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Short communication

## Effects of five years' nitrogen deposition on soil properties and plant growth in a salinized reed wetland of the Yellow River Delta

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## ARTICLE INFO

## Keywords:

Nitrogen  
Plant growth  
Soil enzymes  
Nutrient content  
The Yellow River Delta

## ABSTRACT

Atmospheric nitrogen (N) deposition caused by human activities has been shown to have significant impacts on ecosystem structure and function, but the reported impacts have been inconsistent among studies. To test the effects of N deposition on a salinized reed wetland, we built a long-term nitrogen deposition experimental platform in the Yellow River Delta (YRD), China. The present study investigated soil chemical properties, soil enzyme activities and plant growth parameters after five years of N deposition. Results showed that the concentration of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  increased significantly with the increasing level of nitrogen addition, but no significant influences were observed in total nitrogen (TN), total organic carbon (TOC) and available phosphorus (AP) across N addition treatments. Higher amounts of N addition significantly increased the urease activity (Ure) and alkaline phosphatase activity (Alp), but did not enhance the activity of invertase (Inv). The activities of Ure and Alp were positively correlated with the concentration of soil  $\text{NO}_3\text{-N}$ , TN and TC, respectively, but negatively correlated to soil salt content. Meanwhile, the plant height and productivity were significantly stimulated, and the surface soil salt content decreased significantly. The results suggest that atmospheric N deposition may help improve the function of salinized reed wetland ecosystems in the YRD by increasing soil nutrient content, stimulating soil enzyme activity and enhancing plant production.

## 1. Introduction

The increase in atmospheric nitrogen (N) deposition induced by human activities has been recognized as an important factor that can impact structure and function of terrestrial (Duprè et al., 2010), grassland (Bai et al., 2010) and wetland (Mitsch and Gosselink, 2000) ecosystems. Studies until today have demonstrated that N deposition may have positive, negative or neutral effects on soil enzyme activity, plant diversity and species richness (Song et al., 2014; Stevens et al., 2004; van den Berg et al., 2011; Song et al., 2011). Despite the discrepancy in the impact, it is generally accepted that N enrichment can increase aboveground net primary production in terrestrial ecosystems, grasslands, farmlands and wetlands (Stevens et al., 2004; Moldan and Wright, 2011; Koller et al., 2013; Gai et al., 2016). In addition, meta-analyses on terrestrial ecosystems have shown that N addition tends to enhance microbial processes such as  $\text{N}_2\text{O}$  emission, nitrification, and

$\text{CH}_4$  emissions (Aronson and Helliker 2010, Lu et al. 2011).

Coastal wetlands, are a pivotal part of the coastal zone, and like other ecosystems, they receive a large amount of nitrogenous compounds from terrestrial human activities (Galloway et al., 2008; Howarth, 2008). N deposition has been reported to lower the plant species richness in coastal wetlands (Remke et al., 2009; Pakeman et al., 2016); however, the effects of N addition on soil chemical and biological parameters and vegetation growth have not been well understood (Wolters et al., 2016). We previously investigated the total N deposition rate in the Yellow River Delta (YRD) and found it to be approximately  $22.64 \text{ kg/hm}^2$  in the growing season (May to November), which is among the highest in China. We also identified that  $\text{NO}_3\text{-N}$  was the main nitrogen form in dry deposition, the predominant nitrogen in wet atmospheric deposition was  $\text{NH}_4\text{-N}$ , and the ratio of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  to total inorganic nitrogen were 48% and 52%, respectively (Yu et al., 2014). In the present study, we aimed to determine how the

Abbreviations: TC, total carbon; TOC, total organic carbon; DOC, dissolved organic carbon; TN, total nitrogen; AP, available phosphorus; YRD, Yellow River Delta; Inv, invertase activity; Ure, urease activity; Alp, alkaline phosphatase activity

