Biochar addition reduced net N mineralization of a coastal wetland soil in the Yellow River Delta, China

Xianxiang Luo^{a,b}, Lei Chen^a, Hao Zheng^{a,b,*}, Jingjing Chang^a, Hefang Wang^a, Zhenyu Wang^a, Baoshan Xing^{c,*}

^a *Key Laboratory of Marine Environment and Ecology, Ministry of Education and
College of Environmental Science and Engineering, Ocean University of China,
Qingdao 266100, China*

^b *Laboratory of Marine Ecology and Environmental Science, Qingdao National
Laboratory for Marine Science and Technology, Qingdao 266071, China,*

^c *Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA 01003,
USA*

*Corresponding authors:

Dr. Baoshan Xing; phone: +1-413-545-5212; email: bx@umass.edu

Dr. Hao Zheng; phone: +86-532-6678-1068; email: zhenghao2013@ouc.edu.cn

Abstract

Soil degradation has seriously threatened global soil and food security. Biochar application is an promising management option to remediate the degraded soils. However, extensive application of biochar is limited by lack of understanding the effects of biochar on nitrogen (N) mineralization in the degraded coastal wetland soils. Therefore, the individual or combined effects of biochar, reed stem and urea fertilizer application on N mineralization in a coastal wetland soil were investigated using a 150-days incubation experiment, and the underlying mechanisms were discussed. Biochar addition reduced net N mineralization, but no significant effect was observed between the treatments with different addition rates. The combined addition of the biochar and reed stem had little effect on net N mineralization because of the higher C:N ratio (45.5-49.3). However, biochar addition in combination with the urea fertilizer initially decreased net N mineralization, but slightly increased it later on. The biochar-induced reduction of net N mineralization was mainly ascribed to the increased C:N ratio and decreased urease activity. Therefore, adding N fertilizer to the biochar to enhance the delivery of N prior to its incorporation into soil, which may avoid N immobilization due to N deficiency, could be an effective strategy for remediating the degraded coastal wetland soils.

Key words: biochar; soil degradation; N mineralization; C:N ratio; urease activity

1. Introduction

The dominant soils with nutrients deficiency and high-salt stress in the wetlands of Yellow River Delta, China (Wu et al., 2014), have degraded severely, thus threatening soil health and productivity (Koch et al., 2012). The low primary productivity of the coastal wetland soils is the main ecological problem in the Yellow River Delta. Although several technologies (e.g., amendments from straw residues, addition of freshwater, phytoremediation) have been used to remediate these wetland soils, they all have drawbacks (e.g., high costs, short lasting of ameliorating effects) (Wu et al., 2014). Moreover, the remediated soils are still facing severe stress due to anthropogenic disturbances such as oil exploitation, sea salt extraction and tourist industry in this region (Higgins et al., 2013). Therefore, a cost-effective and sustainable soil amendment with multiple benefits for improving soil quality, restoring primary productivity and conserving ecological functions is urgently required. The promotion of biochar technology has been proposed as one of such solutions (Lehmann and Joseph, 2015).

Biochar is a carbon-rich material produced from heating biomass in the absence of or with limited air to above 250 °C, and is distinguished itself from other carbon (C) products (e.g., charcoal, activated carbon) in that it is intended for use as a soil amendment for soil improvement and carbon sequestration, as well as other functions for environmental management (Lehmann and Joseph, 2015). Biochar has been reported to modify soil characteristics via enhancing soil aggregate stability (Soinne et al., 2014), increasing water and nutrient retention (Abel et al., 2013), and

stimulating soil enzymes activities (Oleszczuk et al., 2014), and thus promoting plant growth and augmenting crop yields (Jones et al., 2012; Reibe et al., 2015). However, biochar application into soils does not always result in positive effects (Jeffery et al., 2011; Spokas et al., 2011; Carvalho et al., 2016). Spokas et al. (2011) reported that approximately 30% of the selected studies ($n = 45$) had no significant differences on crop growth or yield compared to the controls without biochar, and even 20% observed negative results. Nitrogen (N) is a major essential nutrient for plant growth, which is mainly originated from mineralization of soil organic N (SON, accounting for 85-95% of total N (TN)), to ammonium by soil microorganisms (e.g., bacteria, actinomyces) in the coastal wetland soils of the Yellow River Delta. However, the effect of adding biochar on SON mineralization in the coastal soil has received much less attention than C (Sun et al., 2014; Wu et al., 2014). Sun et al. (2014) reported that adding wheat straw biochar significantly decreased C mineralization, but the incorporation of the low-temperature biochar (300 °C) alleviated the drought stress to microbes in the coastal soil. Wu et al. (2014) found that application of furfural biochar decreased the soil pH by 0.3–0.4 due to the use of acidic biochar (pH 4.5) and increased soil organic carbon (SOC) content. Because nutrient (e.g., N, P) deficiency is one of the main reasons for the low primary productivity in these wetland soils, more attention should be paid to the interactions between biochar and nutrient cycling, especially N, which is a crucial limiting factor for vegetation restoration in the degraded coastal soils.

