Soil & Tillage Research 164 (2016) 52–62

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still

The effects of rice-straw biochar addition on nitrification activity and nitrous oxide emissions in two Oxisols

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ARTICLE INFO

Article history:
Received 15 September 2015
Received in revised form 4 May 2016
Accepted 8 May 2016
Available online 24 May 2016

Keywords:
Nitrification kinetic model
Oxisols
Biochar amendment
pH
N_2O emissions

ABSTRACT

Nitrification rates in Oxisols vary with soil pH and substrate availability. Biochar can be used to improve acid soils. The aim of this study was therefore to investigate the interactive impacts of 1% and 5% (*w/w*) rice-straw biochar application on nitrification, ammonia oxidizer populations and nitrous oxide (N_2O) emissions over short periods of microcosm incubation in two agricultural Oxisols derived from granite (RGU) and tertiary red sandstone (RTU), respectively. We measured soil nitrate (NO_3^-) and ammonium (NH_4^+) concentrations during the incubation and used nitrification kinetic model to assess the response of nitrification to biochar addition. We also performed real-time quantitative polymerase chain reaction (qPCR) to quantify the copies of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) genes, and collected N_2O gas at various intervals during the 56-day incubation. The addition of ammonium sulfate ($(NH_4)_2SO_4$-N) stimulated nitrification in both soils. In RGU, biochar treatments altered soil nitrification patterns to a first-order reaction model; this stimulation was more pronounced with the increase of biochar application rates. In RTU, 1% biochar treatment increased nitrification rate constants, and 5% biochar treatment altered nitrification patterns from a zero-order to a first-order reaction model. Treating the two soils with 5% biochar rates significantly increased AOB gene copy numbers up to 7.88- and 14-fold compared with the no biochar controls in RGU and RTU, respectively, while the treatments had little or reduced effect on AOA gene copy numbers. Biochar addition significantly reduced cumulative N_2O emissions up to 37.6% in RGU and 46.4% in RTU, respectively. These results underscore the potential of biochar in the restoration of nitrification and the reduction of greenhouse gas N_2O emission in Oxisols.

1. Introduction

Nitrification is an important part of the soil nitrogen (N) cycle that has long concerned scientists, as this process is the key cause of low fertilizer efficiency, which can lead to groundwater contamination and emissions of the greenhouse gas N_2O. Soils exhibit great variations in nitrification rates (Zhao et al., 2007; Zhao and Xing, 2009). Many factors are involved in regulating soil nitrification, including soil pH, temperature, soil moisture, N-substrate supply, microorganisms and soil type (Che et al., 2015). Understanding the nitrification process and its potential environmental impacts in different soils is crucial for improving soil fertility and environmental protection, which can be achieved by focusing on increasing yields and reducing the environmental risks of this process. Oxisols, the highly weathered soils primarily found in the intertropical regions of the word, occupy about 7.5% of the global land area (Beinroth et al., 1996). Due to the unfavorable space-time distribution of rainfall as well as strong leaching, there are serious losses of chemical fertilizer and nutrient deficiencies in this soil type. Low pH levels, high aluminum toxicity and low cation exchange capacity (CEC) are the main factors that limit plant growth in Oxisols. Many studies have been carried out with the aim of developing new approaches for reducing these obstacles (Roth and Pavan, 1991; Ernani et al., 2002; Anda et al., 2008, 2015; Fageria and Baligar, 2008). Liming materials, which typically include carbonates, oxides or hydroxides of Ca and Mg, are often used to raise soil pH in Oxisols. $CaCO_3$ is a common liming material that is often used to counteract acidification. However, large-scale addition of $CaCO_3$ materials is arduous, and long-term intensive application of $CaCO_3$ can cause soil compaction, formation of

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http://dx.doi.org/10.1016/j.still.2016.05.006
0167-1987/

calcified soils and disequilibrium of Ca, K and Mg levels, thus reducing crop yields (Wang, 1995).

Biochar, which is produced by the thermochemical decomposition of organic material in the absence of oxygen, has been the focus of researchers for the past several years (Karaosmanoglu et al., 2000; Lehmann and Joseph, 2009; Lehmann et al., 2011). Due to its unique structure and composition, land application of biochar can potentially increase carbon (C) sequestration, improve soil and lead to sustainable management of organic waste (Lehmann and Joseph, 2009), a "win-win" scenario. China, a major agricultural country, produces 0.6–0.7 billon tons of straw per year. Converting cheap, abundant crop straws into biochar and applying it to soils may have significant agricultural and environmental benefits (Wang et al., 2013a). The inorganic ash composition of biochar includes metal carbonates, silicates and phosphates, which have significant liming value. Biochar can be used as a soil conditioner to ameliorate acid soils (Yuan and Xu, 2011). We previously found that successive biochar application (22.5 Mg ha^{-1} per season) for one year raised soil pH by 1.11 units and increased wheat yields by 150% in wheat/millet rotation acid Oxisols (Zhao et al., 2014). Since soil pH is believed to be a key factor determining the chemical form, concentration and availability of elements (Kemmitt et al., 2006), biochar addition may disturb the process of soil nitrification. Dai et al. (2014) determined the effects of biochar addition on soil pH and found that biochar alkalinity strongly contributed to the increased pH levels in soil and that the concentration of NO_3^- in soils treated with biochar (at both 1% and 3%) increased with time. Nitrification is mediated by ammonia monooxygenase (AMO), which is derived from AOB and AOA in the soil. Ammonia (NH_3), rather than NH_4^+, serves as the substrate for ammonia oxidizers. High alkalinity shifts the equilibrium between NH_3 and NH_4^+ towards NH_3 and increases substrate availability (Nugroho et al., 2006). Nelissen et al. (2015) found that biochar addition increased gross nitrification rates due to higher substrate availability and the availability of biochar's labile C fraction for nitrifying bacteria (soil pH = 6.4). By contrast, Yang et al. (2015) found that biochar amendment limited nitrification of NH_4^+ into NO_3^- in two soils (pH = 6.31, 5.05) due to the chemical adsorption by biochar despite of the elevated soil pH. These different responses of soil nitrification to biochar addition may be attributed to biochar application rates and the soil types examined. Indeed, more research is needed to evaluate how biochar affects soil nitrification activity.

Intensively managed agricultural soils represent the largest global anthropogenic source of the potent greenhouse gas N_2O. To date, studies examining the effects of biochar addition have revealed both negative and positive effects of this process on N_2O emissions in arable soils, and corresponding mechanisms have yet to be proposed (Cayuela et al., 2013). Reduced N_2O emissions after biochar addition was first reported in a greenhouse experiment by Rondon et al. (2005), who found that N_2O emissions were reduced by up to 80% in a low-fertility Oxisol. By contrast, Singh et al. (2010) observed increased N_2O emissions during the earlier stages of their biochar experiment, which was ascribed to biochar's higher labile N contents. N_2O, a nitrification byproduct, is formed during the incomplete oxidation of hydroxylamine to nitrite (NO_2^-), which accounts for 35–53% of total N_2O emissions in agricultural soils (Huang et al., 2014). N_2O emissions are regulated by a suite of factors that affect the substrate (inorganic N, C source) for nitrification, as well as production pathways (soil pH, temperature, aeration). In acid soils, the addition of alkaline biochar increases soil pH and nitrification (He et al., 2016), which may increase N_2O emissions. According to Ma et al. (2015), the accumulation of the intermediate product of nitrification, NO_2^-, can result in an increase in N_2O emissions. Huang et al. (2014) reported that ammonia-oxidation functions as an engine to generate N_2O. However, Ma et al. (2008) did not detect a relationship between N_2O emissions and the abundance of nitrifiers (AOB) in a dark-brown soil of central Canada derived from loamy unsorted glacial till. It is essential to characterize the dynamics of N_2O emissions and the transformation of NH_4^+ to NO_3^- in order to better predict how biochar will affect N_2O emissions in acid soils.

