

# Do biochar and polyacrylamide have synergistic effect on net denitrification and ammonia volatilization in saline soils?

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## Research Article

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# Abstract

Salt-affected soils have poor structure and physicochemical properties, which affect soil nitrogen cycling process closely related to the environment, such as denitrification and ammonia volatilization. Biochar and polyacrylamide (PAM) have been widely used as soil amendments to improve soil physicochemical properties. However, how they affect denitrification and ammonia volatilization in saline soils is unclear. In this study, the denitrification and ammonia volatilization rates were measured in a saline soil field ameliorated with three biochar application rates (0%, 2% and 5%, w/w) and three PAM application rates (0‰, 0.4‰ and 1‰, w/w) over three years. The results showed that denitrification rates decreased by 23.63%-39.60% with biochar application, whereas ammonia volatilization rates increased by 9.82%-25.58%. The denitrification and ammonia volatilization rates decreased by 9.87%-29.08% and 11.39%-19.42% respectively, following PAM addition. However, there was no significant synergistic effect of biochar and PAM amendments on the denitrification and ammonia volatilization rates. The addition of biochar mainly reduced the denitrification rate by regulating the dissolved oxygen and electrical conductivity of overlying water and absorbing soil nitrate nitrogen. Meanwhile, biochar application increased pH and stimulated the transfer of  $\text{NH}_4^+\text{-N}$  from soil to overlying water, thus increasing  $\text{NH}_3$  volatilization rates. Hence, there was a tradeoff between denitrification and  $\text{NH}_3$  volatilization in the saline soils induced by biochar application. PAM reduced the denitrification rate by increasing the infiltration inorganic nitrogen and slowing the conversion of ammonium to nitrate. Moreover, PAM reduced the concentration of  $\text{NH}_4^+\text{-N}$  in the overlying water through absorbing soil ammonium and inhibiting urea hydrolysis, thereby decreasing  $\text{NH}_3$  volatilization rate.

## 1 Introduction

Soil reclamation plays a crucial role in coastal development in China. From 1985 to 2016, a total of 9 310.6  $\text{km}^2$  of tidal flats were reclaimed in the eastern coastal areas of China, and farmland accounts for 41% of the reclaimed area (Liu & Li 2020). However, soil salinization and alkalization have placed restrictions on the potential exploitation and utilization of these tidal flat resources. High concentration of  $\text{Na}^+$  can cause dispersion and expansion of colloidal particles, decrease soil porosity and cause aggregate destruction, leading to changes in the physicochemical properties of soil and water in coastal reclamation areas, including the nitrogen (N) cycling processes (Dendooven et al. 2010; Fei et al. 2017).

Denitrification and ammonia ( $\text{NH}_3$ ) volatilization are crucial processes of nitrogen (N) cycling and are primary pathways for gaseous N loss, which impedes nutrient use efficiency in fields (Cui et al. 2014). Records maintained by the Chinese Ministry of Agriculture show that the utilization of nitrogen fertilizer is only 30%-35% in the crop growing seasons in China (Ma et al. 2014).  $\text{NH}_3$  volatilization also cause a series of ecological and environmental problems, such as air pollution, acid rain formation and eutrophication in water (Schiermeier 2013; Zhu & Chen 2002). Both denitrification and  $\text{NH}_3$  volatilization processes are affected by many factors, including the physical and chemical properties of water and soil, such as nitrogen concentration, pH, salinity and dissolved oxygen (She et al. 2018; Vega-Jarquín et al. 2003). For example, soil pH strongly controls the  $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$  product ratio of denitrification (Obia et al. 2015). Saline soils always have high salinity and pH values and thereby increase N losses through  $\text{NH}_3$  volatilization (Vega-Jarquín et al. 2003). Salinity negatively affected both denitrifying activity and abundance of denitrifying functional genes (Wang et

al. 2018), which was the limiting factor of denitrification. It is imperative to understand the underlying mechanism of N losses through denitrification and  $\text{NH}_3$  volatilization and therefore increase the N utilization efficiency in coastal saline soils from both the sustainable production and the environmental viewpoints.

Biochar, a highly aromatic refractory solid produced by carbon-rich organic biomass under pyrolysis and anoxic or anaerobic conditions, has been widely reported to improve nutrient availability, fix carbon (Malghani et al. 2013), reduce soil greenhouse gas emissions (Stewart et al. 2013), control soil pollution, and increase crop yields (Quilliam et al. 2013). Biochar has also attracted considerable attention as a soil amendment because it is effective in improving the physiochemical and biological properties of salt-affected soils (Saifullah et al. 2018). The  $\text{NH}_3$  volatilization decreased in silt-loam soil applied with wood-derived biochar (Taghizadeh-Toosi et al. 2012), while increased in clay soil applied with straw-derived biochar (Zhao et al. 2013). The acid functional groups on the surface of biochar contributed to absorb ammonium (Asada et al. 2002), which trend to decrease  $\text{NH}_3$  volatilization. On the other hand, the liming effect of biochar application and its influence on soil nutrient transformation might promote  $\text{NH}_3$  loss due to raised soil pH (Jeffery et al. 2016; Taghizadeh-Toosi et al. 2011). Moreover, the function of biochar as an “electron shuttle” that facilitated the transfer of electrons to soil denitrifying microorganisms, which together with its liming effect would promote the denitrification (Luz Cayuela et al. 2013). Biochar can regulate the microbial activity in soil (Lehmann et al. 2011), pH (Enders et al. 2012), the concentration of available  $\text{NO}_3^-$  and organic matter (Prendergast-Miller et al. 2011), which are all known important factors affecting denitrification. Furthermore, biochar amendment could decrease soil N leaching by elevating soil holding capacity (Sun et al. 2018). Given that the mitigation effects of biochar application are not constant across soil type and land use, the impacts of biochar on coastal agricultural soils remains elusive. Therefore, it is necessary to understand how biochar affects N losses from coastal saline soils with lower organic matter and nutrient contents as well as degraded soil structure and physicochemical properties.

Polyacrylamide (PAM) is a linear water-soluble polymer with a strong water adsorption capacity and strong cohesiveness. It can improve the soil pore structure and increase soil permeability, hinder the formation of soil crusts, and increase the soil infiltration rate (Tang & She 2018). It can also improve soil hydraulic properties by increasing soil aeration and water permeability and reduce soil fertilizer losses (Pathan et al. 2004). In addition, PAM can increase the contents of total nitrogen and inorganic nitrogen in soil (Kay-Shoemake et al. 1998a), which is due to the fact that PAM can provide nitrogen contained in its amide group structure into the soil (Kay-Shoemake et al. 1998b; Kumar 1998). Ren et al. (2007) found that PAM could activate urease and thereby promoting the conversion of urea and organic nitrogen into ammonium nitrogen, which is the substrate of  $\text{NH}_3$  volatilization. Meanwhile, PAM increased the organic matter content of soil (Liu et al. 2012) and provided electron donors for denitrifying microorganisms, which is one of the important factors of denitrification. However, there are few studies about how PAM, especially the combined application of PAM and biochar, influences N losses via denitrification and  $\text{NH}_3$  volatilization in coastal saline soils.