Biochar application can alter soil N dynamics via enhancing nitrification (Ulyett

et al.,2014), decreasing denitrification (Case et al.,2015), adsorbing NH_4^+ and reducing NH_3 volatilization (Mandal et al., 2016; Zheng et al., 2013b), mitigating N_2O emission (Bass et al., 2016; Case et al.,2015), increasing microbial N cycling gene abundances (Ducey et al., 2013) and improving N bioavailability for crops (Mandal et al. 2016). Therefore, it is reasonable to hypothesize that biochar application into coastal soil may affect SON mineralization. However, addition of biochar to soils has been shown to decrease N mineralization (Prayogo et al., 2013; Dempster et al., 2012b), increase N mineralization (Ameloot et al., 2015; Maestrini et al., 2014) or have no effect on mineralization (Dempster et al., 2012a). Maestrini et al. (2014) found that the incorporation of ryegrass-derived pyrogenic organic matter (PyOM, similar to biochar) into a forest Cambisol resulted in a higher gross N mineralization due to the mineral N derived from the pyrogenic organic matter decomposition. While Prayogo et al. (2013) showed that willow biochar suppressed N mineralization when applied at both 0.5% and 2% but the mechanisms were not clear. Moreover, Dempster et al. (2012a) concluded that biochar had very limited impact on the mineralization rate of low molecular weight dissolved organic N compounds in two contrasting agro-ecosystems. These findings indicate that the effects of biochar on SON mineralization are highly variable, depending on the types of biochars and soils, and the interactions between them. Based on the possible interactions of biochar and coastal soil, we hypothesized that addition of biochar alone into the coastal soil could reduce N mineralization due to the increased C:N ratio and decreased urease activity, but the combined addition of biochar and organic N fertilizer could enhance N

mineralization due to the decreased C:N ratio and pH, and the increased urease activity. To test this hypothesis, a 150-days laboratory incubation experiment was setup to evaluate the effects of peanut shell biochar application on net N mineralization in an alkaline soil collected from the coastal wetland of the Yellow River Delta. The specific objectives of this research were to: (1) evaluate the effect of biochar addition alone with different rates on the net N mineralization; (2) investigate the combined effect of adding plant residue and biochar on the net N mineralization; and (3) study the effect of adding exogenous organic N on the net N mineralization in the biochar-amended soil.

2. Materials and methods

2.1. Preparation of soil and biochar

The soil was collected from three 100-m² plots in a reed (*Phragmites australis*) wetland (N 37°49'31.3", E 118°59'47.2") in the Yellow River Delta, China, and within each plot, seven soil samples were collected to a depth of 20 cm. The soil samples were air-dried, ground to pass through a 2-mm sieve after removal of the plant residues and rocks, and then thoroughly homogenized for further analysis. The soil properties are presented in Table 1, indicating that the selected soil was alkaline, and deficient in N.

The biochar sample was produced from peanut shell at 350 °C for 2 h using slow pyrolysis as described by Zheng et al. (2013b). Shandong province, the nation's largest producer of peanut, produced more than 23.3% of the total peanut shell with 4.0 million tons every year (Liu et al., 2010; Wang et al., 2010). However, the

majority of the peanut shell is discarded or burned directly, resulting in serious resource loss and environmental pollution. After charring, the biochar sample was ground to pass through a 2-mm sieve, and thoroughly homogenized for further analysis. The reed stems were collected from the same wetland where the soil was sampled, and dried at 75 °C for 24 h, and then ground to pass through a 2-mm sieve . The properties of the biochar and reed stem are presented in Table 1.

