface area (426.4 m^2 g^{-1}) had the strongest ability to reduce the inhibitory effect of DCD in nearly neutral Cambisol, while biochar from rice straw with a high pH had the greatest influence on acidic Latosol. Increasing pyrolysis temperature and application rates can strengthen the ability of biochar to reduce the inhibitory effect of DCD. Generally, the decrease of the DCD nitrification inhibitory effect on nearly neutral soil was controlled by the surface area of the applied biochar; meanwhile, the rise of soil pH caused by biochar application was also an important influencing factor in acid soil.

Keywords: Biochar; Dicyandiamide (DCD); Nitrification; Soil; S-shaped function

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INTRODUCTION

Agricultural systems have become increasingly dependent on nitrogen (N) application, as natural N fixation is insufficient for maintaining high productivity. Nearly 90% of N fertilizers applied worldwide are in ammonium (NH_4^+) form or NH_4^+-producing form such as urea (Subbarao *et al.* 2012). Because soil colloid and organic matter mainly carry a negative charge, NH_4^+ is resistant to loss from soil on account of strong electrostatic adsorption (Arora and Srivastava 2013). However, the NH_4^+ is easily oxidized into the nitrate (NO_3^-) through nitrification by chemolithoautotrophic bacteria (*Nitrosomonas* spp. and *Nitrobacter* spp.) (McCarty 1999) and ammonia-oxidizing archaea. NO_3^- is a highly labile and mobile N form that can be lost from agricultural systems through leaching and/or runoff, and released in gaseous form (N_2O and NO) through denitrification (Upadhyay *et al.* 2011). Therefore, nearly 70% of the N applied is lost, an effect that leads to many potential environmental problems, such as water pollution, eutrophication, and the greenhouse effect (Hungate *et al.* 2003).

Adding nitrification inhibitors (NIs) has been proven to be an effective management strategy for N fertilizer. NIs can reduce nitrification in soil and further lower the loss of N fertilizer by killing nitrifiers or disturbing their metabolism (Guo *et al.* 2014). Dicyandiamide (DCD; $C_2H_4N_4$), one of the most widely applied NIs in agricultural production worldwide, can increase the content of NH_4^+-N in soil and improve N utilization

rate and crop yield (Cui *et al.* 2011), as well as reduce the leaching of NO_3^- and the emission of N_2O (Monaghan *et al.* 2013). However, the efficacy of DCD is not absolutely stable, and is affected by soil properties such as texture, temperature, pH, water content, and organic matter content (Guiraud and Marol 1992; Kumar *et al.* 2000), as well as application rate and form (Cookson and Cornforth 2002; Chaves *et al.* 2006).

Meanwhile, biochar application as another soil management strategy has become the research focus in recent years. Biochar, a solid carbon-rich residue generated through the thermal decomposition of biomass under the condition of a partial or total absence of oxygen (Sohi *et al.* 2010), has attracted widespread attention as a soil amendment. Application of biochar in agriculture is not only beneficial for the recycling of biomass and mitigation of climate change, but also shows good potential for improving soil fertility. Biochar can improve the physical, chemical, and biological properties of soil, including soil structure, nutrient availability, and water and nutrient retention, thus promoting plant growth (Glaser *et al.* 2002; Taghizadeh-Toosi *et al.* 2012) and increasing crop biomass and yield (Major *et al.* 2010; Liu *et al.* 2013). Moreover, it can reduce N loss from agriculture by regulating soil N cycling and soil-plant-microbe interactions (Castaldi *et al.* 2011; Cheng *et al.* 2012).

One of the important features of biochar is its strong adsorption ability, which is caused by its variety of functional groups, micro-porous structures, and large specific surface area that is available for chemical reactions or as a substrate for microorganisms. This feature enables biochar the ability of nutrient retention and influences the fate and ecotoxicological effects of organic compounds in soil (Park *et al.* 2011; Tsai and Chen 2013). For example, recent studies have shown that the content and biological activity of organic compounds such as phenols, trichloroethylene, atrazine, sulfamethoxazole, phenanthrene, and polycyclic aromatic hydrocarbons (Cornelissen *et al.* 2005; Cao and Harris 2010; Yao *et al.* 2012) in water or soil are reduced by the addition of biochar. Application of the amendments with strong adsorbability into soil may have either a positive or negative impact on the efficacy of purpose-applied inputs of agricultural production. For example, adsorption to solid phase reduces the leaching of soil-applied compounds and protects them from microbial degradation (Jones *et al.* 2011), although the compounds may be inactive through the strong adsorption of biochar (Graber *et al.* 2011). This means that more amendments are required to achieve the expected effect, resulting in unpredictable environmental risks resulting from their accumulation in soil.

Currently, few studies have reported on the effects of biochar application on the efficacy of NIs. Based on the high affinity and adsorption potential of biochar for organic compounds, we hypothesized that the ability of DCD to prevent the biological oxidation of NH_4^+ to NO_3^- would be reduced by biochar application in soil. Therefore, the purposes of this study were: (1) to reveal the effect of biochar application on the efficacy of nitrification inhibition for DCD in different soils; (2) to evaluate the relationship between the effect and feedstocks, pyrolysis temperatures, and application rates of biochars; and (3) to determine the determinant factor for the effect.

EXPERIMENTAL

Materials

Two types of soil (Cambisol and Latosol) used for this study were collected from different climate zones in China and therefore had different chemical and physical

properties. The former was taken from the Experimental Station of Shenyang Agricultural University (temperate zone, 41°49′37″N, 123°34′38″E) and the latter from the Experimental Station of Chinese Academy of Tropical Agricultural Sciences (tropical zone, 19°29′17″N, 109°29′49″E). Soil samples were collected from the arable layer (0 to 20 cm), air-dried, ground, and then sieved (<2 mm) for incubation experiments. The basic chemical and physical properties of the soils used are summarized in Table 1.