In this study, we utilized nitrification kinetic model equations, ammonia oxidizer populations and N_2O emissions dynamics to investigate the effects of biochar produced from rice straw on nitrification in two Oxisols of southern China during a 56-day incubation experiment. We hypothesized that ammonia oxidizers and hence, nitrification activity would be greatly influenced by this alkaline biochar addition in these two soils and that N_2O emissions would increase in response to the probably increased nitrification of both soils. The results of this study can provide insights into the impacts of straw biochar amendment on N transformation in subtropical acid soils of China.

2. Materials and methods

2.1. Soil sampling site description and biochar preparation

Samples of two acid Oxisols (derived from granite [RGU] and tertiary red sandstone [RTU]) were collected from Wanli, Nanchang, Jiangxi province (28°40′N, 115°37′E) and Yingtan Red Soil Ecological Experiment Station (28°15′N, 116°55′E), respectively, which are located in a hilly region of Southeast China. Both soils were classified as Udox according to USDA Soil Taxonomy (Soil Survey Staff, 1999). The properties of these two soils are shown in Table 1. All samples were collected from the surface layer (0–15 cm), air-dried and sieved through a 2 mm screen before use. Biochar was produced from rice straw using pyrolysis at 500 °C at a rate of 5 °C min^{-1}, which was then held constant for 8 h (Wang et al., 2012a). It was passed through a 1 mm sieve prior to the incubation experiment. The biochar had a pH of 9.16, a total C and N content of 620 and 13.3 g kg^{-1}, respectively, and CEC of 18.9 cmol kg^{-1}; the ash content was 276 g kg^{-1} and the Mehlich III extractable-Ca, P, K, Na, Mg, Fe, Mn, Cu and Zn content was 2.63, 1.06, 18.43, 3.94, 1.41, 0.01, 0.55, 0.0016 and 0.05 g kg^{-1}, respectively.

2.2. Aerobic incubation experiment

A 56-day aerobic incubation experiment was performed to investigate the response of nitrification to biochar addition. The treatments included three biochar application rates (0, 1% and 5% (*w/w*), amounting to 0, 22.5 and 112.5 Mg ha^{-1}, respectively) combined with two N fertilizer ($(NH_4)_2SO_4$-N) rates (0 and 110 mg N kg^{-1}, amounting to 0 and 250 kg N ha^{-1}, respectively)

Table 1
Selected physicochemical properties of air-dried soils examined in this study.

Soil name	RGU	RTU
Soil type	Oxisols	Oxisols
Parent material	Granite	Tertiary red sandstone
Annual temperature (°C)	17.7	18.4
Annual rainfall (mm)	1650	1882
Clay (<0.002 mm,%)	38.1	7.63
Silt (0.002–0.05 mm,%)	40.27	20.7
Sand (>0.05 mm,%)	21.63	71.73
CEC (cmol kg^{-1})	8.95	5.86
Total N (g kg^{-1})	1.01	0.43
TOC (g kg^{-1})	9.11	4.72
pH (H_2O)	5.05	4.90

TOC, total organic carbon; CEC, cation exchange capacity.

(Yuan et al., 2005). Thus, the incubation study included six treatments per soil type: CK, CK + N (N: 110 mg N kg^{-1}), 1%BC (biochar: 1%), 1%BC + N (biochar: 1%, N: 110 mg N kg^{-1}), 5%BC (biochar: 5%) and 5%BC + N (biochar: 5%, N: 110 mg N kg^{-1}). Each treatment was replicated three times. Soil samples (15 g on an oven-dry weight basis) and biochar (0, 0.15, 0.75 g for corresponding biochar treatments) were added to a series of 250 mL Erlenmeyer flasks and treated with distilled water to achieve 40% water holding capacity (WHC). The samples were incubated at 25 °C in the dark for 7 days to stabilize microbial activity. During the 56-day incubation, the following treatments were performed: for treatments with N addition (CK + N, 1%BC + N and 5%BC + N for each soil type), 2 mL 0.825 mg mL^{-1} $(NH_4)_2SO_4$-N solution containing 1.65 mg N was added to each flask; for treatments without N addition (CK, 1%BC and 5%BC for each soil type), only deionized water was applied. The final soil moisture content in all flasks was adjusted to 65% of WHC by weighing. The flasks were then covered with polyethylene film punctured with a needle (to maintain aerobic conditions) and incubated in the dark at 30 °C; deionized water was added every 2 or 3 days to compensate for water loss. Six flasks per treatment were destructively sampled at 0, 1, 7, 14, 21, 35, 49 and 56-day intervals, respectively. Three of these flasks were treated with 75 mL 2 M KCl solution to extract NH_4^+ and NO_3^-, while the other three were used to measure soil pH. The concentrations of NH_4^+ and NO_3^- in the KCl-extracted soil solution were measured using a San++ Continuous Flow Analyzer (Skalar, Netherlands). The soil pH was determined in samples diluted 1:2.5 (*w/v*) in deionized water using a pH meter. Three flasks per treatment were destructively sampled on day 7 and 56, subsamples from which were immediately stored at −75 °C for DNA extraction.

2.3. N_2O sampling and measurements

The headspace air was sampled after 1, 5, 12, 15, 21, 28, 35, 49 and 56 days of incubation using a gas-tight syringe. Before gas sampling, the headspace air in the flasks was thoroughly flushed with ambient air for 15 min at a rate of 200 mL min^{-1}. The flask was then capped immediately by sealing with silicone (NQ-704 silicone adhesive sealant) rubber stoppers fitted with butyl rubber septa and incubated for 5 h. For each measurement, gas samples were withdrawn from the flasks through a three-way stopcock using a 25-mL air-tight syringe, after which the flasks were flushed with ambient air and kept open (Wang et al., 2013b). The gas samples were analyzed by gas chromatography (Agilent 7890A, USA) and detected by ECD. The carrier gases were argon-methane (5%) at a flow rate of 40 mL min^{-1} and the column temperature was 40 °C. Compressed air was used as a standard gas with a value of 313 ppbv. Concentrations of N_2O were quantified by comparing the peak areas of samples with those of reference gasses (Nanjing Special Gas Factory).