2.2 Soil incubation experiment

A laboratory incubation experiment was conducted with a total of seven treatments: (1) unamended soil (CK); (2) soil + 1% (w/w) biochar (1%BC); (3) soil +3% biochar (3%BC); (4) soil + 3% reed stem (3%R); (5) soil + 3% reed and 3% biochar (3%R+3%BC); (6) soil + 0.3% urea (0.3%U); (7) soil + 0.3% urea + 1% biochar (0.3%U+1%BC). The rates of reed stem and urea fertilizer supplied sufficient C and organic N to meet the second level of the classification criteria of soil nutrients in China (National Soil Survey Office, 2002). After mixing, a 40-g soil mixture was placed into a polypropylene cup (57 mm in diameter and 47 mm in height), and all the cups were covered with lids punctured with several holes to allow gas exchange but minimize water loss from evaporation. The experiment had a completely randomized block design with seven treatments, and each treatment had thirty replicates for destructive sampling during the incubation. The soil moisture content was kept at 80% of the maximum water holding capacity (WHC) during the whole incubation period. All the cups were incubated in a constant temperature chamber (SHP-450, Jinghong, China) at 30 °C for 150 days. The soils samples were destructively sampled from

three replicate cups at 0, 6, 11, 18, 25, 34, 46, 60, 100 and 150 day for analysis of NH_4^+ -N, NO_3^- -N, available N (AN), TN, TC, pH, electrical conductivity (EC) and urease activity.

2.3 Sample analysis

The pH, TC, TN, and NO_3^- -N and NH_4^+ -N content of the biochar and reed stem samples were measured as reported by Zheng et al. (2013b). Cation exchange capacity (CEC) was determined in sodium acetate extract (pH 7.0) using a flame atomic absorption spectrophotometer (M6, Thermo Elemental, USA). EC was measured in a 1:5 soil to water slurry using a conductivity meter (Cond 3210, WTW, Germany). The NH_4^+ -N and NO_3^- -N contents were determined using a spectrophotometric method with indophenol blue reagent and phenol disulfonic acid, respectively (Zheng et al., 2013a).

The contents of TC and TN in the soil samples were determined using an Elemental Analyzer (FLASH-2000, Elementar, USA). The soil moisture content was measured gravimetrically. The pH was measured in 1:2.5 (w/v) soil to water slurry by a pH-meter (AB15, Fisher Scientific, USA). Urease activities were measured by a colorimetric method using indophenol blue reagent, and expressed as the reduction of milligrams of urea-N per kilogram of dry soil per hour ($\text{mg N}/(\text{kg soil h})$) (Bhaduri et al., 2016).

2.4 Calculation of net mineralization and nitrification

The net N mineralization during the selected period was calculated by subtracting the inorganic N (NO_3^- -N and NH_4^+ -N) concentration of the initial soil

from that of the incubated soil (Lentz et al., 2014; Dempster et al., 2012b). A positive difference indicates net N mineralization, while a negative value shows net N immobilization during the period (Lentz et al., 2014). Net nitrification are defined as the difference in NH_4^+ -N concentrations between the selected periods (Dempster et al., 2012b). The net N mineralization rates were calculated as the difference between inorganic N values of two sampling times divided by the time interval between the sampling periods (Flavel and Murphy, 2006).

2.5 Statistical Analysis

Statistical analysis was performed using the Statistical Product and Service Solutions Software (SPSS, version 18.0). The statistical significance of the differences between samples was determined by Duncan's test at $P = 0.05$. The correlations were analyzed by Pearson test (two-tailed, $P < 0.05$). The multivariate statistical technique of principal components analysis (PCA) was used to investigate the most susceptible variances and to identify the important components explaining most of the variances in a large data set .

3. Results

3.1 Soil N contents

The NH_4^+ -N content in the 1%BC and 3%BC treatments showed a downward trend over the incubation, similar to the CK treatment (Fig. 1a). However, the NH_4^+ -N content increased and then dramatically decreased in the reed stem and urea treatments with or without biochar addition, but the peak time was different, at day 6 and 46 for the reed and urea treatments, respectively (Fig. 1a and 2a). Except for the

urea treatments, the $\text{NH}_4^+\text{-N}$ content was very low (0.02-4.58 mg/kg) in other treatments during the whole incubation period, and close to zero (0.43-1.52 mg/kg) in all the treatments at the end of incubation. Regardless of the treatments, the biochar had no effects ($P < 0.05$) on $\text{NH}_4^+\text{-N}$ content during the whole incubation (Fig. 1a and 2a). On the contrary, the $\text{NO}_3^-\text{-N}$ content in the biochar and urea treatments continuously increased from 1.31-2.57 to 54.7-452 mg/kg during the incubation (Fig. 1b and 2b). However, the reed stem addition reduced $\text{NO}_3^-\text{-N}$ content from 2.06-2.15 to 0.16-0.20 mg/kg, which kept relatively stable during the whole incubation period (Fig. 1b). The addition of biochar alone significantly ($P < 0.05$) reduced $\text{NO}_3^-\text{-N}$ content after 25 days of incubation, but the addition rate had no significant effect on $\text{NO}_3^-\text{-N}$ content (Fig. 1b). The combined addition of the biochar and reed stem or urea had little effect on $\text{NO}_3^-\text{-N}$ content during the whole incubation compared with the corresponding control treatments (Fig. 1b and 2b). The AN content showed the similar trend to the $\text{NO}_3^-\text{-N}$, but biochar alone significantly ($P < 0.05$) decreased AN content at the end of the incubation, and more biochar added, more AN was reduced (Fig. 1c). In the reed treatments, the biochar application significantly decreased ($P < 0.05$) AN content in the first 60 days of incubation, and then had no effect thereafter, similar to the AN content in the urea treatments with the biochar addition (Fig. 2c). In all the treatments, compared with that at the beginning, TN content significantly ($P < 0.01$) decreased at the end of incubation (Fig. 1d and 2d). However, compared to the CK treatment, the additions of all the amendments including biochar, reed stem and urea significantly ($P < 0.01$) increased TN content through the incubation. For example,