Identifying the factors that contribute to the denitrification and  $\text{NH}_3$  volatilization processes is crucial for understanding the N cycling and reducing N losses in saline soils. Because of the complex multivariate responses of denitrification and  $\text{NH}_3$  volatilization, it is challenging to determine how environmental factors directly and indirectly affect denitrification and  $\text{NH}_3$  volatilization processes. Conventional regression and

Pearson linear correlation analyses are often applied to identify multivariate causality, but they seldom meet the statistical assumptions for normality, and there are often complex multivariate interactions among the factors (Hu & Si 2014). Structural equation modeling (SEM) is a powerful statistical approach for testing networks of direct and indirect theoretical causal relationships in complex data sets with intercorrelated dependent and independent variables. SEM enables the integration of unobserved variables such as theoretical variables that are reflected indirectly by several observed variables (Kline 1998). SEM is also an extension of regression and path analysis that can be used to model multivariate relations and to test multivariate hypotheses. In this paper, we hypothesize that SEM can be used to identify the most binding agents of denitrification and  $\text{NH}_3$  volatilization in saline soils amended with biochar and PAM at coastal agricultural reclamation areas. The objectives of our study are (1) to study the synergistic effects of biochar and PAM on denitrification and  $\text{NH}_3$  volatilization rates in saline soils; (2) to characterize how environmental factors interact to regulate denitrification and  $\text{NH}_3$  volatilization processes and investigate the determinants of denitrification and  $\text{NH}_3$  volatilization in coastal agricultural reclamation areas. This research will provide theoretical support for regulation of fertilizer use and environment in saline soils.

## 2 Materials And Methods

### 2.1 Materials

The soils used in this study were randomly collected in May 2015 from three 4 m × 4 m plots (0-100 cm soil layer) of a farmland in Liuzong Village (32°12'N, 120°42'E) in Juegang town, Rudong County, Jiangsu Province (Fig. 1; Sun et al. 2021). This low-lying flat area, 13.35 km long and approximately 2 067 hectares, is located beside the Yellow Sea and mainly used as agricultural land. The area is characterized as a subtropical maritime monsoon climate with an average annual precipitation of 1 044.7 mm, an average annual evaporation of 1 367.9 mm, and a mean annual temperature of 15 °C. The reclamation area has a mean annual sunshine of 2 421.6 hours and 223 frost-free days each year.

The experimental soils were taken to the laboratory, air-dried and sieved to 5 mm. The soil particle size was analyzed using a MasterSizer2000 laser particle size analyzer (Malvern Instruments, UK). The soil organic matter (OC) was determined using the potassium dichromate wet combustion procedure in an externally heated oil bath, the total nitrogen (TN) was determined by the semimicro-Kjeldahl method, and the cation exchange capacity (CEC) was determined by the ammonium acetate method. The soil electrical conductivity ( $\text{EC}_{1:5}$ ) was determined using a DDS-307 conductivity meter (Shanghai Precision Scientific Instrument Co. Ltd., Shanghai) and soil pH was measured at a soil-water mass ratio of 1:2.5 (Malvern instrument, UK). The biochar used in this experiment was commercial wheat straw biochar (Henan Sanli New Energy Co. Ltd.) obtained by thermal cracking carbonization at 550°C. A anionic PAM was used in the experiment with a molecular weight of 12 million. The measurement details for the physicochemical properties of soil, biochar and PAM are provided in our previous study (Fei et al. 2019) and presented in Table 1.

### 2.2 Experimental Design

The in-situ soil amelioration experiment was conducted in 36 randomly arranged cylindrical test pits (9 treatments with 4 replicates), each of approximately 300 L (Fig. 2; upper diameter = 84 cm, height = 85 cm), at

the Water-Saving Park of Hohai University (Nanjing, 31°57'N, 118°50'E, 144 m above sea level) from 2016 to 2018. The three-years field experiment was conducted under rain-shelter conditions, and the environmental conditions were the same as in Sun et al. (2021). The surface soils (0–20 cm) were amended by thoroughly mixing with three rates of biochar application (control treatment B1 = 0%, B2 = 2% and B3 = 5%, w/w) and three rates of PAM application (control treatment P1 = 0‰, P2 = 0.4‰ and P3 = 1‰, w/w) (Fei et al. 2019). The repacked soil test pits were irrigated and then incubated for six months before rice planting. Wu Yunjing 23 rice, a common high-yielding field variety, was selected as the test crop. Rice seeds were grown in a nursery for 24 days and were transplanted on the day before the basal fertilizer was applied each year. The planting density was 18 hills per test pit and two seedlings per hill. Two days prior to transplanting, the surface soils (0–20 cm) were hand plowed and harrowed. The whole rice growth period was divided into five periods: regreening, tillering, jointing, heading and yellow-ripening. The irrigation method was shallow water frequent irrigation, and the specific irrigation indexes were shown in Table S1. The nitrogen application standard for rice was 300 kg ha<sup>-1</sup>. The basal fertilizer was a compound fertilizer (N:P:K of 15:15:15), and the top-dressing was urea (nitrogen content = 46.4%), which was consistent with the fertilization practices of local farmers. The basal fertilizer was applied the day before transplanting, and the tillering fertilizer was applied 10 days after transplanting. The panicle fertilizer was applied in the early period of booting. The fertilization application times were shown in Table S2.

## 2.3 Denitrification rate measurement

The denitrification rates were measured using membrane inlet mass spectrometry (MIMS, Bay Instruments, Easton, MD, USA) according to the protocol by Kana et al. (1994), which quantifies changes in dissolved N<sub>2</sub>:A<sub>r</sub> ratios within the water overlying the soils. More details of these procedures can be found in previous studies (Li et al. 2013; Xia et al. 2018). Generally, undisturbed soil samplers (PVC, inner diameter = 8 cm, outer diameter = 9 cm, height = 30 cm) were used to collect a total of 252 undisturbed soil cores (4 periods in the second year and 3 periods in the third year) in the 0–10 cm soil layer. The sampling times were recorded in Table S2. The samplers were gently immersed in water to collect the soil cores (0–10 cm) and then sealed at the bottom with rubber stoppers. The soil cores were immersed in water and placed vertically in a culture device (Fig. S1) that simulated an in-situ environment, and then equilibrated overnight with gentle aeration from an aquarium air pump. Three water samples from each core container (5 ml from 1 005 ml of overlying water) were collected at 2-hr intervals during the 8-hr incubation (collected at 0, 2, 4, 6 and 8 h) and preserved in Exetainer vials (Labco Limited, Buckinghamshire, UK) with 20 µL of a saturated HgCl<sub>2</sub> solution. Changes in the N<sub>2</sub> concentration were determined by the following Equation for a given temperature and salinity during incubation.

$$F = [(N_2/A_r)_{outlet} - (N_2/A_r)_{inlet}] \times A_r \times V / A$$

where F indicates the N<sub>2</sub> flux (µmol m<sup>-2</sup> h<sup>-1</sup>) of the soils during the denitrification process; A<sub>r</sub> indicates the argon concentration (µmol L<sup>-1</sup>); N<sub>2</sub>/A<sub>r</sub> indicates the ratio of N<sub>2</sub> concentration to argon concentration; V indicates the flow rate (L h<sup>-1</sup>); and A indicates the surface area of the soils (m<sup>2</sup>). The net N<sub>2</sub> flux was

calculated for each sample by extrapolating the rate of  $N_2$  change from a five-point linear regression over the incubation time (Fig. S1).