2.4. DNA extraction and quantification using real-time quantitative PCR

Subsamples (0.5 g) collected on day 7 and 56 during the incubation period from three replicates per treatment were used for DNA extracting using a FastDNA SPIN kit for soil (MP Biomedicals, USA) following the manufacturer's instructions. DNA concentrations were calculated based on absorbance at 260 nm, and purity was checked by examining the absorbance ratio at 260/280 nm and 260/230 nm using a NanoDrop spectrophotometer (NanoDrop Technologies, USA). The qPCR was performed on a CFX96 Optical Real-Time Detection System (Bio-Rad Laboratories, USA). Primer sets Arch-amoA F (SAATGGTCTGGCTTAGACG), Arch-amoA R (GCG-GCCATCCATCTGTATGT) (Francis et al., 2005) and amoA-1F (GGGGTTTCTACT-GGTGGT), amoA-2R (CCCCTTCGGGAAAGCCTTCTTC) (Rotthauwe et al., 1997) were used to determine the copy numbers of archaeal and bacterial amoA genes, respectively. Each real-time PCR reaction was performed (in triplicate) in a 20 μL volume containing 10 μL SYBR Premix Ex Taq (Takara Biotechnology, Otsu, Shiga, Japan), 0.4 μM of each primer and 2 μL of soil DNA. The thermocycling steps were as follows: 95 °C for 30 s, followed by 39 amplification cycles of 95 °C for 10 s, 95 °C for 30 s, and 72 °C for 30 s to confirm repeatability and to minimize PCR bias. The standard for qPCR was developed using an amoA subclone (confirmed by DNA sequencing) obtained from soils using specific primer sets. Blanks were run with sterile water instead of DNA extract. Specific amplification of PCR products was checked by melting curve analysis. PCR products were checked against DNA markers of known size using agarose gel electrophoresis.

2.5. Data analysis

The dynamics of NO_3^- content during the 56-day incubation period were modeled with Michaelis-Menten kinetics (Mary et al., 1998). Linear regression and non-linear regression were applied to the simulation of zero-order reaction and first-order reaction, respectively. If nitrification follows zero-order kinetics, the rate is limited by enzyme activity and independent of added NH_4^+, whereas first-order kinetics indicates that the rate is controlled by the amount of substrate NH_4^+, and added NH_4^+ usually stimulates nitrification (Zhao et al., 2007).

The zero-order reaction model (Eq. (1)) and first-order reaction model (Eq. (2)) were as follows:

$$N_t = K_0 \times t + N_0 \quad (1)$$

$$N_t = N_0 + N_p \times (1 - \exp(-k_1 \times t)) \quad (2)$$

where N_t is the NO_3^- concentration on day t (mg N kg^{-1} soil), N_0 is the initial concentration of NO_3^- (mg N kg^{-1} soil), N_p is the potential net nitrification (mg N kg^{-1} soil) in a first-order reaction model and k_0 (mg N kg^{-1} d^{-1}) and k_1 (d^{-1}) are nitrification rate constants for zero- and first-order reaction models, respectively.

N_2O emission rates, expressed as μg N kg^{-1} soil h^{-1}, were calculated based on the increase in N_2O concentrations in the headspace of each flask (Eq. (3)). Cumulative N_2O emissions were weighted by the time intervals between two sampling dates (Eq. (4)) (Xiang et al., 2015).

$$F = \rho \times \Delta C \times V \times (273/(273 + T) \times W) \quad (3)$$

$$E = \Sigma(f_i + f_{i+1})/2 \times (t_i + t_{i+1}) \quad (4)$$

where F is the emission rate of N_2O (ng N kg^{-1} h^{-1}); ρ is the density of N_2O under standard state (1.25 kg N_2O-N m^{-3}); ΔC is the change in gas concentration between incubation times of 0 and 5 h (part per billion by volume per hour: ppbv); V is the gas space volume in the flasks used in the experiment (m^3); T is the incubation temperature (°C) and W is the dry weight of the soil (kg). E is cumulative N_2O emissions (μg N kg^{-1}) and f_i and f_{i+1} is the emission rate of N_2O at time t_i and t_{i+1}, respectively.

All statistical analyses were performed using SPSS 20.0 (SPSS Inc.) and Origin 8.5 (OriginLab, USA). One-way analysis of variance (ANOVA) was used to check for quantitative differences between treatments. $P < 0.05$ was considered to indicate statistically significant differences. The copy numbers of ammonia oxidizer genes in each sample were log-transformed prior to statistical analysis.