TN content increased by 17.1% and 80.9% in the 1%BC and 3%BC treatments compared with the CK treatment, respectively, indicating that more biochar led to less N lost from the soil.

3.2 Soil N mineralization

The net N mineralization rates of the CK and biochar alone treatments (1%BC and 3%BC) peaked at day 25, and then decreased to non-significant levels after 125 days of incubation (Fig. 3a). Although the net N mineralization rates in the reed stem treatments (3%R and 3%R+3%BC) was negative and much lower (-0.19 to -0.01 mg/(kg d)) than that of the CK treatment (0.09 to 1.14 mg/(kg d)), they increased over the incubation period (Fig. 3a). Conversely, the urea application resulted in a decreasing trend of the N mineralization rates, regardless of whether biochar was added or not, and the rates were 150-166 and 6.87-6.92 times higher than those of the CK treatment at the beginning and end of the incubation, respectively (Fig. 4a). The biochar alone significantly decreased ($P < 0.05$) the cumulative net N mineralization during the whole incubation period, but the rates of biochar addition had no significant effect on the cumulative net N mineralization (Fig. 3b). In the reed stem treatments, biochar had little effect on the cumulative net N mineralization. However, compared with 0.3%U treatment, the biochar addition significantly decreased ($P < 0.05$) the cumulative net N mineralization in the 0.3%U+1%BC treatment during the first 34 days of incubation, but slightly increased the cumulative net N mineralization in the following incubation (Fig. 4b).

3.3 Soil N nitrification

Similar to the net N mineralization rates (Fig. 3a), the net N nitrification rates in the CK, 1%BC and 3%BC treatments also peaked at day 25, then dramatically decreased and stayed at a very low level (0.35-0.42 mg/(kg d)) at the end of incubation (Fig. 5a). In the reed stem treatments, the net N nitrification rates were negative (-0.35 to -0.01 mg/(kg d)), but had an increasing trend during the whole incubation (Fig. 5a), suggesting that the N immobilization occurred. Unlike the net N mineralization in the urea treatments, the net N nitrification rates in the urea treatments peaked at day 100 (3.99 to 4.01 mg/(kg d)) and then decreased by 23.9-24.0% in the following incubation (Fig. 5a). The biochar alone significantly decreased the net N nitrification rates by 14.5-25.2% compared to the CK treatment, while in the reed stem treatments the adding biochar showed little effect, as well as the urea treatments (Fig. 5a). The cumulative net N nitrification increased with increasing incubation time, but it was negative in the reed treatments during the whole incubation (Fig. 5b). Moreover, the biochar additions had an inhibiting effects on the cumulative net N nitrification in the biochar alone treatments, while the biochar additions had little effect in the biochar treatments combined with reed stem or urea (e.g, 3%R+3%BC and 0.1%U+1%BC) (Fig. 5b).

3.4 Principal components (PCs) and factor correlation coefficients

Because of many variables involved in N mineralization and nitrification, PAC was performed to investigate the important components in the large data set, and the different parameters including $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, AN, TN, C:N ratio, pH, EC, urease activity, the cumulative net N mineralization and nitrification at the beginning (day 6)