The feedstocks of biochar were eucalyptus wood, coconut coir, and rice straw, respectively, which are common and readily available from agricultural and forestry wastes on Hainan Island in China. The biochars were prepared with the method recommended by Yuan and Xu (2012). Briefly, biomass was air-dried at room temperature and ground to pass a 2-mm sieve before being placed into a ceramic crucible covered with a fitting lid, then pyrolyzed under oxygen-limited conditions in a muffle furnace. The pyrolysis temperature was raised to selected values of 350, 500, or 650 °C at a rate of approximately 20 °C min^{-1} and held constant for 2 h. After pyrolysis, the biochar was allowed to cool to room temperature and then stored in a polyethylene bag. The labels and basic properties of the five biochars prepared from three different feedstocks are presented in Table 2.

Biochar pH was measured in deionized water using a 1 to 5 w/v ratio (Gaskin *et al*. 2008). The surface area of biochars was determined using a BET meter (ASAP2020, Micromeritics, USA). The cation exchange capacity (CEC) of soil and biochar was determined by a method described by Gillman and Sumpter (1986). Total C and N analyses of soil and biochar were conducted on a solid TC/TN analyzer (Vario EL Ⅲ, Elementar Analysensysteme GmbH, Germany).

Table 1. Basic Chemical and Physical Properties of Soils Used

Soil Type	pH	CEC	Organic C	Total N	NH_4^+-N	NO_3^--N	Sand	Silt	Clay
		(cmol kg^{-1})	(g kg^{-1})		(mg kg^{-1})		(%)		
Cambisol	7.14	14.8	14.83	1.24	1.21	23.62	45.2	35.2	19.6
Latosol	4.83	6.38	7.35	0.74	8.65	13.22	23.2	24.4	52.4

Table 2. Basic Properties of the Biochars Produced from Eucalyptus Wood, Coconut Coir, and Rice Straw

Feedstock	Biochar Label	Pyrolysis Temperature (ºC)	pH	CEC (cmol kg^{-1})	Surface Area (m^2 g^{-1})	Total C (g kg^{-1})	Total N (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
Eucalyptus wood	EW500	500	8.37	8.2	426.4	786	6.4	1.4	0.8
Coconut coir	CC500	500	9.13	17.5	218.7	738	13.9	1.7	2.2
Rice straw	RS350	350	8.22	34.5	6.9	537	15.8	2.4	3.2
	RS500	500	9.85	21.8	72.6	676	14.3	2.6	3.2
	RS650	650	10.24	12.4	214.2	725	11.6	3.1	4.7

Methods

To study the effect of feedstocks, pyrolysis temperatures, and application rates of biochar on the inhibitory efficacy of DCD in soils, incubation experiments with the following combinations of fertilizer, DCD, and biochar were conducted.

No-biochar treatments

1. CK: no N, DCD, or biochar
2. N: 150 mg kg^{-1} NH_4^+-N only
3. DCD: 150 mg kg^{-1} NH_4^+-N and 10 mg kg^{-1} DCD

Feedstocks

4. 1.5%EW500: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 1.5% EW500
5. 1.5%CC500: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 1.5% CC500
6. 1.5%RS500: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 1.5% RS500

Pyrolysis temperatures

7. 2%RS350: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 2% RS350
8. 2%RS500: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 2% RS500
9. 2%RS650: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 2% RS650

Application rates

10. 0.5%CC500: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 0.5% CC500
11. 1%CC500: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 1% CC500
12. 2.5%CC500: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 2.5% CC500
13. 5%CC500: 150 mg kg^{-1} NH_4^+-N, 10 mg kg^{-1} DCD, and 5% CC500

The experiments were carried out in a series of 1-L glass flasks with three replications. Biochar was thoroughly mixed with 200 g of soil and then added to distilled water dissolved $(NH_4)_2SO_4$ and DCD to adjust the soil moisture content to 60% water-holding-capacity. The mixtures were introduced into flasks before being covered with polyethylene film with pinholes to maintain aerobic condition. All samples were incubated at a constant temperature of 25 °C. The evaporation loss of water was adjusted once every three days by adding distilled water throughout the experimental period.

A soil subsample (5 g) was taken after 1, 14, 21, 28, 35, 42, 49, 56, 63, and 70 days of incubation, respectively, to measure the concentration of NH_4^+-N and NO_3^--N. Another 5 g of soil subsample was taken at the same time to measure soil pH. Soil NH_4^+-N and NO_3^--N concentrations were measured using a subsample of moist soil extracted with 2 mol L^{-1} KCl solution. After centrifugation and filtration, the soil extract was analyzed using a continuous flow autoanalyzer (Auto-Analyzer III, BRAN + LUEBBE; Germany). Soil pH was measured in a suspension of soil and water (1:2.5 w/v).