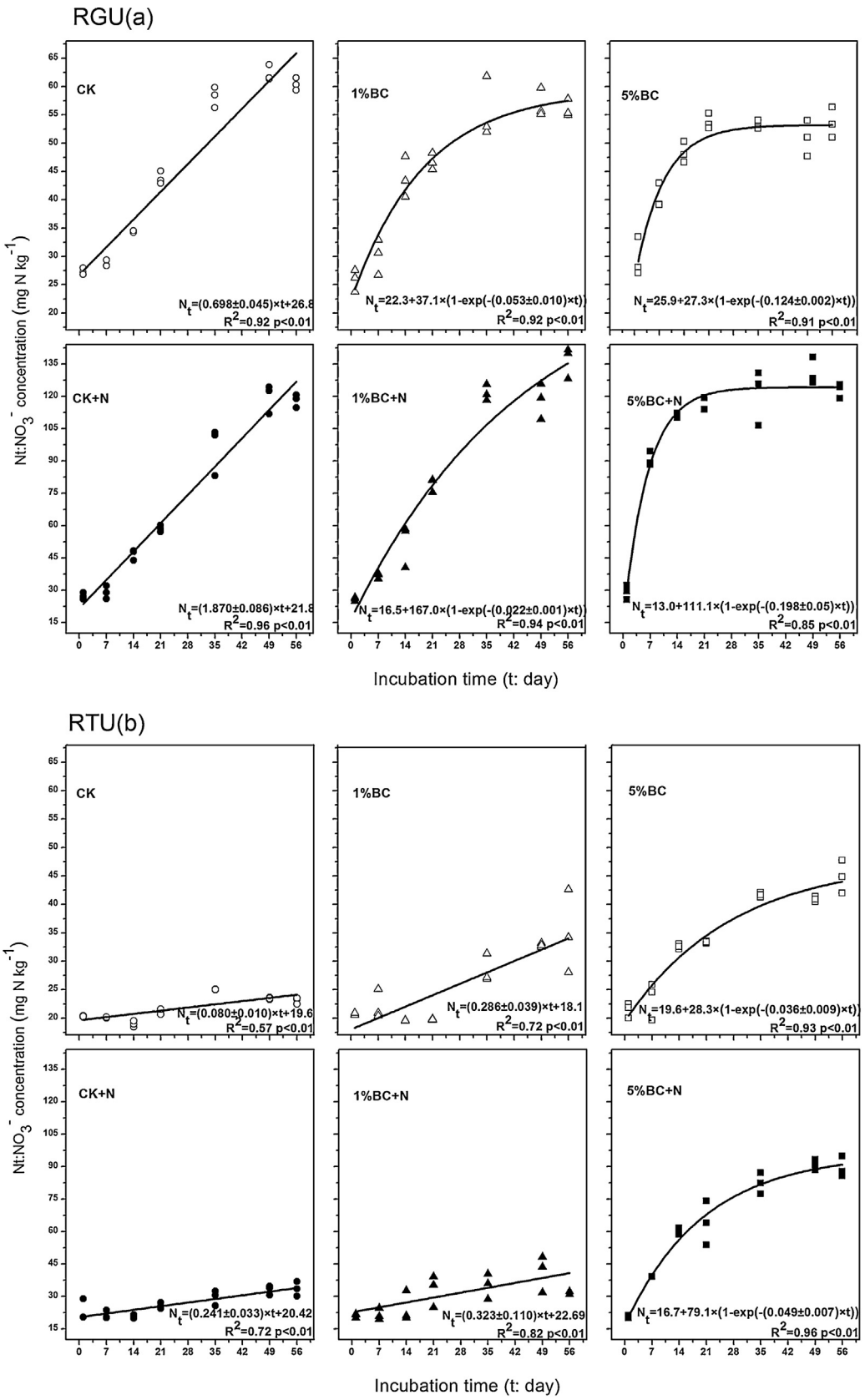

Fig. 1. Nitrification patterns over a 56-day incubation period at 30 °C and 65% water-holding capacity per treatment for RGU and RTU soil. The zero-order kinetic model ($N_t = K_0 \times t + N_0$) and the first-order kinetic model ($N_t = N_0 + N_p \times (1 - \exp(-k_1 \times t))$) were used to simulate changes in NO_3^- concentration over incubation time. N_t, NO_3^- concentration on day t (mg N kg^{-1} soil); N_0, initial concentration of NO_3^- (mg N kg^{-1} soil); N_p, potential net nitrification (mg N kg^{-1} soil) in the first-order reaction model; k_0 (mg N $kg^{-1}d^{-1}$) and k_1 (d^{-1}), nitrification rate constants for the zero- and first-order reaction models, respectively. RGU and RTU, the soils were derived from granite and tertiary red sandstone, respectively. CK, 1%BC and 5%BC represent application rates of 0, 1 g and 5 g rice straw biochar per 100 g soil, respectively. N, 110 mg N kg^{-1} ammonium sulfate. The data points for the corresponding sampling day represent the three replicates.

3. Results

3.1. Nitrification responses

The nitrification levels of the two soils examined in this study were low. The net NO_3^- accumulation rate from the day 1 to day 56 for control treatments was 33.08 mg N kg^{-1} in RGU and only 2.82 mg N kg^{-1} in RTU (Fig. 1). Regression analysis of NO_3^- accumulation (Fig. 1) showed that basal nitrification in the controls during the 56-day incubation varied, with the rate constant of 0.698 and 0.080 mg N kg^{-1} d^{-1} in RGU and RTU, respectively. Biochar treatment altered the nitrification rate in RGU. The net NO_3^- accumulation from the day 1 to day 56 in RGU was 30.17 and 24.01 mg N kg^{-1} for the 1%BC and 5%BC treatments, respectively, while net NO_3^- accumulation from the day 1 to day 56 in RTU was 14.26 and 23.4 mg N kg^{-1} for the 1%BC and 5%BC treatments, respectively. Regression analysis of NO_3^- accumulation showed that the two biochar treatments altered the nitrification patterns in RGU, which followed a first-order kinetic model, and that the rate constants increased with increasing biochar application rate. For RTU, nitrification followed a zero-order kinetic model in the control and 1%BC treatment groups, with the rate constant of 0.080 and 0.286 mg N kg^{-1} d^{-1}, respectively. The 5%BC treatment changed the nitrification pattern to first-order kinetics.

The addition of NH_4^+ stimulated nitrification in both soils to varying degrees. In RGU, net NO_3^- accumulation from the day 1 to day 56 in CK + N was 90.81 mg N kg^{-1}, accounting for 67.6% of the total inorganic N contents on day 0 after the addition of exogenous NH_4^+-N (Fig. 1a), while this value was only 10.25 mg N kg^{-1} in CK + N treated RTU at the end of the incubation (Fig. 1b). Biochar application increased nitrification rates by 17.9% and 3.2% in 1% BC + N- and 5%BC + N-treated RGU, respectively, while net NO_3^- accumulation was almost the same in CK + N and in 1%BC + N-treated RTU, i.e., approximately 10.25 and 10.18 mg N kg^{-1}, respectively. By contrast, 5%BC + N treatment greatly enhanced net NO_3^- accumulation in RTU compared with the control (by 85.0% at the end of the incubation). Regression analysis (Fig. 1) showed that the nitrification pattern in the control with N addition followed zero-order kinetic models in both soils. For RGU, in the biochar treatment groups with N addition, the time course of NO_3^- accumulation pattern changed from a zero-order to a first-order reaction model, with a nitrification rate constant of 0.022 and 0.198 d^{-1} for the 1%BC and 5%BC treatments, respectively. For RTU, 1%BC + N treatments increased the nitrification rate constant (0.286 mg N kg^{-1} d^{-1}) compared with the control (0.080 mg N kg^{-1} d^{-1}). The 5%BC + N treatment altered the kinetic model of nitrification to a first-order reaction model, with a nitrification rate constant of 0.049 d^{-1} and maximum nitrification activity of 79.1 mg N kg^{-1}.

The NH_4^+ concentrations decreased rapidly during the first 49 days in RGU (Fig. 2). For the 5%BC + N treatment, NH_4^+ was almost completely depleted during the initial two weeks of incubation, whereas only 15.7% and 31.5% of NH_4^+ was depleted from the CK + N and 1%BC + N treatment groups, respectively. For RTU, only 12–13% of NH_4^+ was consumed in the CK + N and 1% BC + N treatment groups over the course of incubation, while approximately 78% of NH_4^+ was transformed in the 5%BC + N treatment group during the first 35 days of incubation, after which its levels remained stable until the end of the incubation.

3.2. Changes in soil pH

For both soils, the addition of biochar increased soil pH on day 1; the increase was more pronounced with the increase of biochar application rates. At the end of the incubation, the pH increased by