and end (day 150) of the incubation were introduced as the analysis variables in the PCA (Bhaduri et al., 2016). The results were shown in Fig. 6 and Table 2. From the PCA, the PC1 and PC2 explained 59.5% and 31.1% of the total variance in the soils at the beginning incubation, accounting for 90.6% of the total variance (Table 2, Fig. 6a), indicating that most information about soil properties related with N mineralization and nitrification was contained in the first two PCs (Table 2). The PC1 had significantly positive correlations with the contents of $\text{NH}_4^+\text{-N}$ (0.991), AN (0.995) and TN (0.940), the cumulative net N mineralization (0.998) and nitrification (0.999), and C:N ratio had a significantly negative correlation with PC1 (-0.962). However, pH, EC and $\text{NO}_3^-\text{-N}$ content were positively correlated with PC2 (0.754, 0.752 and 0.958), respectively, but the urease activity was negatively with PC2. At the end of the incubation, the PC1 and PC2 explained 80.1% and 14.2% of the total variance in the soils, respectively, accounting for 94.3% of the total variance (Table 2, Fig. 6b). The PC1 had significantly positive correlations with the contents of $\text{NH}_4^+\text{-N}$ (0.984), $\text{NO}_3^-\text{-N}$ (0.998), AN (0.998) and TN (0.860), and pH and C:N ratio had negative correlations with PC1 (-0.782, -0.965), respectively. However, the PC2 only had a similar positive correlation with the urease activity. The score plots of all treatments were classified as two distinctive groups related to the coefficient of PC1. The first group included the treatments of CK, 1%BC, 3%BC, 3%R+3%BC and provided higher C content, while the second group included the treatments of 0.3%U, 0.3%U+1%BC and provided higher N content. These results suggested that C content, N fractions and contents (e.g., AN, $\text{NH}_4\text{-N}$, TN) and soil properties (e.g., C:N ratio)

significantly affected N mineralization in the biochar-amended soils.

4. Discussion

4.1 Effects of C:N ratio on N mineralization

The N mineralization and nitrification in soils are a function of the C and N pools available to microorganisms (Robertson and Groffman, 2007), which are largely dependent on environmental factors (e.g., soil texture, pH, temperature, moisture) , additions of exogenous substances (e.g., fertilizer, plant residues, bio-solids and contaminants) (Oleszczuk et al., 2014), N fractions and contents (e.g., amino acid N, amino sugar N) in soils (Schomberg et al., 2012) and soil biota (e.g., earthworm, fungi and mycorrhiza) (Clough et al., 2013). Biochar may contain bioavailable C fractions during its production, its mineralization and release will be dependent on how recalcitrant the biochar and soil N and C pools are, and on the soil and biochar C:N ratio (Clough et al., 2013). The C:N ratio, reflecting the content of available C and N for organisms, is a key factor controlling the balance between N mineralization and N immobilization, occurring at the same time within relatively small volumes of soil (Robertson and Groffman, 2007). Generally, materials with a C:N ratio > 25:1 stimulate N immobilization, while those with a C:N ratio < 25:1 stimulate N mineralization (Robertson and Groffman, 2007). The C:N ratios were 34, 44 and 105 for the selected soil, biochar and reed stem, respectively (Table 1), much higher than the ratio of 25:1, implying that the additions of the biochar and reed stem may increase C:N ratio (Fig. 7a), thus reducing N mineralization. This was consistent with our results (Fig. 3). In the biochar and reed stem treatments, the C:N ratio

significantly increased during the incubation (Fig. 7a). On the contrary, the urea addition significantly decreased the C:N ratio from 32.8-33.8 to 7.09-11.3, resulting in the increased N mineralization due to the sufficient quantity of N supply (Fig. 4). A similar result was reported by Pereira et al. (2015), who found that adding 3% of holm oak biochar produced at 650 °C to a composting mixture of poultry manure and barley straw favored N mineralization due to the improved physical properties of the mixture by preventing the formation of clumps larger than 70 mm and the enhanced microbiological activity. These results demonstrated that the increased C:N ratio was the one of the main reasons for the decreased N mineralization in the biochar-amended soils, confirming by the negative correlation between the C:N ratios and the cumulative net mineralized N ($r = -0.850$, $n = 63$, $P < 0.01$). Sigua et al. (2016) reported that application of switchgrass biochars produced at 250 and 500 °C caused N immobilization in 50 days of incubation because of the higher C:N ratio ranging from 129:1 to 250:1 for the biochars. This was similar to the decay of plant litters, generally resulting in N immobilization in soils (Moreno-Cornejo et al., 2014). This was also why the decreased N mineralization occurred in the 3%R and 3%R+3%BC treatments, which had the highest C:N ratio ranging from 45.5 to 52.8 (Fig. 7a). Therefore, C:N ratio of the labile organic matter fraction in biochars, biosolids or composts needs to be considered when applying them into soils because it could affect crop growth and productivity as a result of potential N immobilization (Clough et al., 2013; Rigby et al., 2016).