## 2.4 Ammonia volatilization measurement

A static semi-opened chamber technique was used to measure the  $NH_3$  volatilization rate (2 periods in 2017 and 2018) in the water-soil systems (Martines et al. 2010). After the application of tillering fertilizer and panicle fertilizer in each year, a collection chamber (a PVC tube, inner diameter = 15 cm, height = 20 cm) was inserted 3–5 cm deep into the soils. Two polyethylene foam discs (thickness = 2 cm, diameter = 15 cm, density =  $0.03 \text{ g cm}^{-3}$ ) were soaked with 15 mL phosphoglycerin solution (50 mL phosphoric acid and 40 mL glycerin, to 1 000 mL with distilled water). One disc was placed on a wire mesh that was welded 6 cm under the top of each tube to absorb the  $NH_3$  volatilized from the overlying water, and the wire mesh was 4 cm above the water surface. The other disc, which was placed 2 cm above the first disc, was used to protect the first disc and absorb  $NH_3$  in the atmosphere (Fig. S2). After 24 hours, the first foam disc was put into a 500-mL plastic bottle, and then 300 mL  $2 \text{ mol L}^{-1}$  KCl solution was added to fully immerse the disc. The bottle was oscillated for 1 h (after being covered) to extract the  $NH_3$  that had accumulated in the foam disc. Finally, 30 mL of extracting solution was analyzed for  $NH_4^+$ -N using a flow injection analyzer (Skalar Analytical, Breda, the Netherlands).

## 2.5 Environmental factor measurements

A total of 252 (9 treatments with 4 replicates, 4 periods in the second year and 3 periods in the third year) data sets, including the overlying water samples (100 ml each) and well-mixed soil samples (0–5 cm layer, approximately 20 g each), were collected at the sampling time (Table S2). The overlying samples were placed on ice and transported to the laboratory, filtered ( $0.7 \mu\text{m}$  Whatman GF/F filters), and frozen until analysis. The soil samples were air-dried and grinded for determination. For each treatment, the elements in the driving factor components (overlying water and soil) were measured. The dissolved oxygen (DO) content of the overlying water was measured using a portable parameter detector (Hach Company, Loveland, CO). The EC and pH of the overlying were measured by a DDS-307 conductivity meter and PHSJ-4F pH meter (Shanghai Precision Scientific Instrument Co. Ltd., Shanghai), respectively. The well-grinded soil samples (20 g) were mixed with 50 mL of  $2 \text{ mol L}^{-1}$  KCl and were shaken, filtered and frozen until analysis. The  $NO_3^-$ -N and  $NH_4^+$ -N of the filtered water and soil extracts were analyzed using a flow injection analyzer (Skalar Analytical, Breda, The Netherlands).

## 2.6 Data analysis

Statistical analysis, including one-way ANOVA, multiple comparisons (LSD,  $P = 0.05$ ), Pearson linear correlation analysis and determination of normality tests were conducted using SPSS (version 22.0). Bivariate relationships between each independent variable and denitrification/ $NH_3$  volatilization rates were assessed to identify if there were potentially nonlinear relationships among the variables.

Structural equation modeling (SEM) was used to further gain a multivariate perspective about the relative influences of the overlying water and soil on the denitrification/ $NH_3$  volatilization rate. SEM analysis can divide the net effect of an experimental factor into direct and indirect effects through other aspects of the

system (Xia et al. 2018), and estimate the strengths of these multiple effects. Moreover, SEM can evaluate the causal relationships between exploratory variables by introducing intermediate variables, which are indirect effects. The indirect effects were calculated by multiplying the involved standardized path coefficients (Grace 2006). We evaluated two SEMs to investigate the control of net denitrification rates and  $\text{NH}_3$  volatilization rates, respectively, using observed variables. The denitrification SEM included three environmental variables of overlying water and three variables of nitrogen, the  $\text{NH}_3$  volatilization SEM included two environmental variables of overlying water and two variables of ammonium. These two SEMs provided a detailed perspective of the relationships and related the important components of driving factors to denitrification/ $\text{NH}_3$  volatilization rates. AMOS software (version 24) was used for the SEM implementation. The maximum likelihood estimation method was used to calculate the model fit parameters. The adequacy of each model was determined using a  $\chi^2$  test and the correlation coefficient.

## 3 Results

### 3.1 Changes of denitrification rates with biochar and PAM amendments

The net denitrification rates of the soils in 2017 and 2018 were 21.12-158.67  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$  and 48.74-163.27  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ , respectively (Fig. 3). Denitrification rates were high in the tillering and jointing periods of rice growth and then gradually declined in the heading and yellow-ripening periods, which was consistent with the patterns of N concentration in the soil and water during each growth period. The application of biochar and PAM had significantly negative effects on the denitrification rates in each rice growth period (Fig. 3; Table S3). The denitrification rates differed significantly ( $p < 0.05$ ) between the B1 treatment and the B2 or B3 treatments, but there were no significant differences between the B2 and B3 treatments ( $p > 0.05$ ). The application of biochar was shown to inhibit denitrification processes in the saline soil. Compared with the treatment without biochar, the average denitrification rates in 2017 and 2018 after the application of 2% biochar decreased by 25.47% and 39.60% respectively, the average denitrification rates in 2017 and 2018 after the application of 5% biochar decreased by 23.63% and 30.17% respectively (Fig. 3).

The effects of PAM addition on denitrification were consistent with that of biochar amendment treatments. Compared with the control treatment (P1), the average reduction of N losses through denitrification in saline soil after adding 0.4‰ PAM was 9.87% in 2017 and 16.38% in 2018, the average reduction in N losses after adding 1‰ PAM was 10.63% in 2017 and 29.08% in 2018 (Fig. 3). The application of PAM inhibited soil denitrification, but the inhibitory effect did not increase significantly with an increase in PAM content. Moreover, there was no significant synergistic effect of biochar and PAM amendments on soil denitrification rates (Table S3).

### 3.2 Changes of $\text{NH}_3$ volatilization rates with biochar and PAM amendments

Due to the application of N fertilizer in the tillering and jointing periods, the ammonium concentrations in the water and soil remained high, which dropped sharply during the heading and yellow-ripening periods (data not

shown). Therefore, this study only measured the  $\text{NH}_3$  volatilization rates at tillering and jointing periods. As shown in Fig. 4 and Table S4, biochar application had a significantly positive effect on the rate of  $\text{NH}_3$  volatilization ( $p < 0.05$ ), while PAM application had the opposite effect on  $\text{NH}_3$  volatilization rate. There was no significant synergistic effect of biochar and PAM amendments on  $\text{NH}_3$  volatilization rates ( $p > 0.05$ ).

For the B1 treatment, the  $\text{NH}_3$  volatilization rates in 2017 and 2018 were between 327.31- 489.41  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$  and 223.13- 578.65  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ , respectively. For the B2 treatment, the  $\text{NH}_3$  volatilization rates ranged from 376.93 to 648.81  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$  in 2017, and in 2018, it ranged from 232.03 to 610.46  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ . For the B3 treatment, the  $\text{NH}_3$  volatilization rate in 2017 was between 379.67 and 661.14  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$  and was between 269.84 and 617.38  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$  in 2018 (Fig. 4). The  $\text{NH}_3$  volatilization rates from the B2 and B3 treatments were significantly higher than those from the B1 treatment ( $p < 0.05$ ), but the difference between the B2 and B3 treatments was not significant.

Compared with P1 treatment, the average  $\text{NH}_3$  volatilization rates without biochar addition of the P2 and P3 treatments decreased by 18.20% and 19.42%, respectively; the average  $\text{NH}_3$  volatilization rates with 2% biochar addition of the P2 and P3 treatments decreased by 11.39% and 15.06%, respectively; the average  $\text{NH}_3$  volatilization rates with 5% biochar addition of the P2 and P3 treatments decreased by 16.07% and 16.39%, respectively (Fig. 4). There was significant difference in the  $\text{NH}_3$  volatilization rates between the P2 or P3 treatments and the P1 treatment ( $p < 0.05$ ), but there was no significant difference between the P2 and P3 treatments ( $p > 0.05$ ).