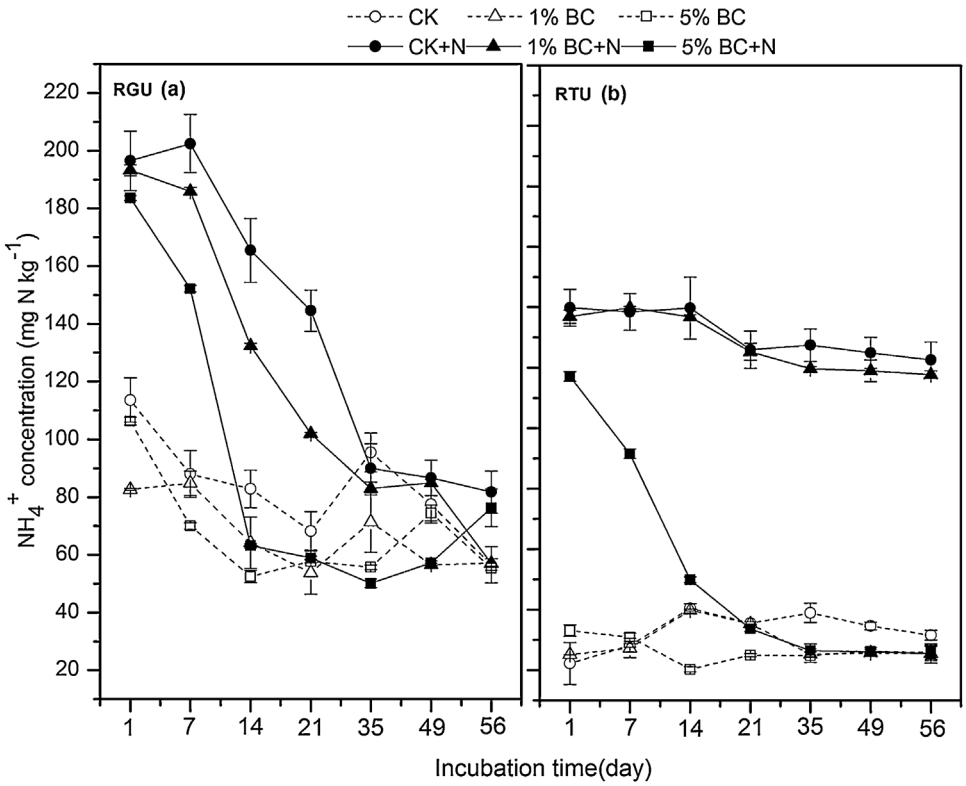

Fig. 2. Time courses of NH_4^+ concentrations over a 56-day incubation at 30 °C and 65% water holding capacity per treatment for RGU (a) and RTU (b) soil. RGU and RTU, the soils were derived from granite and tertiary red sandstone, respectively. CK, 1% BC and 5% BC represent application rates of 0, 1 g and 5 g rice straw biochar per 100 g soil, respectively. N, 110 mg N kg^{-1} ammonium sulfate. Error bars indicate standard deviation of replicates (n = 3).

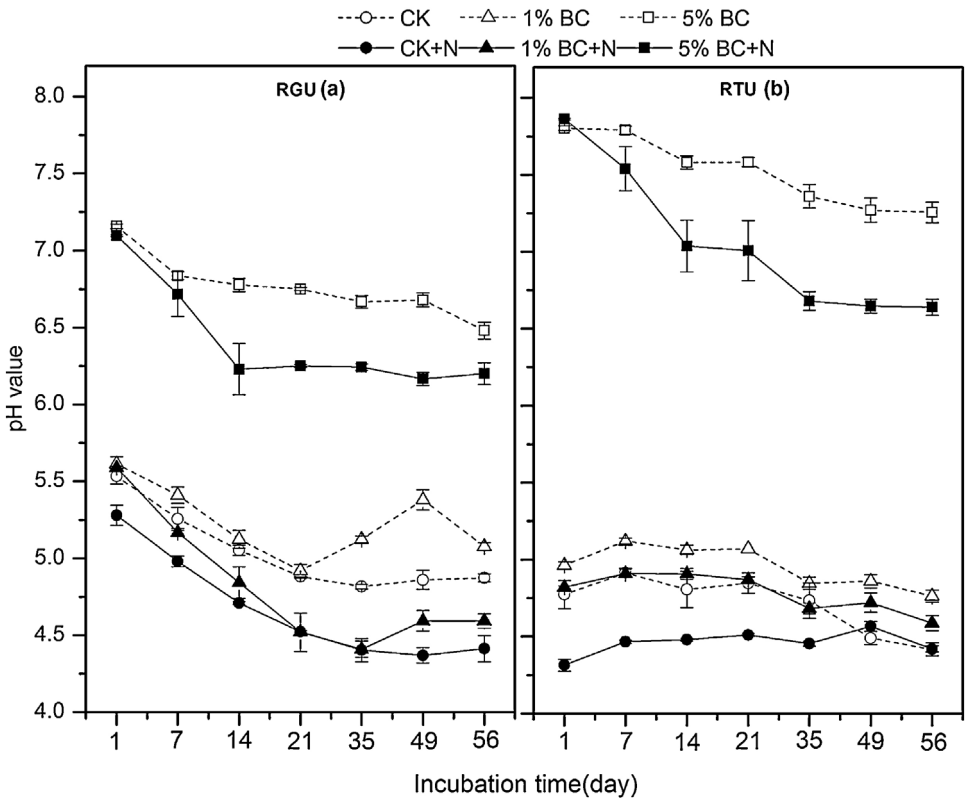

Fig. 3. Changes in soil pH over a 56-day incubation at 30 °C and 65% water holding capacity per treatment for RGU (a) and RTU (b) soil. RGU and RTU, the soils were derived from granite and tertiary red sandstone, respectively. CK, 1%BC and 5%BC represent application rates of 0, 1 g and 5 g rice straw biochar per 100 g soil, respectively. N, 110 mg N kg^{-1} ammonium sulfate. Error bars indicate standard deviation of replicates (n = 3).

0.21 and 1.61 units and 0.35 and 2.84 units in the 1%BC- and 5%BC-treated groups (compared to the control) for RGU and RTU, respectively. The addition of NH_4^+-N reduced the initial soil pH compared to the corresponding treatments without N addition (Fig. 3); the higher the biochar application rate, the greater the initial soil pH increased. At the end of the incubation period, soil pH values in biochar treated-samples were higher than those of the corresponding samples without biochar addition.

3.3. Soil ammonia oxidizer population

To characterize ammonia oxidizer population dynamics during the incubation, we quantified the dynamics of AOA and AOB gene copy numbers (Fig. 4). For RGU treatments without N addition, the AOA gene copy numbers in the CK group ranged from 8.80×10^6 copies g^{-1} soil on day 7 to 1.43×10^8 copies g^{-1} soil on day 56 (Fig. 4a). The abundance of AOA genes in the two biochar-treated groups increased with increasing incubation time but was lower than that of the control at the end of the incubation, ranging from 2.52×10^7 to 3.95×10^7 copies g^{-1} soil for the 1%BC- and 5%BC-treated groups, respectively. For treatments with N addition, the copy numbers of AOA gene in the control were significantly higher than those in the two biochar treatment groups on day 7 and 56. For RTU treatments without N addition (Fig. 4b), this value was significantly higher in the 5%BC treatment group than in the other groups. In the CK + N treatment group, the highest AOA gene copy number (1.70×10^7 copies g^{-1} soil) occurred on day 7, while the lowest value (3.08×10^6 copies g^{-1} soil) was detected on day 56. The 5%BC treatment groups (both with and without N addition) had the highest AOA gene copy numbers at the end of the incubation period.

For RGU (Fig. 4c), the AOB gene copy number in the CK fluctuated from 1.10×10^7 to 1.12×10^7 copies g^{-1} soil during both sampling periods. Among treatments without N addition, the 5%BC treatment significantly increased the AOB gene copy number (up to 7.2-fold) compared to the control on day 7. The addition of NH_4^+ did not significantly increase AOB gene copy number in the control at either sampling period. By contrast, in the biochar-treated samples, N addition considerably increased AOB gene copy numbers; this positive trend was more significant with increasing rate of biochar addition. Interestingly, the copy numbers of AOB genes in the corresponding treatment groups were lower in RTU than in RGU regardless of incubation period. For the RTU treatment groups without N addition (Fig. 4d), both biochar treatments significantly increased AOB gene copy numbers (up to 7.24-fold compared with the control) on day 7; the addition of N increased the abundance of AOB genes in the corresponding treatment groups to varying degrees, except for the 1%BC treatment groups.