Data Processing and statistics

During long-term incubation, the accumulation of NO_3^--N in soil (nitrification) follows an S-shaped function because there is a delay phase before rapid nitrification, followed by the maximal rate phase, until nitrification reaches a plateau with the exhaustion of NH_4^+ (Hadas *et al.* 1986). The S-shaped curve can be described by the logistic model described by De Neve *et al.* (2004) in Eq. 1,

$$N(t) = \frac{N_A}{1+\beta e^{-kt}} \quad (1)$$

where $N(t)$ (mg kg^{-1}) is the amount of NO_3^--N accumulation in soil at the incubation time of t(d), N_A (mg kg^{-1}) is the potential amount of N nitrified, β is a constant of dimensionless quantity that determines the initial rate of nitrification, and k (d^{-1}) is the nitrification rate constant. The slope of tangent at the inflection point is the maximum value, defined as the maximal nitrification rate and represented with K_{max} (mg kg^{-1} d^{-1}) in Eq. 2,

$$K_{\max} = k \times \frac{N_A}{4} \quad (2)$$

The node between the straight line of maximal nitrification rate and time-axis is defined as the delay time of nitrification, represented by T_d (d) in Eq. (3):

$$T_d = \frac{\ln\beta - 2}{k} + \frac{4}{k(\beta+1)} \quad (3)$$

The time when the accumulated amount of NO_3^--N reaches $N_A/2$ is defined as the half-oxidation time of NH_4^+, represented by $T_{0.5}$ (d) in Eq. 4:

$$T_{0.5} = \frac{\ln\beta}{k} \quad (4)$$

All data were analysed statistically with one-way ANOVA and the Duncan test ($p<0.05$) using SPSS 18.0 (IBM, USA). Linear and nonlinear regression analysis and curve-fitting were performed using Origin Pro 7.0 (Origin Lab, USA).

RESULTS

No-biochar Treatments

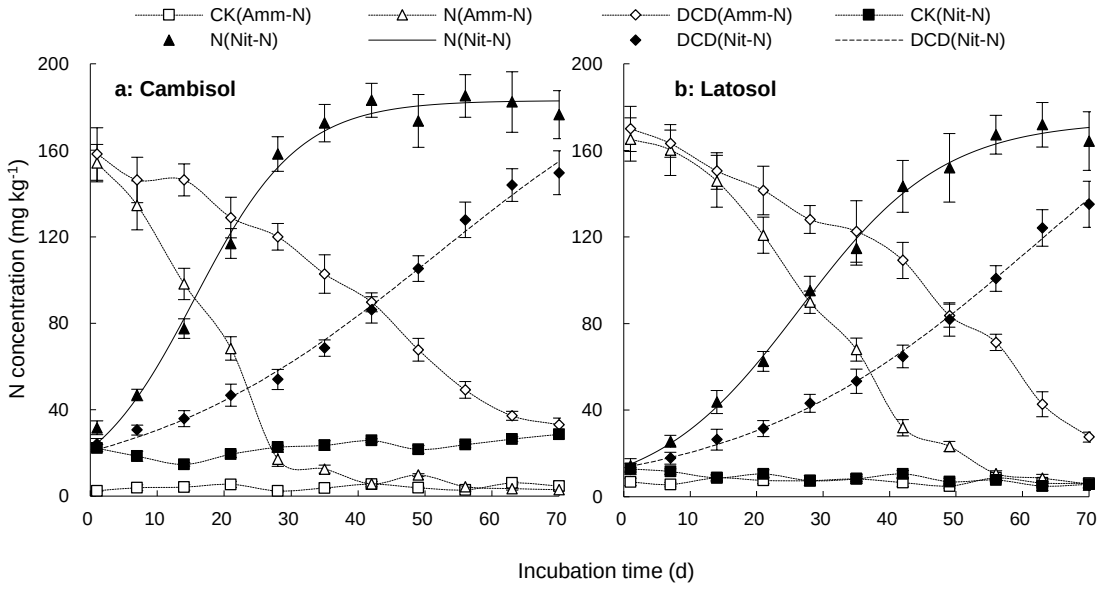

Fig. 1. Dynamics of NH_4^+-N and NO_3^--N concentration in (a) Cambisol and (b) Latosol during the incubation of no-biochar-application treatments (CK, N, and DCD). Curves represent the S-shaped function fitted to the nitrification data, error bars represent standard errors (n=3), and Amm and Nit represent NH_4^+ and NO_3^-, respectively

The NH_4^+-N and NO_3^--N concentration in the CK soils, which were not applied with N fertilizer, DCD, or biochar, fluctuated and remained at a low level during the 70-day incubation period (Fig. 1) because of the balance of mineralization, nitrification, and denitrification. The concentration of NH_4^+-N decreased very quickly in the soils treated with only 150 mg kg^{-1} N as $(NH_4)_2SO_4$. Approximately 90% of NH_4^+-N disappeared after 28 days of incubation in Cambisol, and 80% of NH_4^+-N was oxidized after 42 days of incubation in Latosol (Fig. 1).

Correspondingly, the accumulation rates of NO_3^--N were very rapid. This indicates that nitrification occurred rapidly, and NH_4^+ was oxidized to NO_3^-quickly in the soils without amended-DCD. Fitting with the S-shaped function, the nitrification parameters K_{max}, T_d, and $T_{0.5}$ of N treatments in Cambisol and Latosol were 5.86 and 3.99 mg kg^{-1} d^{-1}, 3.73 and 8.35 d, and 15.63 and 26.64 d, respectively (Table 3).

Table 3. Parameters of the S-shaped Function Fitted to the Nitrification Data of the N-application Treatment Soils