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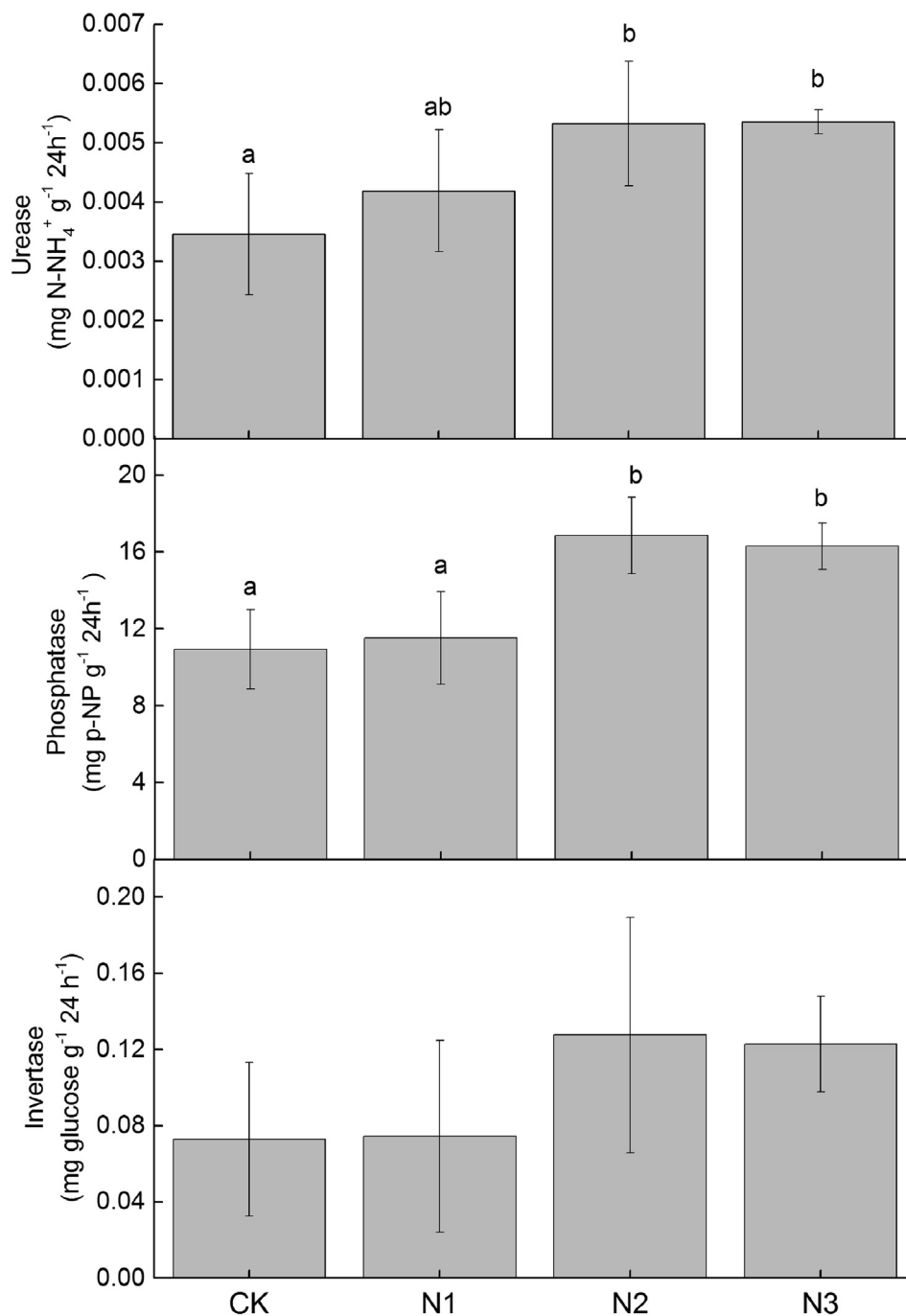
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<https://doi.org/10.1016/j.ecoleng.2019.06.016>

Received 21 February 2019; Received in revised form 2 June 2019; Accepted 24 June 2019

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**Fig. 1.** Effects of nitrogen addition on soil urease, phosphatase and invertase activities in a salinized reed wetland in the Yellow River Delta. (In each column, the data markers identified with the same letters are not significantly different ( $P > 0.05$ ) between treatments according to a least significant difference test. Bars without letters mean no significant differences. CK: control plots without N addition; N1: plots with  $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  addition amount; N2: plots with  $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  addition amount; N3: plots with  $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  addition amount.)