3.4. N_2O emissions

In RGU, N_2O emissions varied significantly with incubation time, as shown in Fig. 5a. Rapid emissions of N_2O from soils occurred immediately after NH_4^+-N addition. The greatest N_2O-N emission (up to 388 ng kg^{-1} h^{-1}) occurred on day 1 following CK + N treatment; during the earlier stage of incubation, N_2O emissions from 5%BC + N-treated soil were higher than those of the other treatments, while they continued to decline until day 21, after which the levels fluctuated through the end of the incubation. Beginning on day 12, in the CK + N treatment group, N_2O emissions climbed rapidly and remained higher than those of the other treatments throughout the remaining incubation period. Statistical

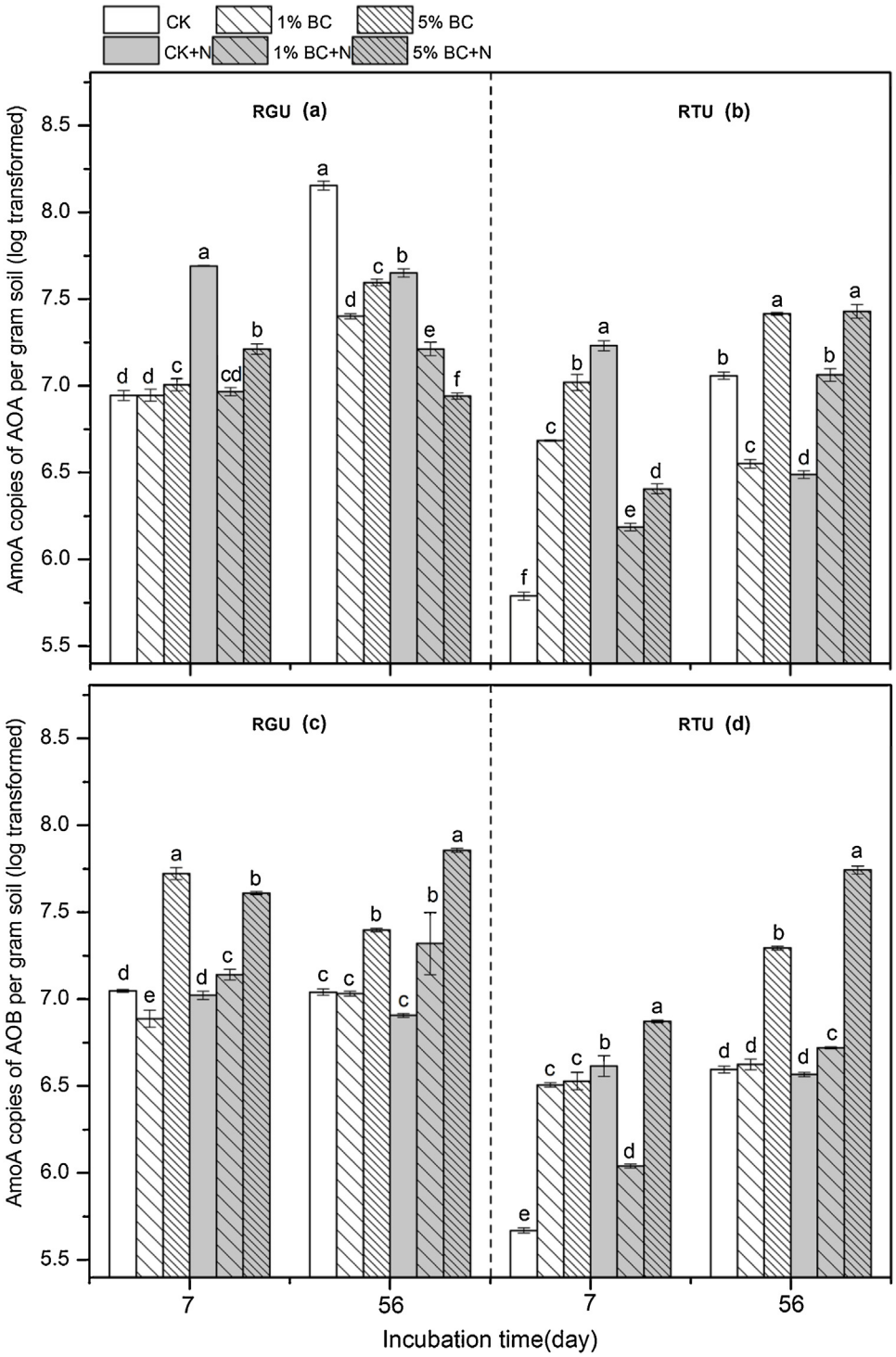

Fig. 4. Archaeal (a, b) and bacterial (c, d) amoA gene copy numbers over a 56-day incubation at 30 °C and 65% water holding capacity per treatment for RGU and RTU soil. RGU and RTU, the soils were derived from granite and tertiary red sandstone, respectively. CK, 1% BC and 5% BC represent application rates of 0, 1 g and 5 g rice straw biochar per 100 g soil, respectively. N, 110 mg N kg^{-1} ammonium sulfate. Error bars indicate standard deviation of replicates (n = 3) for each treatment. Letters above bars indicate a significant difference ($p < 0.05$) between treatments for each soil type.

analysis indicated that N_2O emissions from RGU were influenced by N addition and biochar application (Fig. 5c). In treatment groups without N addition, cumulative N_2O emission levels were 87.6, 79.7 and 76.7 μg kg^{-1} soil for the control, 1%BC and 5%BC treatment groups, respectively (Fig. 5c). When N was added, both biochar treatments significantly reduced cumulative N_2O emissions (by 33.6% and 37.6% for the 1%BC and 5%BC treatment groups, respectively) compared with the control.

In RTU, N_2O emissions peaked in the middle of the incubation period followed by steady, small fluctuations throughout the remaining incubation period. The highest N_2O-N emission level, 227.6 ng kg^{-1} h^{-1}, occurred in response to CK + N treatment