### 3.3 The performance of SEM models for denitrification and $\text{NH}_3$ volatilization

Table 2 and Table 3 show the results of the bivariate relationships between the denitrification/  $\text{NH}_3$  volatilization rates and the environmental factors. The denitrification rates were significantly correlated with the  $\text{NO}_3^- \text{-N}$ ,  $\text{NH}_4^+ \text{-N}$ , DO, EC, and pH in the overlying water; and the  $\text{NH}_4^+ \text{-N}$ ,  $\text{NO}_3^- \text{-N}$  in soil.  $\text{NH}_3$  volatilization rates were significantly correlated with the  $\text{NH}_4^+ \text{-N}$  concentration in overlying water and soil as well as pH in the overlying water. The two models fitting results from AMOS24 are shown in Fig. 5 and Fig. 6. The denitrification model showed that the denitrification rate could be adequately explained by the effects of various environmental factors of overlying water on nitrogen level ( $\chi^2 = 26.869$ ,  $R^2 = 0.78$ ; the standardized path coefficients are shown in Fig. 5). The  $\text{NH}_3$  volatilization model explained 75% of the variance in the  $\text{NH}_3$  volatilization rate ( $\chi^2 = 2.726$ ,  $R^2 = 0.75$ ; the standardized path coefficients are shown in Fig. 6). From a statistical perspective, the constructed SEMs were acceptable for modeling the denitrification/ $\text{NH}_3$  volatilization rate in saline soils.

In the SEM of denitrification, the influencing observed variables included nitrogen levels and overlying water environment. The  $\text{NO}_3^- \text{-N}$  concentration showed great direct effect on the net denitrification rate, and the direct path coefficients of  $\text{NO}_3^- \text{-N}$  in soil and overlying water on denitrification were 0.57 and 0.49, respectively. The SEM results showed that nitrogen concentrations were significant negatively correlated with DO concentrations and positively correlated with salinity of overlying water. The salinity and DO of overlying water

had indirect effects on denitrification rate through nitrogen concentrations, with path coefficients of 0.34 and -0.27, respectively. The negative effect of DO on denitrification mainly occurred because denitrification is an anaerobic reaction that generally occurs in anaerobic or anoxic environments. Wang et al. (2019) found a decreasing trend in denitrification products with an increase in oxygen content. Therefore, increasing DO in the early growth stage of rice can effectively inhibit denitrification and reduce nitrogen loss. In addition to nitrate concentration, the soil ammonium also had a positive indirect effect on the denitrification rate, indicating that some of the substrates used during soil denitrification came from nitrification and that coupled nitrification-denitrification occurred in soil.

In the SEM of  $\text{NH}_3$  volatilization, the observed variables from overlying water included the  $\text{NH}_4^+$ -N concentrations, pH and salinity, and the  $\text{NH}_4^+$ -N concentrations in soil was also an observed variable. Figure 6 shows that the overlying water  $\text{NH}_4^+$ -N concentration made the largest direct positive contribution to the  $\text{NH}_3$  volatilization rate, with a path coefficient of 0.62, and the  $\text{NH}_4^+$ -N concentration in soil had a negative effect on  $\text{NH}_3$  volatilization, with a path coefficient of -0.35. The pH of the overlying water had indirect positive effect on  $\text{NH}_3$  volatilization through  $\text{NH}_4^+$ -N concentrations of overlying water and soil. The EC of the overlying water indirectly negatively influenced  $\text{NH}_3$  volatilization through the concentration of  $\text{NH}_4^+$ -N in the overlying water and soil. Moreover, the salinity of overlying water had a direct positive effect on contribution to  $\text{NH}_3$  volatilization. These results suggested that the  $\text{NH}_3$  volatilization rates in the saline paddy soils were mainly affected by the overlying water especially the  $\text{NH}_4^+$ -N concentration. In paddy fields,  $\text{NH}_3$  volatilization occurs mainly at the interface between the overlying water and the atmosphere. Nitrogen migrates downwards, thus increasing the nitrogen content of the soil, decreasing the substrates available for  $\text{NH}_3$  volatilization in the overlying water. This resulted in a reduction in  $\text{NH}_3$  volatilization. The results showed that nitrogen losses in paddy fields can be reduced by adjusting environmental factors and increasing soil ammonium nitrogen retention capacity through appropriate field management or soil amendments.

## 4 Discussion

### 4.1 The influence mechanism of biochar and PAM on denitrification in saline soils

Biochar was shown to affect the denitrification rates negatively in saline soils. The results of two-year test-pit experiments showed that the denitrification rates in saline soil decreased significantly after the addition of biochar ( $p < 0.05$ ). Previous studies have shown that biochar application has an inhibitory effect on denitrification in different soils (Singh et al. 2010). Due to the porous and low density of biochar, the biochar amendment reduced the soil bulk density (Oguntunde et al. 2008), improved soil aeration (Karhu et al. 2011), thus decreased soil anoxic condition and increased the dissolved oxygen content (Zhang et al. 2010), which inhibited the denitrification of nitrogen microorganisms through inhibiting the synthesis or activity of denitrifying enzymes under the anaerobic condition (Penn et al. 2016). Denitrification process is a reduction reaction with electron transfer and electrical conductivity is a signal of electron transport capacity and the appropriate EC may facilitate the transfer of electrons to soil denitrifying microorganisms, thereby promoting the denitrification process (Fig. 5; Liu et al. 2019). Meanwhile, salinity in the overlying water may affect the

denitrification negatively by influencing the availability of oxygen (Fig. 5; Noe et al. 2013; Zhou et al. 2017). Previous studies demonstrated that biochar may improve leaching of soluble salts to decrease soil EC (Yue et al. 2016). Some studies have shown that biochar application had no significant effect on denitrification rates, and it was even shown to increase denitrification rates in some cases (Clough et al. 2010; Karhu et al. 2011). The effects of biochar on denitrification vary depending on the properties of feedstock and pyrolysis condition (Van Zwieten et al. 2015; Wang et al. 2017). Biochar mainly plays a role in adsorption effect or liming effect in denitrification according to its feedstock (Liu et al. 2018). In this study, biochar could effectively absorb soil  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  predominantly (Fig. S3) because of its high surface area and strong capacity for ion absorption (Kizito et al. 2015; Xu et al. 2016; Zheng et al. 2013), thereupon then inhibiting nitrogen losses and reducing the denitrification rate (Fig. 5). There was no significant difference in denitrification between the biochar application rates of 2% and 5% ( $p > 0.05$ ). Thus, increasing the biochar application rate did not further inhibit the denitrification rate.

As a soil amendment, PAM can promote the formation of soil water-stable aggregates, improve soil structure, increase soil water infiltration, and reduce nutrient losses (Li et al. 2014a). In this study, PAM was shown to significantly reduce denitrification rates in saline soil of a paddy field ( $p < 0.05$ ). The mechanisms by which PAM application reduces denitrification rates may be related to the influence of nitrogen redistribution in soil. PAM increased the internal porosity, total porosity and capillary porosity of the soil (Fei et al. 2019). As a result, the soil infiltration rate also significantly increased (Yu et al. 2003), which lead to an increase in the infiltration of inorganic nitrogen in the overlying water and soil (Fig. S3) and reduce the denitrifying substrate (Li & Wang 2016), thus reducing the denitrification rate. Furthermore, the application of PAM can effectively improve the adsorption capacity of soil for ammonium and slow down the conversion of  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_3^-\text{-N}$  in soil (Deng et al. 2009). This is consistent with the result that the concentration of  $\text{NH}_4^+\text{-N}$  in soil increased and the concentration of  $\text{NO}_3^-\text{-N}$  decreased after PAM application (Fig. S3), thereby reducing the crucial substrate for denitrification (Fig. 5; Li et al. 2014b; Zhao et al. 2014). However, increasing the amount of PAM had no significant effect on reducing the denitrification rate, which may be due to the saturation of  $\text{NH}_4^+\text{-N}$  adsorption by PAM. The results of this study showed that the effects of biochar and PAM on the denitrification rates were significant ( $p < 0.05$ ), and the effect of biochar addition on nitrogen losses via denitrification was greater than that from PAM addition.