The N embodied in the plant derived biochar has previously been assumed to be

of low availability due to its low content and heterocyclic structures (Clough et al., 2013; Zheng et al., 2013b), thus the contribution of N released from biochars in N mineralization was often ignored. As reported in the present study, the NH_4^+ -N and NO_3^- -N contents in the biochar were 3.17 and 0.94 mg/kg, respectively, much lower than those in the selected soil (Table 1). Consequently, it was recommended that adding nutrients to the biochar to enhance the delivery of N prior to incorporation into soil, which may avoid N immobilization due to N deficiency (Sigua et al., 2016). This was why the urea fertilizer was added in combination with the biochar (0.3%U+1%BC) in this study (Fig. 4). However, Sarkhot et al. (2012) prepared nutrient enriched biochar by shaking the biochar with dairy manure effluent for 24 h, which increased the C and N content of the biochar by 9.3% and 8.3%, respectively. When the untreated biochar and N enriched biochar were added to a soil in an eight week incubation, the reduction in available NH_4^+ -N and NO_3^- -N content was observed, suggesting the possibility of N immobilization. Still, N enriched biochar could be used as a slow release N fertilizer (Sarkhot et al., 2012). In addition, biochar may contain bioavailable N fractions, their mineralization and release will be dependent on how recalcitrant the biochar is (Clough et al., 2013). Wang et al. (2012) reported that total hydrolysable N including amino acids and amino sugars shown to represent the labile N pool in the manure-derived biochars. However, most of these biochars showed a progressive net N immobilization from the beginning of the incubation but the mechanism was unclear. Moreover, NH_4^+ -N and NO_3^- -N could represent the labile N pool in biochars in short term, but the heterocyclic N could be potentially available

in long term (Heike, 2010; Hilscher and Knicker, 2011; Knicker, 2011), which was not addressed in the present study. Therefore, further tracer studies with N-labelled biochar are required to fully understand the effects of various biochar with different N fractions on SON mineralization and to determine if the effects observed are of relatively short duration or more long-term (Clough et al., 2013).

4.2 Effects of pH on soil N mineralization

Biochar may increase soil pH due to its inherent alkalinity, and thus it has become a promising amendment for acidic soils (Ducey et al., 2013). The optimal pH range for many soil microbial oxidizers is pH 7–9 (Robertson and Groffman, 2007). Within the acidic to slightly alkaline range ($< \text{pH } 8$), raising the soil pH value increased N mineralization rate in biosolids-amended soil (Rigby et al., 2016). Therefore, more N immobilization was observed (Fig. 3) because the pH decreased from 8.00–8.15 in the CK treatment to 7.72–7.87 in the reed treatments (3%R and 3%R+3%BC), confirming that the reed stem-induced pH reduction was responsible for the N immobilization. Similarly, compared to the CK treatment, the urea application had no effect on soil pH for the first 25 days of incubation, but significantly decreased pH to 7.52–7.59 at the end of incubation (Fig. 7b). This is another mechanism responsible for the enhanced N mineralization and nitrification (Fig. 4–5) in the urea treatments besides the lower C:N ratio (Fig. 7a), which was confirmed by the significantly negative correlations between pH values and the cumulative net N mineralization in the urea treatments (3%BC: $r = -0.709$, $P < 0.05$; 0.3%U+1%BC: $r = -0.753$, $P < 0.05$). However, the soil pH increased by 0.01–0.11 in

the 1%BC and 3%BC treatments compared to the CK treatment (Fig. 7b), but N mineralization was weakened, suggesting that the biochar-induced pH increase is unlikely to be a major factor controlling N mineralization in the biochar alone amended soils. In addition, the biochar-induced higher soil pH would result in significant ammonia volatilization from the soils (Mandal et al., 2016; Schomberg et al., 2012), probably explaining the decreased mineralized N in the 1%BC and 3%BC treatments (Fig. 1a-b). But Mandal et al. (2016) found that ammonia volatilization was reduced by approximately 70% with macadamia nut shell and poultry litter biochars because of NH_3 adsorption/immobilization. These opposite results were attributed to the lower pH of their soils (5.50-5.80).