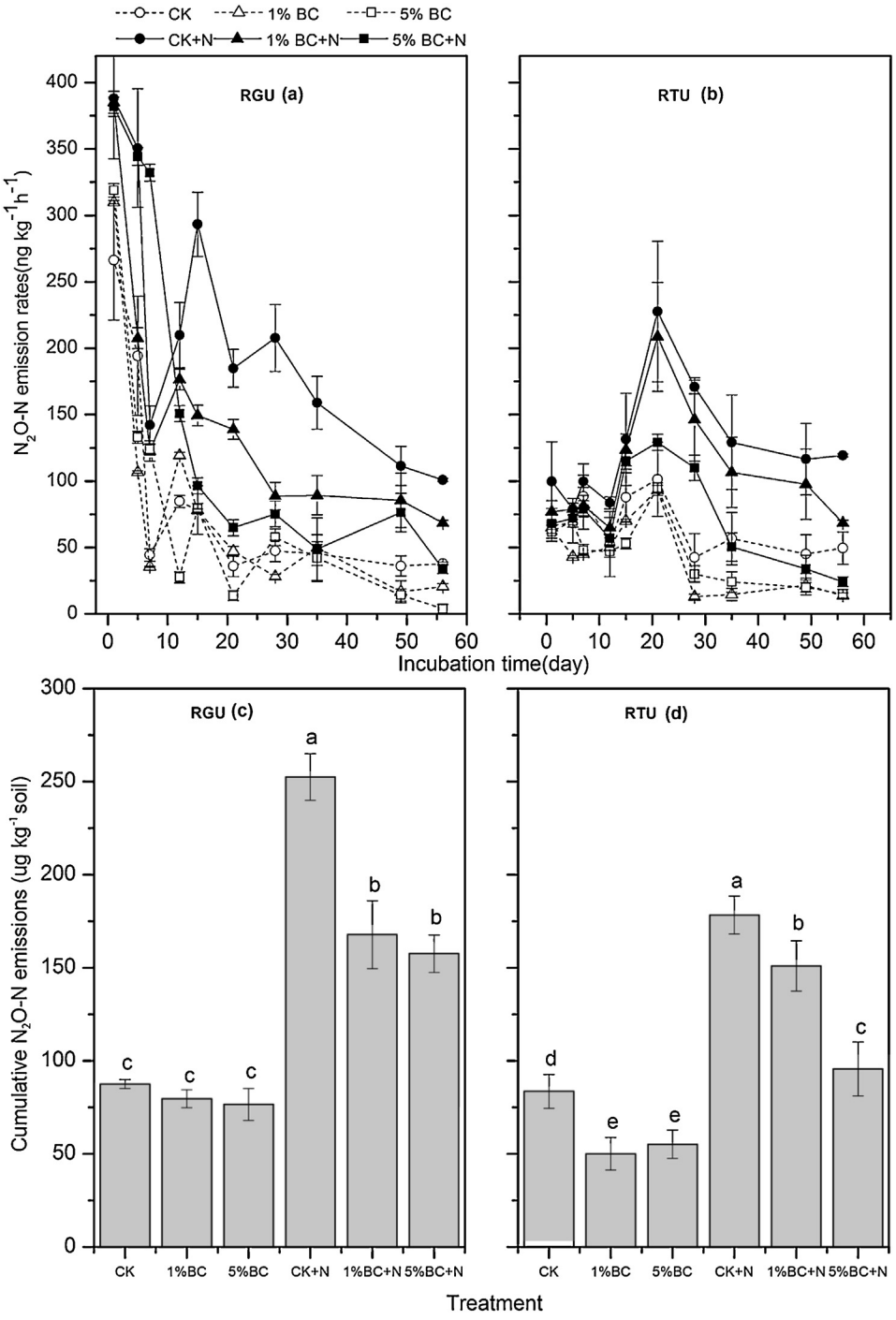

Fig. 5. Dynamics of N_2O emissions rates (a, b) and cumulative N_2O emissions from RGU (a, c) and RTU (b, d) over a 56-day incubation at 30 °C and 65% water holding capacity per soil treatment. RGU and RTU, the soils were derived from granite and tertiary red sandstone, respectively. CK, 1%BC and 5%BC represent application rates of 0, 1 g and 5 g rice straw biochar per 100 g soil, respectively. N, 110 mg N kg^{-1} ammonium sulfate. Emission rates are expressed as average values from three replicates. Error bars indicate the standard deviation of replicates (n = 3) for each treatment. Letters above bars indicate a significant difference ($p < 0.05$) between treatments for each soil type.

(Fig. 5b) on day 21, followed by 1%BC + N and 5%BC + N treatments. For the treatments without N addition, N_2O emissions peaked on day 21, followed by a rapid decline, reaching steady levels that were lower than those of the other treatments. N_2O emissions from RTU were also affected by N addition, biochar application and their interaction (Fig. 5d). N_2O emissions were substantially reduced by these treatments, with reductions of 15.3% and 46.4% for the 1% BC + N and 5%BC + N treatments, respectively, compared with the control.

4. Discussion

4.1. Biochar addition consistently increases nitrification activity in two Oxisols

In the current study, soil nitrification of NH_4^+ in both soils was weak, and net NO_3^- accumulation in the control was low throughout the incubation period, presumably due to high soil acidity and the absence of the substrate NH_4^+ (Robertson, 1982;

Nugroho et al., 2006), which did not favor nitrification activity (Fig. 1). The time course of NO_3^- accumulation under control treatments in both soils followed zero-order kinetics (Fig. 1), with low reaction rate constants (0.698 and 0.080 mg N kg^{-1} d^{-1} in RGU and RTU, respectively), indicating that the nitrification rates were greatly limited by enzyme activity to various degrees (Zhao et al., 2007). de Gannes et al. (2014) demonstrated that soil clay contents significantly modulated the pH effect, as the nitrification potential of an acidic clay soil was ca. eight-folder greater than that of sandy loam soil with a similar pH value, which was attributed to the ability of high-CEC minerals to enable localized exchange of NH_4^+ and protons, thereby buffering acidity. In the current study, the clay contents of RGU soil was five times as that of RTU (Table 1), RGU had higher nitrification rates than RTU soil; moreover, the gene copies of ammonia oxidizers of the control in RGU during the incubation was higher than the control in RTU, and almost up to 18 times higher at the day 7 (Fig. 4). The approximately eight-fold higher nitrification rate constant of RGU versus RTU (Fig. 1) may also be related to soil organic C content (Table 1), as the quantity and quality of organic C amendments play an important role in regulating soil nitrification, and higher soil total N and organic C contents always result in higher rates of nutrient release (Strauss and Lamberti, 2002).

Biochar amendment increased soil nitrification activity in both soils, and 5%BC treatment changed the soil nitrification pattern to first-order kinetics (Fig. 1), suggesting that nitrifier activity was gradually restored in these soils and that enzyme activity was not the limiting factor; substrate availability ultimately controlled the nitrification rate (Mary et al., 1998; Zhao et al., 2007). Enhanced nitrification activity following biochar amendment is supported by the qPCR results. Biochar application increased both AOA and AOB amoA gene abundance on day 7 of incubation (Fig. 4): the greater the biochar application rate, the greater the increase of gene copy number. These results are consistent with the findings that Terra Preta soils (which have high organic matter content due to the pre-Colombian practice of slash and char agriculture, making them exceptionally fertile) contain higher amoA gene copy numbers than the adjacent soils (non-Terra Preta) (Taketani and Tsai, 2010). The positive response of ammonia oxidizers to biochar application may be related to its mineral components, including several macro- and micro-nutrients which are essential for microbial growth, as well as increased nutrient retention and availability in the soil and the adsorption of organic matter to the biochar surface (Lehmann and Joseph, 2009). Biochar may greatly improve soil structure and aeration due to its high porosity and low bulk density (Joseph et al., 2010), thereby providing favorable conditions for nitrification, an aerobic oxidation process. Nitrification in acid soils depends on a range of factors such as pH, moisture, temperature, substrate-NH_4^+ and soil type (De Boer and Kowalchuk, 2001; Nugroho et al., 2009). Due to the liming activity of biochar and its high pH value (pH = 9.16), biochar addition can improve the adverse acidic soil environment (Fig. 3; Yuan and Xu, 2011). As our incubation experiment was conducted in a consistent environment of moisture and temperature, soil pH might be a key factor influencing the effectiveness of biochar on nitrification. In this study, biochar treatments raised the pH values of the two soils (Fig. 3) and increased their nitrification responses (Fig. 1). The positive correlation of nitrification and soil pH observed in the present study is consistent with Sahrawat (1982), who found that the amounts of NO_3^--N increased with the soil pH in five acid soils.