N deposition would affect vegetation community stability and soil nutrient characteristics in the salinized wetlands of the YRD, the response of which will largely depend on whether N deposition can alleviate salt stress-induced impacts.

The Yellow River Delta is one of the most active deltas with significant land-ocean interactions in the world and an important habitat for migrating birds because of its geographic position. During the last several decades, the delta has experienced significant changes attributed to human activities and climate change. Massive sediment has been transported from the Loess Plateau, resulting in the expansion of the coastal wetland by  $20\text{--}25 \text{ km}^2$  per year (Ren and Walker 1998);

meanwhile, the non-tidal wetland is degrading due to the high evaporation rate and soil salinization. *Phragmites australis*, a typical plant in the delta, has decreased by 17% from 1986 to 2001 (Wang et al., 2006). Therefore, the YRD has become a unique area to study the effects of environmental factor change on the structure and function of wetland ecosystem. In May 2012, we built a long-term N deposition experimental platform on a salinized reed wetland of the YRD. The objectives of the study were to answer three questions: (1) does N deposition have an impact on soil nutrient concentrations? (2) how do soil enzyme activities respond to different levels of N deposition? (3) how do N deposition affect plant growth and above-ground biomass in a saline

**Table 1**

Effects of nitrogen addition on soil chemical properties in a salinized weed wetland in the Yellow River Delta. Different letters indicated significant differences ( $P < 0.05$ ) between treatments according to a least significant difference test.

Index	CK	N1	N2	N3
NH <sub>4</sub> -N (mg/kg)	114.63 ± 1.77 a	128.50 ± 16.98 ab	134.85 ± 13.02 ab	145.10 ± 22.63 b
NO <sub>3</sub> -N (mg/kg)	5.19 ± 2.24 a	7.36 ± 0.97 ab	10.92 ± 2.00 bc	14.83 ± 3.86 d
TN (g/kg)	0.54 ± 0.12 ns	0.57 ± 0.08 ns	0.66 ± 0.08 ns	0.68 ± 0.06 ns
TC (g/kg)	15.81 ± 1.43 a	16.69 ± 1.13 ab	18.21 ± 1.33 b	18.47 ± 1.18 b
TOC (mg/l)	13.35 ± 2.60 ns	11.16 ± 2.78 ns	11.80 ± 4.32 ns	12.14 ± 2.25 ns
C/N	29.11 ± 4.81 ns	29.09 ± 2.52 ns	27.54 ± 3.42 ns	27.30 ± 2.21 ns
AP (mg/kg)	2.69 ± 0.81 ns	2.74 ± 0.61 ns	3.01 ± 0.89 ns	2.65 ± 0.00 ns
pH	7.58 ± 0.11 ns	7.61 ± 0.20 ns	7.54 ± 0.26 ns	7.59 ± 0.17 ns
Salinity (%)	0.69 ± 0.10 a	0.64 ± 0.09 a	0.50 ± 0.03 b	0.49 ± 0.04 b

wetland ecosystem?

## 2. Materials and methods

### 2.1. Sites, vegetation and environmental conditions

The study sites are located in the Yellow River Delta Ecology Research Station of Coastal Wetland in the northern part of Shandong Province (37°45'50"N, 118°59'24"E) (Fig. 1). The climate of the region belongs to warm temperate continental monsoon climate. The annual average temperature is 11.7–12.8 °C. The annual evaporation is 1900–2400 mm, and the annual precipitation 530–630 mm, of which 70% is rainfall that occurs between July and September. It is typified by distinct wet (in general, July to September) and dry (in general, October to June) seasons and remains inundated throughout the wet season.