## 4.2 The influence mechanism of biochar and PAM on $\text{NH}_3$ volatilization in saline soils

In traditional paddy field, urea and ammonium nitrogen fertilizers are the main nitrogen fertilizers. Urea hydrolysis promotes  $\text{NH}_3$  volatilization in flooded layer (Wang et al. 2012). The  $\text{NH}_3$  volatilization was significantly correlated with the concentration of ammonium nitrogen in the overlying water (Table 3, Fig. 6). The N loss of  $\text{NH}_3$  volatilization is mainly concentrated in the tillering and jointing periods after applying fertilizer. Higher  $\text{NH}_4^+\text{-N}$  and lower  $\text{NO}_3^-\text{-N}$  concentrations of overlying water under biochar application were observed at tillering and jointing periods (Fig. S3) consistent with the result of Feng et al. (2017). Many studies have investigated the effect of biochar on  $\text{NH}_3$  volatilization, but the results are varied (Sha et al. 2019). The application of biochar has been shown to decrease (Esfandbod et al. 2017; Mandal et al. 2016), increase

(Wang et al. 2017) or have no effect (Feng et al. 2017) on  $\text{NH}_3$  volatilization. The ammonium adsorption capacity of biochar could tend to reduce the  $\text{NH}_3$  volatilization (Asada et al. 2002). But then, biochar application had a liming effect caused by basic salts of biochar (Nguyen & Lehmann 2009), resulting in an increase in soil pH, which is considered conducive to  $\text{NH}_3$  volatilization (Sha et al. 2019; Taghizadeh-Toosi et al. 2011). pH promoted the transfer of ammonium in the soil to the overlying water (Fig. 6), the decrease of soil  $\text{NH}_4^+$ -N concentration was accompanied by the increase of  $\text{NH}_4^+$ -N concentration in the overlying water, which contributed to furtherance of  $\text{NH}_3$  volatilization (Fig. 6). Salinity has a complicated influence on  $\text{NH}_4^+$ -N in soil. On the one hand, salinity-induced reduction in soil ammonium adsorption capacity (Rysgaard et al. 1999), which accelerates the release of ammonium. Nevertheless, the release of ammonium in soil is very small under low salt condition (Rysgaard et al. 1999), there was no significant correlation between the salinity of overlying water and  $\text{NH}_3$  volatilization rate in this study (Table 3). On the other hand, salinity significantly affects the community and activity of nitrifying bacteria (Wang et al. 2018) and reduces the nitrification rate, thus increasing the content of ammonium in soil (Fig. 6). Studies have proved that biochar addition affected the dynamic of ammonium and nitrification process in microcosms of coastal alkaline soil (Song et al. 2014). In this study, the rate of  $\text{NH}_3$  volatilization in saline paddy field soil increased significantly after applying biochar, which was consistent with the results of Sun et al. (2017). However, there were no significant differences in the  $\text{NH}_3$  volatilization rate between the 2% and 5% biochar application rates. Increasing the amount of biochar did not significantly increase the  $\text{NH}_3$  volatilization rate, which may have been due to the increased absorption of ammonium by more biochar (Seredych & Bandosz 2007) .

Studies have demonstrated that PAM application can absorb non-point source contaminants by electrostatic force for cationic charge sites (Li & Wang 2016), thereby reducing concentrations of N in irrigation runoff (Krauth et al. 2008). The experimental results of Entry & Sojka (2003) showed that application of PAM greatly reduced the content of  $\text{NH}_4^+$  in runoff. Smith & Harrison (1991) found that the application of PAM delayed the diffusion and release of nitrogen. PAM can be absorbed on the soil surface after applying into soil, the anionic charges on PAM may absorb the  $\text{NH}_4^+$  in soil (Entry & Sojka 2003), which has strong affinity for soil clay, so the PAM application should lower  $\text{NH}_4^+$  concentrations in overlying water (Fig. S3; Li & Wang 2016). Moreover, the application of PAM has a certain inhibitory effect on soil urease activity, which plays a critical role in the conversion of urea in soil and directly affects the volatilization rate of  $\text{NH}_3$ . Nyord et al. (2012) found that after the addition of PAM, the  $\text{NH}_3$  volatilization rate significantly decreased, which was consistent with our results. However, there was no significant change in the rate of  $\text{NH}_3$  volatilization with increasing PAM content. Excessive PAM application may result in the increase of large-sized aggregates and reduce the adsorption of nitrogen by soil. PAM application decreased the rates both of denitrification and  $\text{NH}_3$  volatilization, meanwhile, the infiltration of organic nitrogen increased, which may cause the risk of groundwater pollution. This problem should be further investigated in the follow-up study on the effect of PAM amendment on soil nitrogen cycling.

## 5 Conclusions

Based on three-years field experiments, we studied the effects of biochar and PAM amendments on the denitrification and  $\text{NH}_3$  volatilization rates of saline soils in a coastal agricultural reclamation area. Nitrogen loss from denitrification and  $\text{NH}_3$  volatilization mainly occurred at tillering and jointing periods. Compared with

that in the control, biochar and PAM application significantly reduced the denitrification rate in saline soil by 23.63%-39.60% and 9.87%-29.08%, respectively ( $p < 0.05$ ). The application of biochar significantly increased  $\text{NH}_3$  volatilization rates by 9.82%-25.58%, while PAM amendment significantly reduced  $\text{NH}_3$  volatilization rates by 11.39%-19.42% ( $p < 0.05$ ). During the growth periods of rice, the synergistic effect of biochar and PAM on denitrification rates was not significant ( $p > 0.05$ ), and the effect of biochar addition on the denitrification rate was greater than the effect of PAM addition. SEM showed that the denitrification rates were negatively affected by DO and positively affected by EC in the overlying water. The addition of biochar mainly reduced the denitrification rate by increasing the dissolved oxygen in the water and absorbing soil nitrate nitrogen. PAM reduced the rate of denitrification by increasing the infiltration inorganic nitrogen and slowing the conversion of ammonium to nitrate. The concentration of  $\text{NH}_4^+\text{-N}$  in the overlying water directly determines  $\text{NH}_3$  volatilization rate, and pH is the most critical environmental factor affecting  $\text{NH}_3$  volatilization rate. Biochar application increased pH and stimulated the transfer of  $\text{NH}_4^+\text{-N}$  from soil to overlying water, thus increased  $\text{NH}_3$  volatilization rate. PAM reduced the concentration of  $\text{NH}_4^+\text{-N}$  in the overlying water through absorbing soil ammonium and inhibiting urea hydrolysis, thereby decreasing  $\text{NH}_3$  volatilization rate. Biochar decreased the denitrification rate while stimulated volatilization of  $\text{NH}_3$ . Hence, there was a tradeoff between denitrification and  $\text{NH}_3$  volatilization in paddy field of saline soils induced by biochar application. However, PAM application decreased the rates both of denitrification and  $\text{NH}_3$  volatilization.

## Declarations

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**Authors' contributions** Yongchun Pan performed the data analysis and interpretation of the results, and wrote and organized the article. Dongli She, the project leader, conceived and designed the analysis; contributed for data analysis; contributed to the final version of the manuscript. Zhenqi Shi and Xinyi Chen performed the experiments and data analysis, performed the interpretation of the results. Yongqiu Xia contributed to the experimental design and contributed with critical revision of the manuscript.