The amoA gene abundance of AOA and AOB in both soils responded differently to NH_4^+-N and biochar addition. For both soils, AOA gene copy numbers were lower in N-treated soils than in soils without N addition at the end of incubation, while AOB gene copy numbers were higher in N-treated soils than in soils without N addition (Fig. 4). The copies numbers of AOB genes were higher than those of AOA genes in biochar-treated soils, which also had higher nitrification activity, suggesting that AOB play an important role in biochar-enhanced nitrification (Fig. 4). Gubry-Rangin et al. (2010) reported that AOB favored nutrient-rich environments and were responsible for the ammonia oxidation supplied by nitrogen fertilizer, while AOA may play an important role in NH_4^+-poor environments (Gubry-Rangin et al., 2010). Our previous pot study revealed that three-year successive biochar application (22.5 Mg ha^{-1} per crop season) could affect the diversity of both AOA and AOB in a wheat/millet rotation Oxisol, and consistently increased their abundance compared with untreated control soil (He et al., 2016), whereas in the present short-term study with biochar–blending microcosm, 5%BC treatment of RGU significantly reduced AOA gene copy numbers (by 80.4%) versus the control. Nitrification, a metabolic microbial process involving multiple species, is influenced by microbial adaptation and functional redundancy. The physicochemical properties of biochar are altered with increasing application time (Cheng et al., 2008), leading to different responses in terms of soil factors and nitrification. More studies investigating the succession patterns of nitrifiers are needed to predict nitrification activity and the potential environmental effects of N fertilizer application and soil C sequestration, which will enhance efforts to develop new fertilization strategies.

4.2. Biochar reduces N_2O emissions across nitrification processes in both Oxisols

Throughout the incubation, cumulative N_2O emissions in the two soils were significantly reduced by biochar addition in treatments with N addition and the reduction was more pronounced with the increase of biochar application rates. Generally, N_2O is produced in soil mainly via microbial activity through nitrification, denitrification, and nitrifier denitrification (Gillam et al., 2008; Huang et al., 2014). In the current soil aerobic incubation, N_2O production from nitrification is thought to be the dominant source. Due to its low bulk density and unique structure characteristics, biochar has been observed to affect soil physical properties, which may suppress N_2O production by improving aeration of the soil or absorbing water from the soil (Yanai et al., 2007; Lehmann and Joseph, 2009). Cayuela et al. (2015) and Quin et al. (2015) reported the molar H:Corg ratio of biochar, which is related to its redox activity and adsorption properties, was also a key factor in mitigating N_2O emissions; the molar H:Corg ratio <0.3 (produced temperature 200–700 °C) had more effect on the reduction of N_2O emissions. The biochar used in this study was produced at 500 °C for 8 h, it may have a high degree of aromatic condensation (Wang et al., 2012a). The generally high H:Corg ratio in biochars (José et al., 2014) produced at 500 °C may provide high redox activity and adsorption properties and reduced N_2O emission in two biochar-treated soils. In addition, biochar poses negatively charged surface, it has shown a strong affiliation to various chemicals, especially cations, including NH_4^+, and thus affect the transformation of chemicals in soils (Cheng et al., 2008; Wang et al., 2012b). According to Clough et al. (2010) and Yang et al. (2015), the sorption of NH_4^+/NH_3 by biochar can reduce the availability of substrate for nitrification and N_2O formation. In our study, the concentration of inorganic N (NH_4^+ plus NO_3^-) in biochar treatments was decreased by 9.2%–13.5%, and 4.5%–26.4% for RGU and RTU, respectively, compared with the controls at the end of incubation (data not shown), suggesting that added N was likely adsorbed or fixed by biochar in the current enclosed experiment system without crop planting, and thus N_2O emissions of the two soils were reduced. This observed decrease in inorganic N was also probably due to stimulated volatilization of NH_3 as soil pH increased with biochar addition (Fig. 3; Zhao et al., 2013).

It is well known that N fertilization increase N_2O emissions in agricultural soils (e.g., Xiong et al., 2002; Wang et al., 2013b). We observed large peaks in N_2O emissions after the addition of exogenous NH_4^+ into the two soils (Fig. 5), agreeing well with previous studies (Yan et al., 2001; Ma et al., 2015). The key mechanism for this is that the high NH_4^+ concentration usually inhibits the NO_2^- transformation to NO_3^- and stimulates NO_2^- accumulation, thus resulting in an increase in N_2O emissions (Ma et al., 2015; Venterea et al., 2015). Biochar addition greatly accelerated the consumption of NH_4^+ by enhancing nitrifying activity of the two soils, as compared to the treatments with only N addition (Figs. 1 and 2). This phenomenon was more obvious for RTU, where NH_4^+ concentration was extremely high and relatively stable under the CK + N treatment over the course of 56-day incubation but declined quickly to a low level in 35 days under the 5%BC + N treatment group (Fig. 2b). Therefore, accelerated nitrification in biochar treatments may mitigate the inhibitory effect of high NH_4^+ concentration on the conversion of NO_2^- to NO_3^-, and thereafter the formation of N_2O in the two soils. Venterea et al. (2015) also reported the soil which displayed greater nitrification rates and NO_3^- levels produced 2–10 times less N_2O than the soil which displayed smaller nitrification rates. However, further studies by using ^{15}N tracer methods are needed to directly investigate the microbial dynamics of NO_2^- formation and N_2O emission during nitrification process in biochar-amended soils.

5. Conclusions

In this study, we combined the soil NH_4^+ transformation process with analysis of nitrification kinetics and ammonia oxidizer populations to examine the short-term effects of biochar on nitrification activity of two subtropical Oxisols from southern China. The application of biochar enhanced nitrification in both agricultural soils, and 5%BC treatments changed the soil nitrification patterns to a first-order reaction model, suggesting that nitrifier activity was gradually restored in soils following biochar addition, enzyme activity was no longer the limiting factor for nitrification. As demonstrated by qPCR, AOB play an important role in biochar-enhanced nitrification. Although biochar addition enhanced nitrification, the levels of its intermediate product, N_2O, were substantially reduced in the short term. Since the results are based on a short-term incubation in microcosm without growing plants and we did not consider the possible plant-soil-biochar interaction under field conditions, additional long-term field studies of the effects of biochar on other N processes (denitrification, mineralization, N_2-fixation) are needed to evaluate the effects of biochar on N fate.

Acknowledgments

This study was financially supported by the National Fund for Transformation of Agricultural Scientific and Technological Achievements, China (2013GB24910678), the National Natural Science Foundation of China (Grants 41271312), and the Young Foundation of State Key Laboratory of Soil and Sustainable Agriculture, Chinese Academy of Sciences (Y412010006).

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