More than 85% of the natural vegetation species are salt tolerant plants and aquatic plants, and the most dominant plant is *Phragmites australis* (common reed). The dominant soil types are classified as Calcaric Fluvisols, Gleyic Solonchaks and Salic Fluvisols (FAO), which developed on loess material carried by water from the Loess Plateau. The total N, total carbon, NH<sub>4</sub>-N and NO<sub>3</sub>-N of the top 10 cm soils in the study sites were 0.55 ± 0.08 g kg<sup>-1</sup>, 15.7 ± 0.21 g kg<sup>-1</sup>, 119.3 ± 11.2 mg kg<sup>-1</sup> and 3.45 ± 0.50 mg kg<sup>-1</sup>, respectively, and the total salt content and pH 6.01 ± 0.53 g kg<sup>-1</sup> and 7.75 ± 0.10, respectively.

### 2.2. Simulated nitrogen deposition

The experiment (initiated in 2012) had 16 plots (6 m × 4 m) with a randomized block design. We established four levels of N fertilization with four replicate plots per treatment, including: control 0 kg N ha<sup>-1</sup> yr<sup>-1</sup> (CK); N1, 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>; N2, 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>; N3, 200 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The fertilization levels were selected based on the current N deposition rate and the potential amount that is anticipated to occur in the future (Yu et al., 2014). For each fertilization event, we first dissolved NH<sub>4</sub>NO<sub>3</sub> in pure water and then evenly sprayed the solution over the area of target; the control treatment area was sprayed using the same amount of pure water without N addition. The fertilization began in May 2012 and was performed once a month until May 2017. In order to prevent horizontal movement and lateral loss of the added N, each plot was separated by a one meter of buffer zone.

### 2.3. Soil sampling and chemical property and enzyme activity analysis

In May 2017 (after five years of treatment), we randomly sampled an area of 0.5 m × 0.5 m in each plot, collecting aboveground vegetation biomass and taking soil cores (0–15 cm depth) with a 3.5-cm-diameter soil corer. Each soil core was cut vertically, half of which was used to test soil enzyme activity, and the other half natural wind dried for soil biochemical properties analysis.

Soil salt content was determined by weight loss of 1:5 (soil: water by weight) extract after oven-drying at 105 °C to constant weight. Soil pH was also determined in a 1:5 (soil: water) solution (w/v) with a digital pH meter (LE438, Shanghai, China). Nitrate (NO<sub>3</sub><sup>-</sup>-N) and ammonium (NH<sub>4</sub><sup>+</sup>-N) were extracted with 2 M KCl, and measured by an AutoAnalyser III continuous Flow Analyzer (SEAL, Australia). Available phosphorous (AP) was extracted with 0.5 M NaHCO<sub>3</sub>, and measured by a UV-Vis spectrophotometer (T6 new century, China). Total organic carbon (TOC) was determined by a TOC analyzer (Shimadu, Japan) using a 1:4 (soil: water) solution (w/v). Soil total N (TN) and total carbon (TC) contents were measured by an elemental analyzer (Elementar Vario MACRO, Germany).

Activities of urease (Ure), alkaline phosphatase (Alp) and invertase (Inv) were determined according to the handbook of Bao (2005). For determination of urease activity, 1.5 ml toluene, 20 ml citrate buffer (pH 6.7) and 1 ml of 10% urea substrate solution were added to 5 g of soil sample, and the samples were then incubated for 24 h at 37 °C. The formation of ammonium was determined spectrophotometrically at 578 nm, and results were expressed as mg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> soil 24 h<sup>-1</sup>.