**Data availability** The datasets analyzed during the current study are available from the corresponding author on reasonable request.

## Compliance with ethical standards

**Competing interests** The authors declare that they have no competing interests.

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## Tables

**Table 1** Basic physicochemical properties of soil, biochar and PAM

	OC	TN	pH	EC (dS·m <sup>-1</sup> )	BD	CEC	Size composition (%)			Texture
	(g·kg <sup>-1</sup> )	(g·kg <sup>-1</sup> )			(g·cm <sup>-3</sup> )	(cmol·kg <sup>-1</sup> )	sand	silt	clay	
<b>Soil</b>	3.1	0.41	8.0	4.0	1.38					
						4.99	32.37	62.75	4.88	silt loam
<b>Biochar</b>	467.2	5.9	9.9	1.0	0.69	Ca (g·kg <sup>-1</sup> )	Cl (g·kg <sup>-1</sup> )	Inter-porosity (%)	Intra-porosity (%)	particle density (g·cm <sup>-3</sup> )
						0.0016	1.44	13.2	49.3	1.83
<b>PAM</b>	Property	molecular weight								
	anionic	12 million								

Note: OC, organic carbon; TN, total nitrogen; EC, electrical conductivity; BD, bulk density; CEC, cation exchange capacity

**Table 2** Correlations between the denitrification rate and environmental factors (252 samples)

		Denitrification rate		Overlying water				Soil	
		NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N	DO	EC	pH	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N	
Denitrification rate	1	0.729**	0.557**	-0.773**	0.798**	0.471**	0.680**	0.827**	
Overlying water	NO <sub>3</sub> <sup>-</sup> -N	1	.492**	-0.600**	0.782**	0.513**	0.580**	0.654**	
	NH <sub>4</sub> <sup>+</sup> -N		1	-0.435**	0.582**	0.625**	0.168*	0.522**	
	DO			1	-0.780**	-0.441**	-0.810**	-0.710**	
	EC				1	0.599**	0.774**	0.864**	
	pH					1	0.311**	0.402**	
Soil	NO <sub>3</sub> <sup>-</sup> -N						1	0.730**	
	NH <sub>4</sub> <sup>+</sup> -N							1	

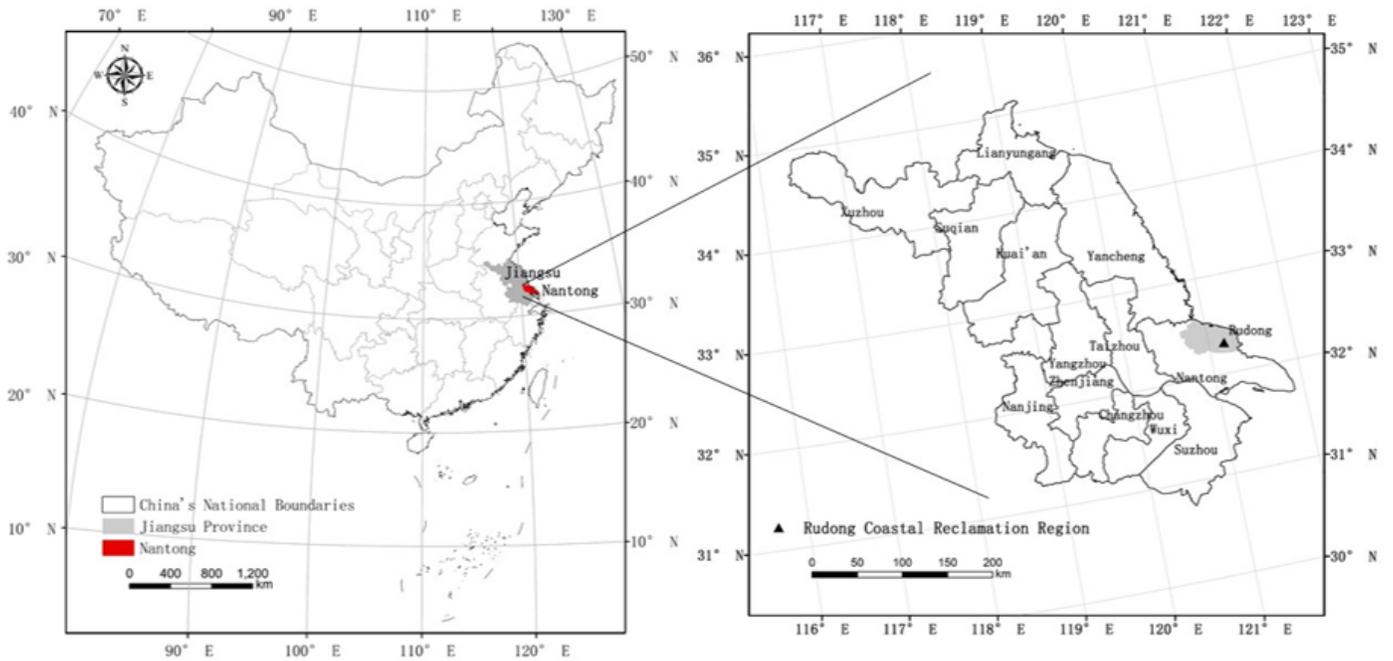
\*\* indicates a significant correlation at the 0.01 level (both sides); \* indicates a significant correlation at the 0.05 level (both sides)

**Table 3** Correlations between the NH<sub>3</sub> volatilization rate and environmental factors (144 samples)

		NH <sub>3</sub> volatilization rate		Overlying water				Soil	
		NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N	EC	pH	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N		
NH <sub>3</sub> volatilization rate	1	0.253**	0.641**	0.046	0.696**	-0.357**	-0.681**		
Overlying water	NO <sub>3</sub> <sup>-</sup> -N	1	0.492**	0.782**	0.513**	0.580**	0.654**		
	NH <sub>4</sub> <sup>+</sup> -N		1	0.582**	0.625**	0.168*	0.522**		
	EC			1	0.599**	0.774**	0.864**		
	pH				1	0.311**	0.402**		
Soil	NO <sub>3</sub> <sup>-</sup> -N					1	0.730**		
	NH <sub>4</sub> <sup>+</sup> -N						1		

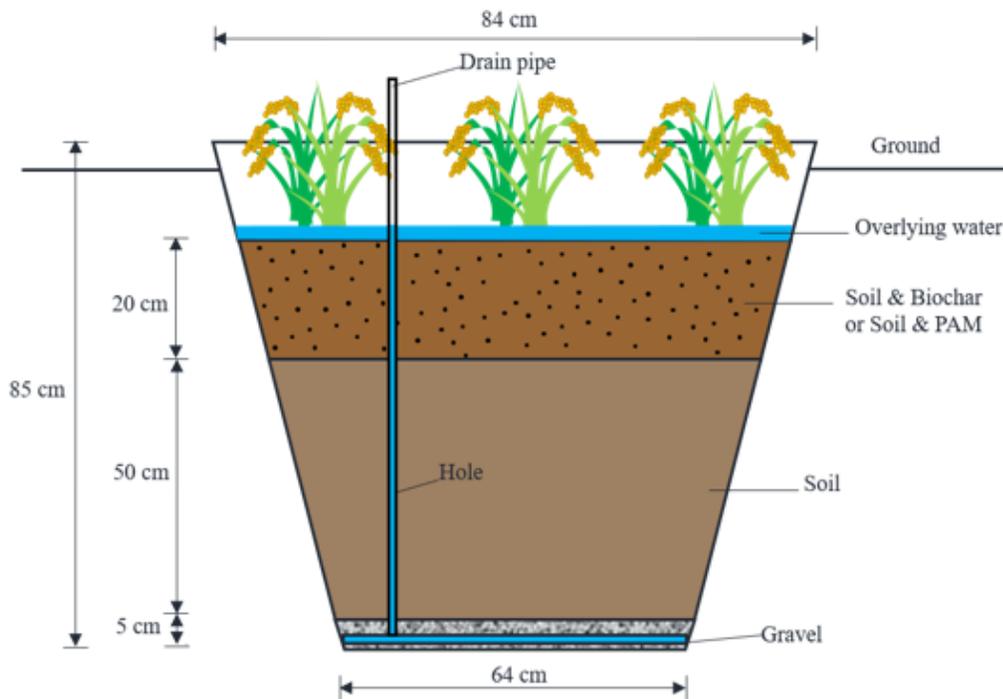
\*\* indicates a significant correlation at the 0.01 level (both sides); \* indicates a significant correlation at the 0.05 level (both sides)