Soil Type	Experiment	Treatment	N_A (mg kg^{-1})	β	k (d^{-1})	R^2	K_{max} (mg kg^{-1} d^{-1})	T_d (d)	$T_{0.5}$ (d)
Cam-bisol	No-biochar	N	183.0(1.0)	7.39(0.24)	0.128(0.011)	0.992	5.86	3.73	15.63
		DCD	224.4(6.8)	9.87(0.92)	0.044(0.004)	0.994	2.47	14.94	52.03
	Feedstock	1.5%EW500	184.2(0.2)	11.25(0.86)	0.109(0.012)	0.986	5.02	6.85	22.21
		1.5%CC500	187.3(0.3)	9.67(0.38)	0.082(0.006)	0.993	3.84	7.85	27.67
		1.5%RS500	185.8(0.5)	9.33(0.99)	0.066(0.005)	0.994	3.07	9.40	33.84
	Pyrolysis temperature	2%RS350	221.8(4.0)	8.63(0.80)	0.046(0.005)	0.992	2.55	12.40	46.85
		2%RS500	191.1(0.4)	8.81(0.93)	0.069(0.005)	0.995	3.30	8.46	31.53
		2%RS650	188.5(0.1)	7.27(0.85)	0.085(0.006)	0.994	4.01	5.50	23.34
	Application rate	0.5%CC500	211.3(1.4)	8.27(0.41)	0.047(0.003)	0.998	2.48	11.58	44.95
		1%CC500	196.6(1.0)	9.18(0.76)	0.066(0.004)	0.997	3.24	9.24	33.59
		2.5%CC500	182.9(0.8)	7.73(0.72)	0.089(0.005)	0.997	4.07	5.66	22.98
		5%CC500	179.5(0.7)	7.13(0.91)	0.109(0.007)	0.994	4.89	4.19	18.02
Latosol	No-biochar	N	173.6(0.2)	11.60(0.41)	0.092(0.005)	0.996	3.99	8.35	26.64
		DCD	234.5(7.9)	16.42(0.39)	0.045(0.003)	0.997	2.64	22.85	62.19
	Feedstock	1.5%EW500	178.0(1.0)	9.53(0.73)	0.063(0.004)	0.997	2.80	10.07	35.78
		1.5%CC500	173.0(0.1)	10.49(0.39)	0.062(0.002)	0.992	2.68	11.27	37.91
		1.5%RS500	165.9(0.9)	13.01(0.44)	0.090(0.005)	0.995	3.73	9.46	28.51
	Pyrolysis temperature	2%RS350	177.4(0.7)	11.80(0.08)	0.058(0.004)	0.986	2.57	13.46	42.55
		2%RS500	171.8(0.9)	11.13(0.81)	0.092(0.003)	0.995	3.95	8.04	26.19
		2%RS650	164.9(0.5)	9.48(0.30)	0.111(0.007)	0.993	4.58	5.68	20.26
	Application rate	0.5%CC500	241.4(2.1)	13.82(0.92)	0.044(0.003)	0.998	2.66	20.36	59.68
		1%CC500	216.6(8.7)	11.45(0.28)	0.048(0.006)	0.991	2.60	15.82	50.79
		2.5%CC500	167.7(0.8)	10.23(0.92)	0.084(0.004)	0.997	3.52	8.11	27.68
		5%CC500	174.6(0.2)	6.56(0.66)	0.085(0.005)	0.995	3.71	4.82	22.13

Values in brackets are standard errors of the fitting parameters (n=11).

The nitrification rate in soil was significantly reduced by DCD application. During the incubation period, NH_4^+-N concentration in DCD-treated soils was always higher than that in N-only soils, whereas NO_3^--N concentration was lower (Fig. 1). At the end of the 70-day incubation, NH_4^+-N concentration in DCD-treated soils was significantly higher

($p<0.05$) than that in CK and N-only soils, and the NO_3^--N concentration did not reach the potential amount (N_A). This suggests that DCD application of 10 mg kg^{-1} could effectively reduce the rate of oxidation from NH_4^+-N to NO_3^--N, making N present in NH_4^+ form. Compared with N treatments, the K_{max} of DCD treatments in Cambisol and Latosol were decreased by 57.8% and 33.8%, T_d were extended by 11.21 and 14.50 d, and $T_{0.5}$ were increased by 36.40 and 35.55 d (Table 3).

Feedstocks

The dynamics of NH_4^+-N and NO_3^--N concentration during incubation in two soils, treated with fertilizer, DCD, and three biochars (EW500, CC500, and RS500) produced from different feedstocks are given in Fig. 2. Table 3 summarizes the fitted results of nitrification using the S-shaped function. The results showed that the NH_4^+-N concentration of biochar-treated soil was higher than that of N-treated soil, whereas it was lower than that of DCD-treated soil. This indicates that the inhibitory effect of DCD on nitrification was reduced by biochar application, resulting in the oxidation rate of NH_4^+ and accumulation rate of NO_3^--N being higher than those in soil treated with DCD. The parameters of the S-shaped function fitted to the nitrification data showed a similar conclusion: The values of K_{max}, T_d, and $T_{0.5}$ of biochar-treated soil ranged between that of N-treated soil and DCD-treated soil.