For determination of alkaline phosphatase activity, 1.5 ml toluene, 10 ml phosphate buffer (pH 7.1) and 10 ml of 0.02 M p-nitrophenyl phosphate solution were added to 2 g soil sample, and the samples were incubated for 24 h at 37 °C. The formation of p-nitrophenol was determined spectrophotometrically at 510 nm and results were expressed as mg p-nitrophenol g<sup>-1</sup> soil 24 h<sup>-1</sup>.

Invertase activity was determined by incubating 5 g soil with 15 ml of 8% sucrose solution and 5 ml of phosphate buffer (pH 5.5) at 37 °C for 24 h and by measuring the reduced sugars as glucose spectrophotometrically at 508 nm with 3, 5-dinitrosalicylic acid. The results were expressed as mg glucose equivalents g<sup>-1</sup> soil 24 h<sup>-1</sup>.

### 2.4. Statistical analysis

Data were analyzed using the software package SPSS 20.0 for Windows and the Origin 9.1 and were presented as mean ± standard deviation (n = 4). The one-way analysis of variance (ANOVA) was performed to determine the effect of different levels of N deposition on soil chemical properties, enzymatic activities and plant growth. The correlations of soil biochemical properties were analyzed with the Pearson test (two-tailed). Significance for all statistical analyses was accepted at  $P = 0.05$  level.

## 3. Results

### 3.1. Effects of N addition on soil chemical properties

Soil NH<sub>4</sub>-N and NO<sub>3</sub>-N contents were significantly affected by N addition ( $P < 0.05$ ) (Table 1). The NH<sub>4</sub>-N concentration in treatment N3 was significantly higher than that of CK. No significant differences were observed between CK, N1 and N2. The NO<sub>3</sub>-N concentration increased significantly with the increasing level of N addition. When

**Table 2** Pearson correlation between the selected soil biochemical parameters in a salinized reed wetland of the Yellow River Delta.

	pH	Salt	NH <sub>4</sub> -N	NO <sub>3</sub> -N	TN	TC	C/N	TOC	AP	Inv	Ure	Alp
pH	1											
Salt	0.088 (0.745)	1										
NH <sub>4</sub> -N	-0.322 (0.224)	-0.455 (0.077)	1									
NO <sub>3</sub> -N	-0.107 (0.694)	-0.691** (0.003)	0.669** (0.005)	1								
TN	-0.345 (0.191)	-0.450 (0.081)	0.429 (0.098)	0.609* (0.012)	1							
TC	-0.150 (0.578)	-0.602* (0.014)	0.466 (0.069)	0.749** (0.001)	0.854** (0.000)	1						
C/N	0.424 (0.102)	0.178 (0.509)	-0.296 (0.266)	-0.357 (0.175)	-0.891** (0.000)	-0.544* (0.029)	1					
TOC	0.189 (0.483)	0.029 (0.915)	0.016 (0.953)	-0.014 (0.959)	0.107 (0.692)	-0.113 (0.678)	-0.172 (0.525)	1				
AP	-0.769** (0.000)	-0.068 (0.802)	0.252 (0.347)	0.250 (0.350)	0.489 (0.055)	0.308 (0.245)	-0.571* (0.021)	-0.393 (0.132)	1			
Inv	-0.070 (0.797)	-0.334 (0.206)	0.320 (0.227)	0.445 (0.084)	0.674** (0.004)	0.720** (0.002)	-0.499* (0.049)	-0.126 (0.642)	0.217 (0.420)	1		
Ure	0.028 (0.918)	-0.557* (0.025)	0.548* (0.028)	0.595* (0.015)	0.679** (0.004)	0.776** (0.004)	-0.472 (0.065)	-0.276 (0.301)	0.227 (0.398)	0.719** (0.002)	1	
Alp	-0.025 (0.928)	-0.688** (0.003)	0.431 (0.096)	0.637* (0.008)	0.665** (0.005)	0.795** (0.000)	-0.400 (0.125)	-0.255 (0.340)	0.322 (0.223)	0.559* (0.024)	0.846** (0.000)	1

Note: n = 4. \*P < 0.05; \*\*P < 0.01. The correlation coefficients are in the brackets.

compared with CK, N2 and N3 had significantly higher NO<sub>3</sub>-N concentrations, with an average increase of 110.40% and 185.74%, respectively. However, N addition did not significantly change soil TN. Correlation analysis showed that the concentration of NO<sub>3</sub>-N was positively correlated to NH<sub>4</sub>-N (r = 0.669, P < 0.01) and TN (r = 0.609, P < 0.05) concentrations in soil (Table 2).