4.3 Effects of urease activity on soil N mineralization

Highly variable effect of biochar on soil enzymatic activities depends on the interaction between biochar and the target substrate for each specific enzyme (Bailey et al., 2011; Bhaduri et al., 2016). Urease, one of the important soil enzyme associated organic N transformation, can hydrolyze urea to ammonium, and its activity may reflect the soil microbial processes and N supply capacity (Nannipieri et al., 2012). In the present study, the biochar additions significantly decreased urease activity by 13.0%-49.8% in the first 34 days of incubation, and had no significant effect thereafter (Fig. 7c), consistent with that the N mineralization was reduced due to biochar addition (Fig. 3). Thus the inhibited urease activity was one of the reasons for the short-term N immobilization. Similarly, Wu et al. (2012) also reported that wheat straw biochar addition significantly decreased urease activities but the mechanism

was not clear. Conversely, the urease activity of the biochar-blended compost was significantly enhanced despite the lower amount of microbial biomass, probably due to protection of urease by its association with organic complexes of high molecular weight (e.g., humic polymers and clay surfaces) (Jindo et al., 2012). For the 0.3%U and 0.3%U+1%BC treatments, compared to the CK treatment, the greatly stimulated N mineralization (Fig. 4) was attributed to the markedly increased (by 16.6%-247%) urease activities (Fig. 7c). Likewise, the biochar addition also significantly decreased the urease activity in the 0.3%U+1%BC treatment compared with the 0.3%U treatment in the first 46 days of incubation, but had no significant effect after day 46 (Fig. 7c). This changing trend of urease activity, similar to the cumulative net N mineralization (Fig. 4b), reconfirms that the biochar-induced reduction of urease activity was responsible for the decreased N mineralization. However, the urease activities significantly increased in the 3%R and 3%R+3%BC treatments compared with the CK treatment (Fig. 7c), probably because of the protection and stabilization of urease resulted from enzyme-organic interaction (Nannipieri et al., 2012). It is important to recognize that N mineralization and immobilization are occurring at the same time within relatively small volumes of soil, and net N mineralization is the balance between the two processes (Robertson and Groffman, 2007). Therefore, although the N mineralization was also enhanced due to the reed stem application (e.g., 3%R treatment), the N immobilization resulted from the extremely high C:N ratio exceeded the enhanced N mineralization (Fig. 3).

Soil enzyme activities control the rate of soil organic matter decomposition and nutrient cycling processes (e.g., N mineralization and nitrification) (Nannipieri et al., 2012). However, the response of soil enzymes to biochar additions and the main factors that drive their consequent behavior are not clear, which has drawn increasing attention from scientists (Demisie et al., 2014; Jindo et al., 2012; Oleszczuk et al., 2014; Bhaduri et al., 2016). As reported in the present study, application of biochar has generally been reported to reduce the soil enzymatic activities associated with soil C and N mineralization, but increased the soil enzymatic activities involved in P cycling (alkaline phosphatase) (Oleszczuk et al., 2014). The biochar-induced enhancement of urease activity was also observed (Jindo et al., 2012; Bhaduri et al., 2016). The mechanisms underlying the biochar-induced reduction of enzymatic activity are unknown because of the complicated properties of biochar and soil enzyme, as well as the interactions between them. The reduction of enzymatic activities in the biochar-amended soils were possibly attributed to: (1) sorption of enzyme onto biochars via H-bonding and cation exchange, resulting in conformational change and reduction of their active sites; (2) sorption of substrates onto biochars, reducing the substrate availability for enzymes; and (3) biochar-induced alteration of soil conditions (e.g., pH, moisture, Ca^{2+} and Mg^{2+} content), which were not suitable for enzymatic reactions. Still, anticipations for biochar-induced increased enzymatic activity and enhanced enzymatic reactions, favoring nutrients utilization and contamination remediation of soils/sediments, have been reported (Bhaduri et al., 2016). Therefore, more studies are needed to further examine the response of soil

enzymes to biochar additions and the main factors that drive their consequent behavior in nutrient cycling.

5. Conclusion

The net N mineralization of the coastal wetland soil in the Yellow River Delta was reduced by the peanut shell biochar additions, but no significant effect was observed between the treatments with different addition rates. Biochar in combination with the plant residues (reed stem) had little effect on net N mineralization, while in combination with the organic N fertilizer (urea) decreased net N mineralization at the initial stage of incubation, but slightly increased net N mineralization later on. The biochar-induced reduction of net N mineralization was mainly ascribed to the increased C:N ratio and decreased urease activity. These results suggested that biochar effects on N mineralization are specific to individual biochar or combinations with other amendments (e.g., N fertilizer, plant residues). It must be noted that these findings were obtained under a controlled lab experiment, and although they are valid for the studied scenario, further studies should be conducted under field conditions before remediating the degraded coastal soil using biochars.

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Figure caption

Fig. 1. Effects of biochar additions on soil N contents: (a) $\text{NH}_4^+\text{-N}$; (b) $\text{NO}_3^-\text{-N}$; (c) available N (AN); and (d) total N (TN). CK: unamended soil; 1%BC: soil + 1% biochar; 3%BC: soil +3% biochar; 3%R: soil + 3% reed stem; 3%R+3%BC: soil + 3% reed and 3% biochar. Error bars represent standard errors of the means ($n = 3$).