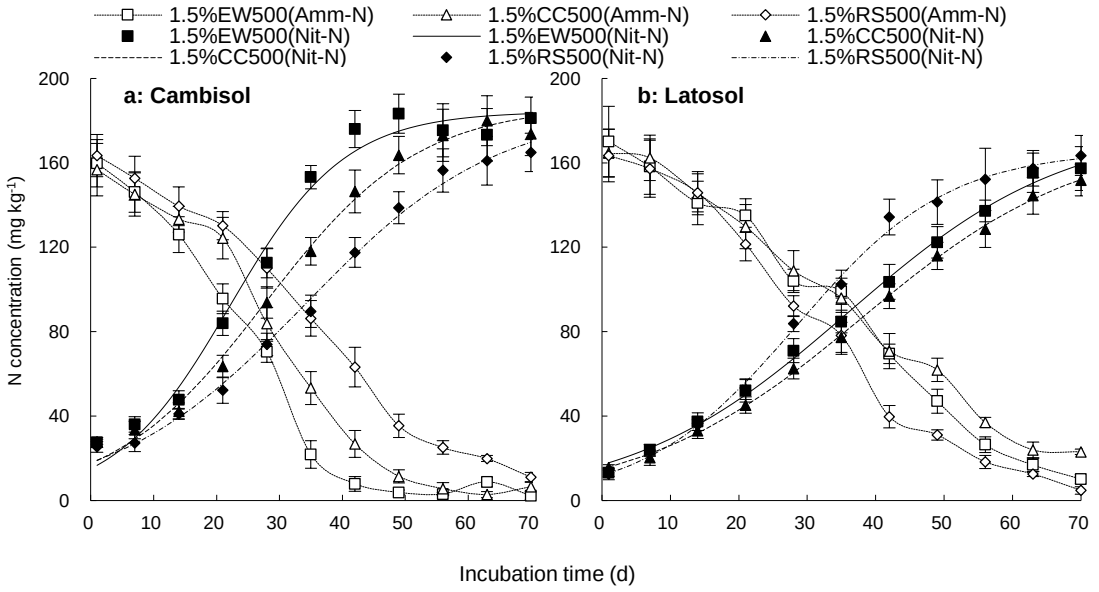

Fig. 2. Dynamics of NH_4^+-N and NO_3^--N concentration in (a) Cambisol and (b) Latosol treated with $(NH_4)_2SO_4$, DCD, and biochars produced from various feedstocks (1.5%EW500, 1.5%CC500, and 1.5%RS500) during incubation. Curves represent the S-shaped function fitted to the nitrification data, error bars represent standard errors (n=3), and Amm and Nit represent NH_4^+ and NO_3^-, respectively

Comparing nitrification in soils treated with biochars pyrolyzed from three types of feedstocks at 500 ºC applied with the same rate (1.5% of soil), it can be observed that K_{max} as a nitrification parameter in Cambisol complied with 1.5%EW500 > 1.5%CC500 > 1.5%RS500, while T_d and $T_{0.5}$ showed the opposite trend and complied with 1.5%RS500 > 1.5%CC500 > 1.5%EW500. The data suggested that EW500 biochar had the strongest

negative effect on the inhibitory efficacy of DCD, followed by CC500, while RS500 had the weakest effect. As for Latosol, the sequence of K_{max} was 1.5%RS500 > 1.5%EW500 > 1.5%CC500, and that of T_d and $T_{0.5}$ was 1.5%CC500 > 1.5%EW500 > 1.5%RS500. In other words, RS500 biochar had the strongest negative effect on the efficacy of DCD in Latosol, followed by EW500 and CC500.

Pyrolysis Temperatures

The dynamics of NH_4^+-N and NO_3^--N concentration in Cambisol and Latosol treated with $(NH_4)_2SO_4$, DCD, and rice straw biochars produced at various pyrolysis temperatures (2%RS350, 2%RS500, and 2%RS650) during incubation are shown in Fig. 3, and the parameters fitted by the S-shaped function are presented in Table 3. Similarly to the feedstock experiment, the NO_3^--N accumulation rate in biochar-treated soil was significantly higher than that in DCD-treated soil ($p<0.05$), indicating that the efficacy of DCD was reduced by biochar application to different extents. Furthermore, different pyrolysis temperatures for biochar had a distinct degree of influence on the efficacy of DCD. Generally, as pyrolysis temperature increased, the inhibitory ability of DCD on nitrification was decreased by rice straw biochar, with K_{max} increasing and T_d and $T_{0.5}$ decreasing. It is worth mentioning that K_{max} in Latosol treated with 2%RS650 was larger than that in N-only soil, whereas T_d and $T_{0.5}$ were smaller than that in N-only soil. This suggests that the nitrification inhibitory effect of DCD was counteracted by biochar application, and even that nitrification in the soil was promoted.

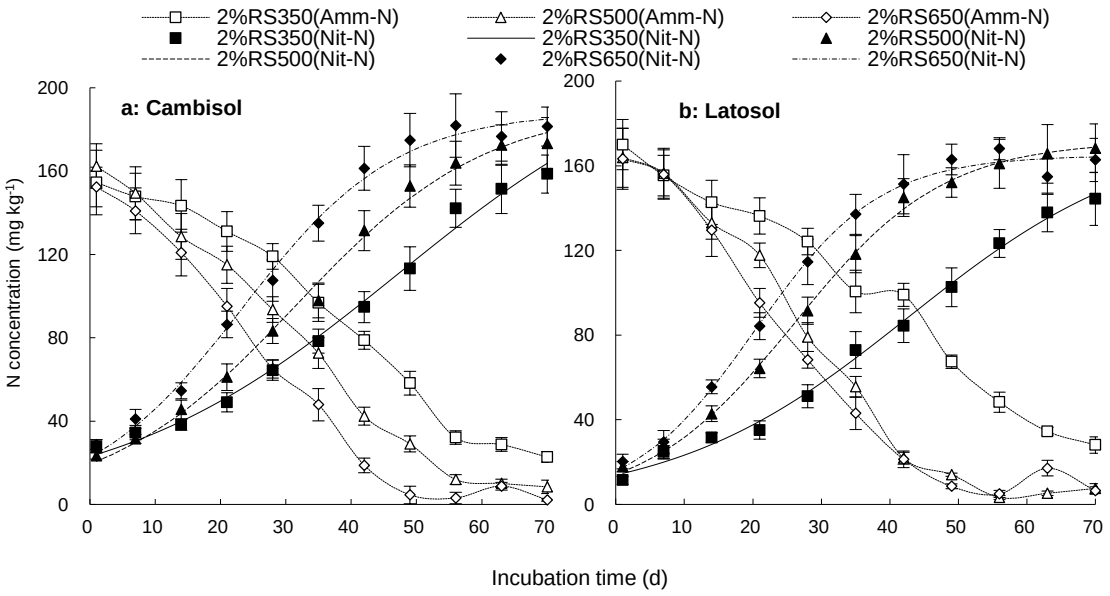

Fig. 3. Dynamics of NH_4^+-N and NO_3^--N concentrations in (a) Cambisol and (b) Latosol treated with $(NH_4)_2SO_4$, DCD, and rice straw biochars produced at various pyrolysis temperatures (2%RS350, 2%RS500, and 2%RS650) during incubation. Curves represent the S-shaped function fitted to the nitrification data, error bars represent standard errors (n=3), and Amm and Nit represent NH_4^+ and NO_3^-, respectively