## Figures



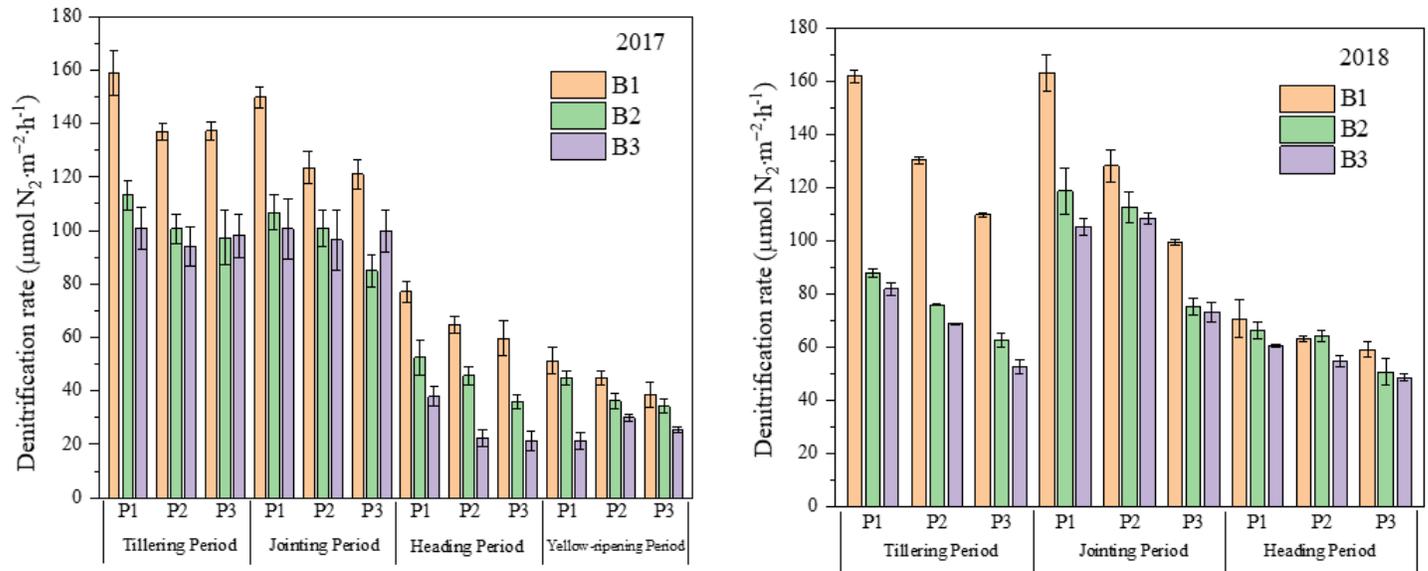
**Figure 1**

Location of the sampling site (Figure quoted from Sun et al. (2021)). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



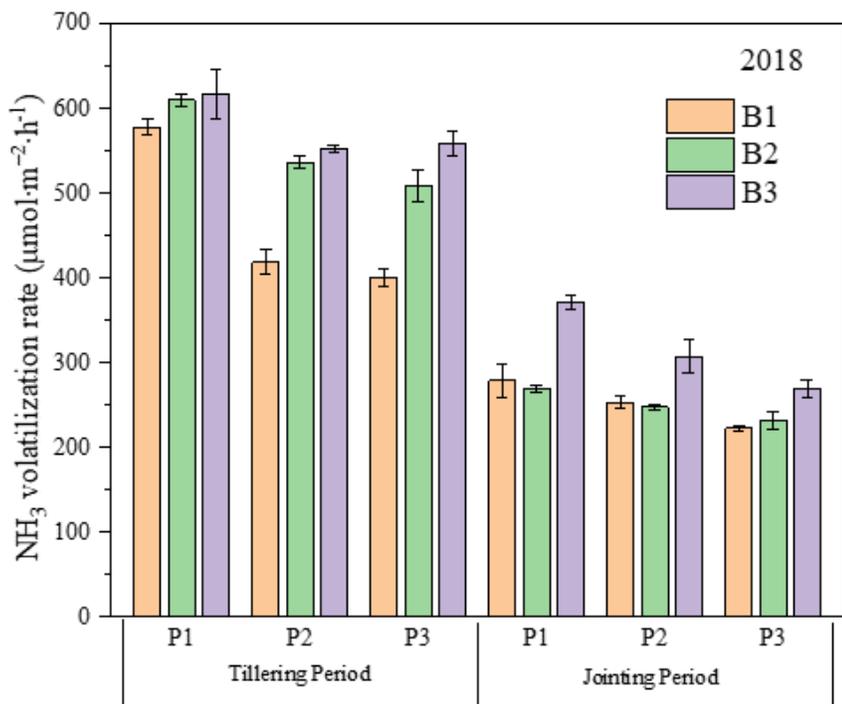
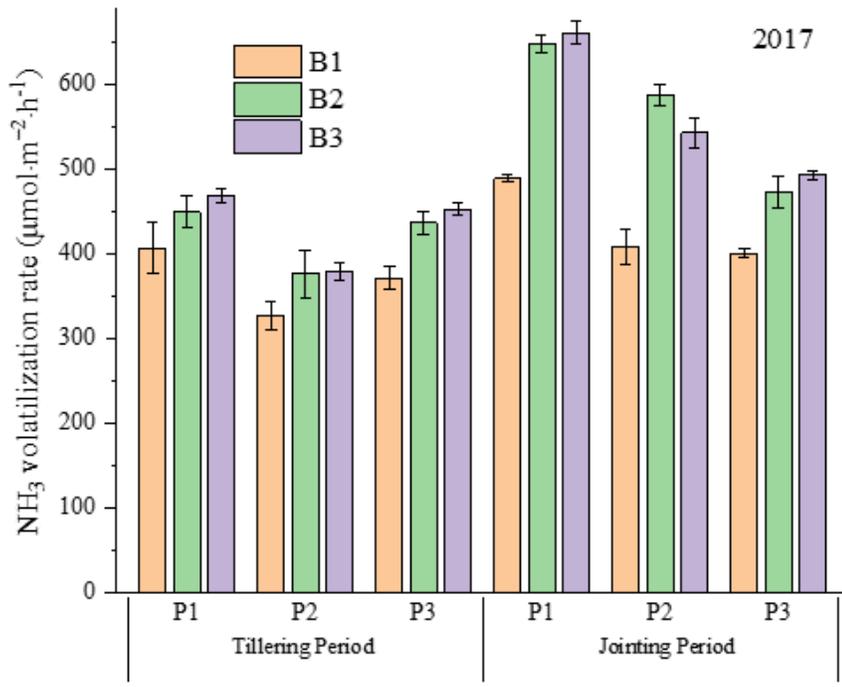
**Figure 2**

Conceptual diagram of the field experiment (Figure quoted from Sun et al. (2021)).