Fig. 2. Effects of biochar and urea additions on soil N contents: (a) $\text{NH}_4^+\text{-N}$; (b) $\text{NO}_3^-\text{-N}$; (c) available N (AN); and (d) total N (TN). CK: unamended soil; 1%BC: soil + 1% biochar; 0.3%U: soil + 0.3% urea; 3%U+1%BC: soil + 0.3% urea + 1% biochar. Error bars represent standard errors of the means ($n = 3$).

Fig. 3. Effects of biochar additions on (a) net N mineralization rates and (b) cumulative net N mineralization. CK: unamended soil; 1%BC: soil + 1% biochar; 3%BC: soil +3% biochar; 3%R: soil + 3% reed stem; 3%R+3%BC: soil + 3% reed and 3% biochar. Error bars represent standard errors of the means ($n = 3$).

Fig. 4. Effects of biochar and urea additions on (a) net N mineralization rates and (b) cumulative net N mineralization. CK: unamended soil; 1%BC: soil + 1% biochar; 0.3%U: soil + 0.3% urea; 3%U+1%BC: soil + 0.3% urea + 1% biochar. Error bars represent standard errors of the means ($n = 3$).

Fig. 5. Effects of biochar additions on (a) net N nitrification rates and (b) cumulative net N nitrification. CK: unamended soil; 1%BC: soil + 1% biochar; 3%BC: soil +3% biochar; 3%R: soil + 3% reed stem; 3%R+3%BC: soil + 3% reed and 3% biochar; 0.3%U: soil + 0.3% urea; 3%U+1%BC: soil + 0.3% urea + 1% biochar. Error bars represent standard errors of the means ($n = 3$).

Fig. 6. Score and loading plots for the principal components: (a) day 6 and (b) day

150. CK: unamended soil; 1%BC: soil + 1% biochar; 3%BC: soil +3% biochar; 3%R:

soil + 3% reed stem; 3%R+3%BC: soil + 3% reed and 3% biochar; 0.3%U: soil + 0.3%

urea; and 3%U+1%BC: soil + 0.3% urea + 1% biochar.

Fig. 7. Effects of biochar additions on soil properties: (a) C:N ratio; (b) pH; and (c)

urease activity. CK: unamended soil; 1%BC: soil + 1% biochar; 3%BC: soil +3%

biochar; 3%R: soil + 3% reed stem; 3%R+3%BC: soil + 3% reed and 3% biochar;

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represent standard errors of the means ($n = 3$).

Table 1. Selected characteristics of the soil, biochar and reed samples

Samples	pH	TC (%)	TN (%)	C:N ratio	H:C atomic ratio	CEC (cmol/kg)	EC (μS/cm) ^a	NH ₄ ⁺ -N (mg/kg)	NO ₃ ⁻ -N (mg/kg)
Soil	8.09±0.02	1.13±0.15	0.033±0.001	34±0.37	ND	9.77±0.14	1548±62(1:5)	4.58±0.12	1.41±0.07
Biochar	8.54±0.03	44.0±0.5	1.01±0.01	44±0.69	0.31	14.9±0.3	5256±132(1:25)	3.17±0.14	0.94±0.17
Reed	4.62±0.02	42.9±2.1	0.41±0.02	105±0.93	1.59	ND ^β	ND	ND	ND

^aEC: electrical conductivity; the EC values were measured in 1:5 and 1:2.5 soil to water slurry using a conductivity meter (Cond 3210, WTW, Germany) for the soil and biochar samples, respectively.

^βND: not determined.

Table 2. Rotated factor correlation coefficients between measured parameters and PCs (PC1 and PC2) for the selected soil

Parameter	6 day		150 day	
	PC1	PC2	PC1	PC2
Total variance	59.5%	31.1%	80.1%	14.2%
pH	0.272	0.754*	-0.782	-0.531
EC	0.346	0.752*	0.995*	-0.044
NH ₄ ⁺ -N	0.991*	-0.125	0.984*	0.079
NO ₃ ⁻ -N	-0.211	0.958*	0.998*	0.041
AN	0.995*	-0.096	0.998*	0.043
TC	-0.561	-0.694	-0.617	0.601
TN	0.940*	-0.259	0.860*	0.414
C:N	-0.962*	-0.233	-0.965*	0.238
Urease activity	0.468	-0.861	-0.447	0.822*

*The asterisks after the data indicate the significant correlations analyzed by means of Statistical Product and Service Solutions Software (SPSS, version 18.0).

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