Application Rates

With increasing application rates of coconut coir biochar pyrolyzed at 500 ºC, the decrease of NH_4^+-N concentration in Cambisol and Latosol was sped up, and the

accumulation of NO_3^--N increased (Fig. 4), with K_{max} increasing and T_d and $T_{0.5}$ decreasing (Table 3). This indicates that the nitrification inhibitory effect of DCD in soil can be reduced with increasing application rates of biochar. When the application rate of coconut coir biochar (CC500) in Latosol was 5% of the soil, T_d and $T_{0.5}$ were lower than that in N treatment soil by 42.3% and 16.9%, respectively. The nitrification inhibitory effect of DCD was fully covered by the promotion of biochar.

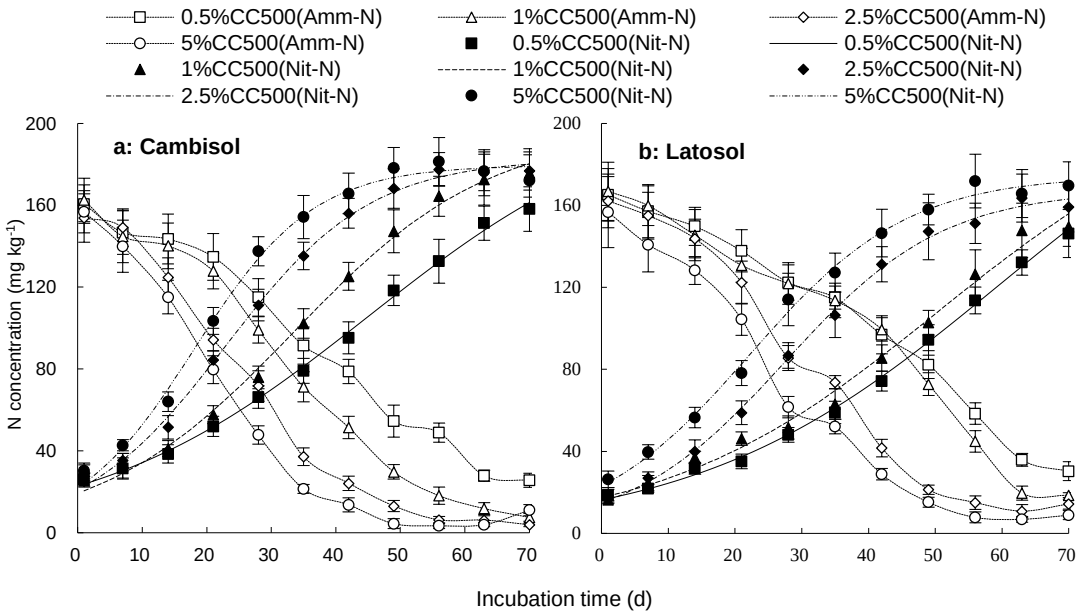

Fig. 4. Dynamics of NH_4^+-N and NO_3^--N concentration in (a) Cambisol and (b) Latosol treated with $(NH_4)_2SO_4$, DCD, and various coconut coir biochar application rates (0.5%CC500, 1%CC500, 2.5%CC500, and 5%CC500) during incubation. Curves represent the S-shaped function fitted to the nitrification data, error bars represent standard errors (n=3), and Amm and Nit represent NH_4^+ and NO_3^-, respectively

DISCUSSION

The NO_3^--N concentration in most of the treatment soils reached or approached the potential amount of nitrification N_A during the 70-day incubation (Table 3). It took about 35 days for the accumulation of NO_3^--N in N treatment to reach 90% of N_A in Cambisol. Because of the rich amorphous iron oxides, heavy texture, and lower pH of Latosol, the nitrification rate was lower than for other types of soil (Burns *et al.* 1996). It took 56 days for NO_3^--N to reach 90% of N_A in Latosol. The oxidization of NH_4^+ was effectively inhibited by DCD application, resulting in a nitrification rate below 2 mg kg^{-1} d^{-1} during incubation in both soils, and a relatively long T_d and $T_{0.5}$. Therefore, similar to the conclusion obtained by other researchers (Slangen and Kerkhoff 1984), N transformation in soil was efficiently suppressed by DCD, maintaining fertilizer N in the form of NH_4^+ in soil for a long time, and preventing N loss and environmental problems caused by the excessive accumulation of NO_3^--N.

As we presumed, the nitrification inhibitory effect of DCD was suppressed by almost all types of biochar at any application rate in this research. Compared with DCD treatment, N_A was reduced in biochar treatments, probably because the strengthened

nitrification caused higher N loss by denitrification. Meanwhile, the two feature times, T_d and $T_{0.5}$, were also reduced to different extents, whereas the K_{max} was accelerated. A similar result was reported in the forest soil of *Pinus ponderosa* with a lower nitrification potential due to the plant secondary metabolites such as dissolved phenols and terpenes, which inhibit soil nitrifying activity (Arora and Srivastava 2013). Applying biochar into the soil could significantly reduce the content of such compounds and improve nitrification potential and net nitrification (DeLuca *et al.* 2006; Ball *et al.* 2010). This conclusion was further supported by the present study by showing that the content of NO_3^--N in soil was promoted by biochar application compared with DCD-only treatment.