**Figure 3**

Rates of denitrification under different biochar and PAM application conditions. B1, B2, B3 represent 0%, 2% and 5% (w/w) biochar addition, respectively; P1, P2, P3 represent 0‰, 0.4‰ and 1‰ PAM addition, respectively.



**Figure 4**

Rates of NH<sub>3</sub> volatilization under different biochar and PAM application conditions. B1, B2, B3 represent 0%, 2% and 5% (w/w) biochar addition, respectively; P1, P2, P3 represent 0‰, 0.4‰ and 1‰ PAM addition, respectively.

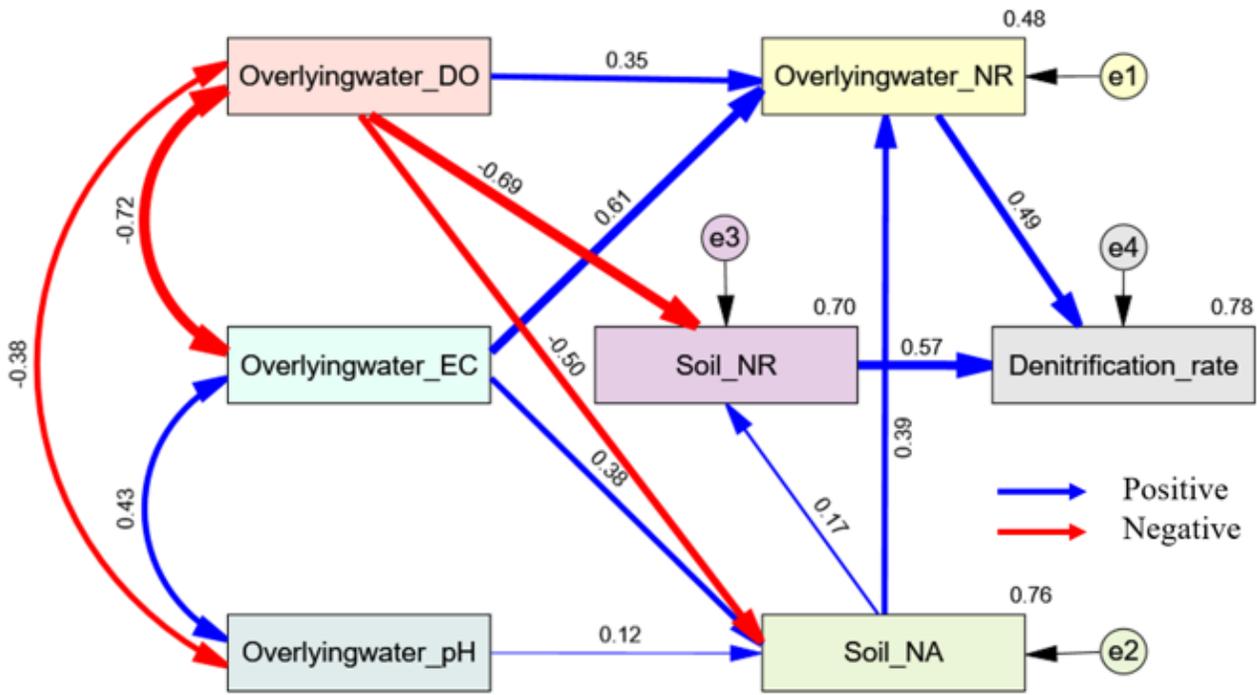
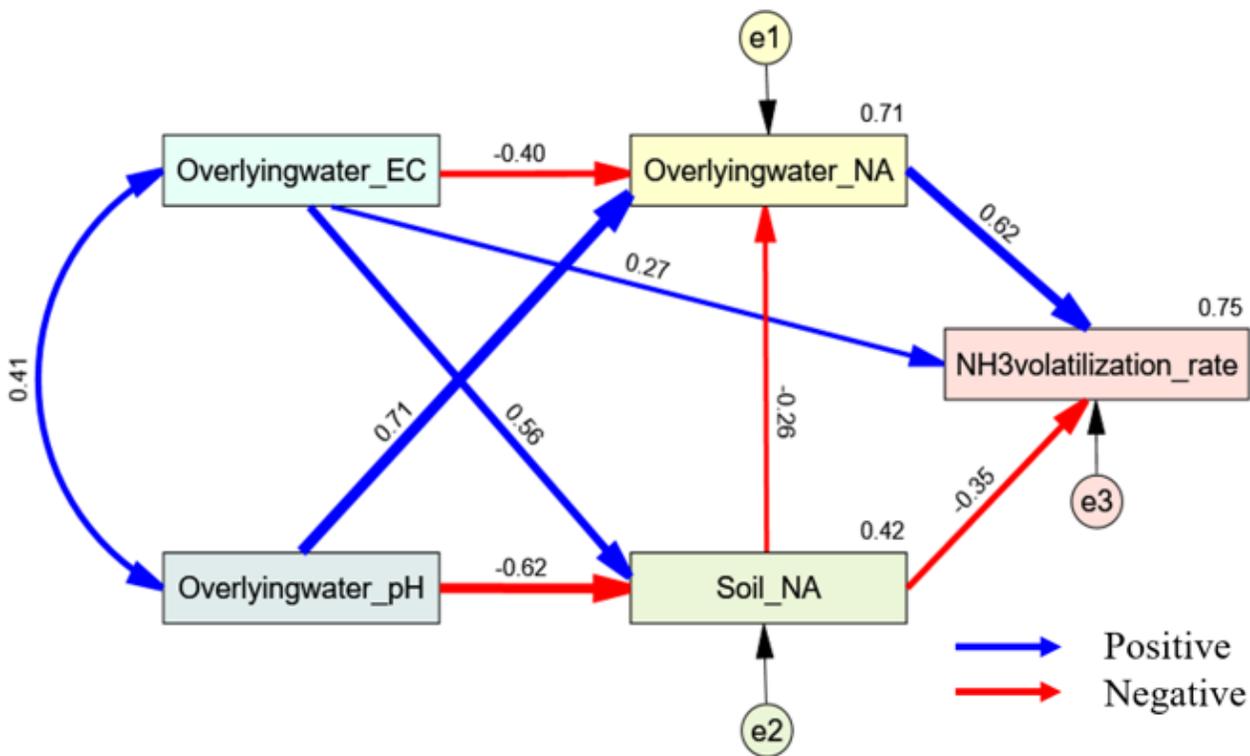


Figure 5

Standardization model that examines the multivariate effects on denitrification rates. The double-headed arrows indicate covariance between related variables. The direction of the arrows indicates the hypothetical direction of causation. The number of adjacent to each arrow are standardized path coefficients that reflect the effect size of the relationship. DO represents dissolved oxygen; EC represents electrical conductivity; NR represents the concentration of NO<sub>3</sub>-N; NA represents the concentration of NH<sub>4</sub>+N.



## Figure 6

Standardization model that examines the multivariate effects on NH<sub>3</sub> volatilization rates. The double-headed arrows indicate covariance between related variables. The direction of the arrows indicates the hypothetical direction of causation. The number of adjacent to each arrow are standardized path coefficients that reflect the effect size of the relationship. EC represents electrical conductivity; NR represents the concentration of NO<sub>3</sub>-N; NA represents the concentration of NH<sub>4</sub>+N.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryinformation.docx](#)