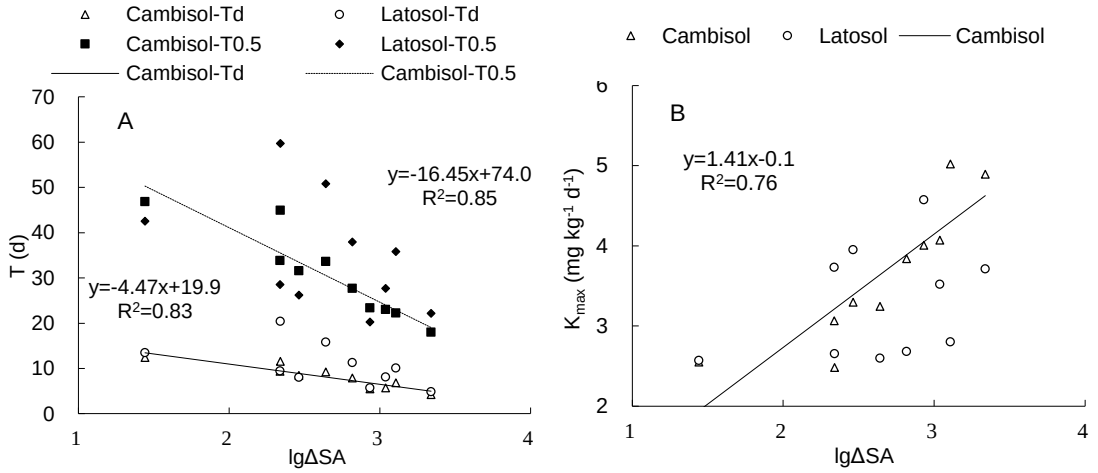

Fig. 5. The relationship between the logarithm of biochar surface area (lgΔSA) and the parameters T_d (A, $p<0.001$), $T_{0.5}$ (A, $p<0.001$), and K_{max} (B, $p=0.001$) of nitrification in soils applied with DCD ($n=10$). ΔSA (m^2) is the surface area of biochar applied to 200 g of soil

Furthermore, data analysis suggested that the ability of biochar to reduce the nitrification inhibitory effect of DCD in Cambisol increases along with the rise of the surface area of applied-biochar, *i.e.*, the parameters T_d, $T_{0.5}$, and K_{max} of nitrification were significantly linearly correlated with the logarithm of surface area ($lg\Delta A_s$) ($p<0.05$) of the applied biochar (Fig. 5). In Latosol, although the parameters of nitrification changed with the surface area of biochar applied, the statistical significance has not been reached (Fig. 5). Because of its varied surface functional groups, micro-porous structure, and large specific surface area, biochar has a strong affinity and adsorption ability for organic substances. The ability of biochar to reduce the efficacy of NIs may generate from the adsorption, with the same mechanism as its inhibition to bioavailability for heavy metals and pesticides in soil (Cui *et al.* 2012; Graber *et al.* 2012). Additionally, biochar seems to assist microbial activity by having a porosity that provides a favourable microhabitat for soil microorganisms, acting as a refuge site and preventing the interference of DCD to nitrifiers (Pietikäinen *et al.* 2000).

Biochar with different properties can be produced from different feedstocks under various conditions (Guerrero *et al.* 2005). Feedstock is the key factor controlling the physico-chemical properties of biochar, while pyrolysis temperature is the most important process parameter (Kwapinski *et al.* 2010). Usually, biochar produced from woody plants with higher lignin contents has a larger output, total C content, and surface area than that from herbal plants and manure (Demirbas 2006). For instance, the surface area of EW500

is 1.9 times that of CC500, and 5.9 times that of RS500 (Table 2). Therefore, EW500 has the strongest influence on the efficacy of DCD in Cambisol compared with the other two biochars. The crystallinity of its carbonaceous structure is an important factor affecting the adsorption ability of biochar, which increases with increasing pyrolysis temperature (Lua *et al.* 2004). Moreover, as pyrolysis temperature rises, -OH and -CH moieties gradually are reduced, C=C moieties increase, and the transformation from amine-N to pyridine-N increases as well (Bagreev *et al.* 2001). The porosity of biochar increases significantly with the rise of pyrolysis temperature, leading to the rapid increase of surface area. In the present study, the surface area of biochar changed from 10 m^2 g^{-1} at a pyrolysis temperature of 350 °C (RS350) to 72.6 m^2 g^{-1} at 500 °C (RS500), and then up to 214.2 m^2 g^{-1} at 650 °C (RS650).

In addition, the adsorptive ability of biochar for the organic substances in soil was also affected by application rates. Generally, the amount of sorption sites and surface area increased with the increment of applied sorbent. The research of Tsai and Chen (2013) showed that the residual equilibrium concentration of paraquat, a type of cation organic herbicide, was lowered in water solution with the increment of pig-manure biochar application. Graber *et al.* (2011) indicated that the dosage of soil fumigant 1,3-dichloropropene for nematodes should be doubled to reach full activity when the soil is amended with biochar of 26 t ha^{-1}. The present research also suggested that the capacity of biochar for reducing the efficacy of DCD drastically changed when applied CC500 increased from 0.5% to 5% of the soil, *i.e.*, the value of K_{max} was doubled, and T_d and $T_{0.5}$ were decreased by 63.8% and 59.9%, respectively, in Cambisol.

Some detailed information from the data on Latosol is worth noting: The capacity of 1.5%RS500 to reduce the efficacy of DCD exceeded that of 1.5%EW500 and 1.5%CC500; the inhibitory effect of DCD was lost entirely in the treatment 2%RS600 and 5% CC500, and the nitrification was even effectively promoted. Moreover, the loss of the DCD inhibitory effect did not show a significant ($p<0.05$) relationship with the surface area of applied biochar in correlation analysis. The authors speculate that the inhibitory effect of DCD could be affected by some other factors in addition to adsorption, among which the changes in soil pH was probably the most important. The subsequent analysis verified the correctness of this inference: The acidity of Latosol was effectively reduced with the application of high-pH biochars (>9.0), and a significant ($p<0.05$) linear correlation was found between soil nitrification parameters T_d, $T_{0.5}$, and K_{max} and the pH values of soils applied with DCD and pH>9.0 biochar (Fig. 6). The pH of acid Latosol returned to neutral and the inhibitory effect of DCD declined with the increasing alkalinity of biochar and application rates. A similar performance did not occur in Cambisol, since there was no significant change in soil pH with biochar application because the initial soil pH was close to neutral (Fig. 6).

The efficacy of DCD in acid soil can be greatly reduced by alkaline biochar, possibly because of the following reasons: (1) Adsorption of biochar for DCD (as explained before); (2) Effect of increasing soil pH on nitrification; autotrophic nitrifying bacteria prefer less acidic soil conditions. The most suitable pH value for nitrification is about 8.5 (Slangen and Kerkhoff 1984). As soil pH increases in the range of 4 to 8.5, the activity of the nitrifying microorganisms in soil will be enhanced (AciegoPietry and Brookes 2008). The soil pH also affects the substrate supplied to the rate-limiting enzyme (ammonia monooxygenase, AMO) for oxidation from NH_3 to hydroxylamine during nitrification by disturbing the NH_4^+/NH_3 equilibrium (pKa=9.25) (Nicol *et al.* 2008). It has been demonstrated that the substrate of AMO is NH_3, rather than NH_4^+ (McCarty 1999); and (3)

Effect of increasing pH on the degradation rate of DCD. The research of Godgers *et al.* (1985) showed that about half of applied DCD in neutral soil had been mineralized after 60 days, while the rate of mineralization in acid soil was only 10 to 25% of that in neutral soil.

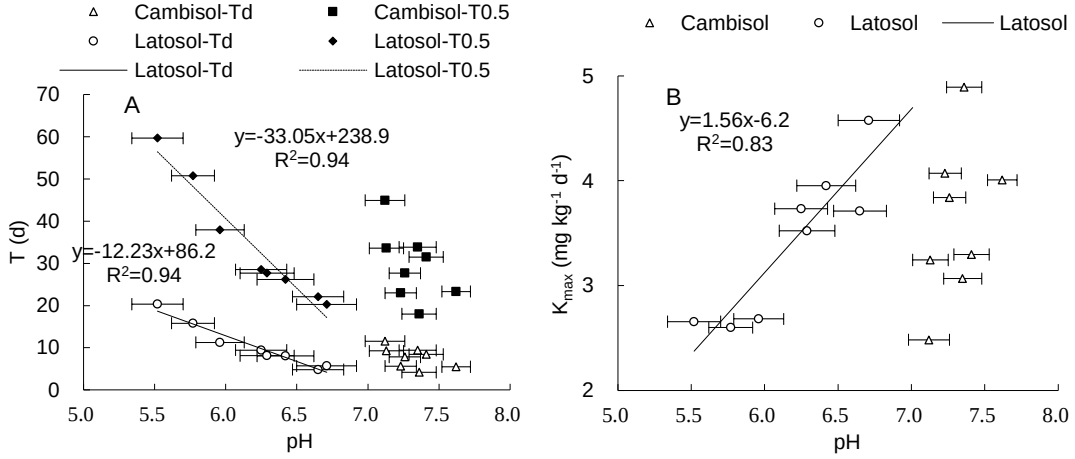

Fig. 6. The relationship between the pH of soil applied with DCD and biochar (pH>9.0) and soil nitrification parameters T_d (A, p<0.001), $T_{0.5}$ (A, p<0.001), and K_{max} (B, p=0.002) (n=8). X-axis represents the average of soil pH during incubation; error bars represent standard errors (n=33)

The various feedstocks from mineral-poor woody materials to mineral-rich crop residues led to highly variable pH values of biochar products, ranging from 4 to 12 (Lehmann 2007). Typically, biochar with a high content of mineral ash has a higher pH than biochar with low ash content. The pH of biochar usually increases with increasing pyrolysis temperature, since high temperature is beneficial for the fusion of alkaline minerals (Lehmann *et al.* 2011). The present results were also consistent with this general rule. The pH of EW500 produced from woody plants was 8.37, the pH of RS350 produced from rice straw pyrolyzed at a low temperature (350 °C) was merely 8.22, while the pH of all other biochars exceeded 9.0 (Table 2). Jeffery *et al.* (2011) noticed that biochars applied in soils with an extensive distribution of pH values could raise the soil pH by 0.1 to 2.0 units. The present research also showed that the rise of pH in neutral Cambisol did not exceed 0.5, while the average rise of the pH in acid Latosol reached about 1.4 for biochar-treated soils.

CONCLUSIONS

1. Biochar application reduced the nitrification inhibitory effect of DCD in soils. The extent of decrease varied with feedstocks. Increase of pyrolysis temperature of rice straw biochar and application rate of coconut coir biochar strengthened the effect of biochar on the inhibitory ability of DCD.
2. The decrease of DCD's nitrification inhibitory ability in soil close to neutral was mostly controlled by the surface area of the applied biochar, while the rise of the soil pH value caused by biochar application was also an important factor affecting the inhibitory efficacy of DCD in acid soil.

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