A small but statistically significant change in soil total carbon (TC) was observed after five years of N addition. However, the soil organic carbon (TOC), C/N ratio and AP were not significantly altered by N addition. The TC was positively correlated to NO<sub>3</sub>-N (r = 0.749, P = 0.001) and TN (r = 0.854, P < 0.001) concentrations in soil. The AP was negatively correlated to pH (r = -0.769, P < 0.001).

Soil pH was not significantly affected by N addition, but the soil salt content decreased significantly as the N addition level increased. Correlation analysis showed that the salt content was negatively correlated to NO<sub>3</sub>-N concentration (r = -0.691, P < 0.01) and TC content (r = -0.602, P < 0.05).

### 3.2. Effects of N addition on soil enzymatic activities

Fig. 2 shows the effect of N addition on soil Ure, Alp and Inv activities. Compared with CK, N2 and N3 significantly increased the Ure activity by an average of 54.00% and 54.66%, respectively (P < 0.05). No significant difference was observed between CK and N1. Similar trends were also found for Alp, with an average increase of 54.05% and 49.01% in N2 and N3, respectively (P < 0.05). There were no significant differences in soil Inv between CK and N addition treatments.

The activity of soil Ure was significantly correlated with soil NH<sub>4</sub>-N (r = 0.548, P = 0.028), NO<sub>3</sub>-N (r = 0.595, P = 0.015), TN (r = 0.679, P = 0.004) and TC (r = 0.776, P = 0.000), respectively; it was negatively correlated to soil salt content (r = -0.557, P = 0.025). Similarly, the activity of soil Alp was negatively correlated to soil salt content (r = -0.688, P = 0.003), but positively correlated with NO<sub>3</sub>-N (r = 0.637, P = 0.008), TN (r = 0.665, P = 0.005) and TC (r = 0.795, P = 0.000), respectively. Strong positive correlations were also found between the activities of soil Ure and Alp (r = 0.846, P = 0.000), Ure and Inv (r = 0.719, P = 0.002), and Alp and Inv (r = 0.559, P = 0.024), respectively.

### 3.3. Effects of N addition on plant height, density and productivity

After five years of N addition, plant height and productivity were enhanced significantly. The N addition increased plant height by an average of 23.36% (N1), 35.62% (N2) and 70.32% (N3), respectively, but only the highest N addition (i.e., N3) showed a significant increase when compared with CK (P < 0.05). No significant differences were observed in plant density. The plant productivity, measured as above-ground biomass, increased significantly with the increasing level of N addition, being 60% (N2) and 77% (N3) higher when compared to CK (Fig. 2).

## 4. Discussion

In the present study, soil TN content did not change after five years of N addition. However, the soil N availability (NH<sub>4</sub>-N and NO<sub>3</sub>-N), which highly depends on the amount of N addition, increased significantly with the increasing level of N addition. Our previous field investigation has revealed that the soil N content in the coastal wetlands of the Yellow River Delta is considerably lower compared to other coastal wetlands, such as those in the Yangtze Estuary (southern China) and the Mississippi River Delta (Yu et al., 2016). Therefore it is highly sensitive to N enrichment, especially for soil available N (NH<sub>4</sub>-N and NO<sub>3</sub>-N) that can be utilized directly by plant in N deficiency environments (Song et al., 2013; Zhang et al., 2015). Several studies have demonstrated that N addition tends to accelerate litter decomposition rate, and thus may cause substantial losses of soil C in wetlands (Melillo

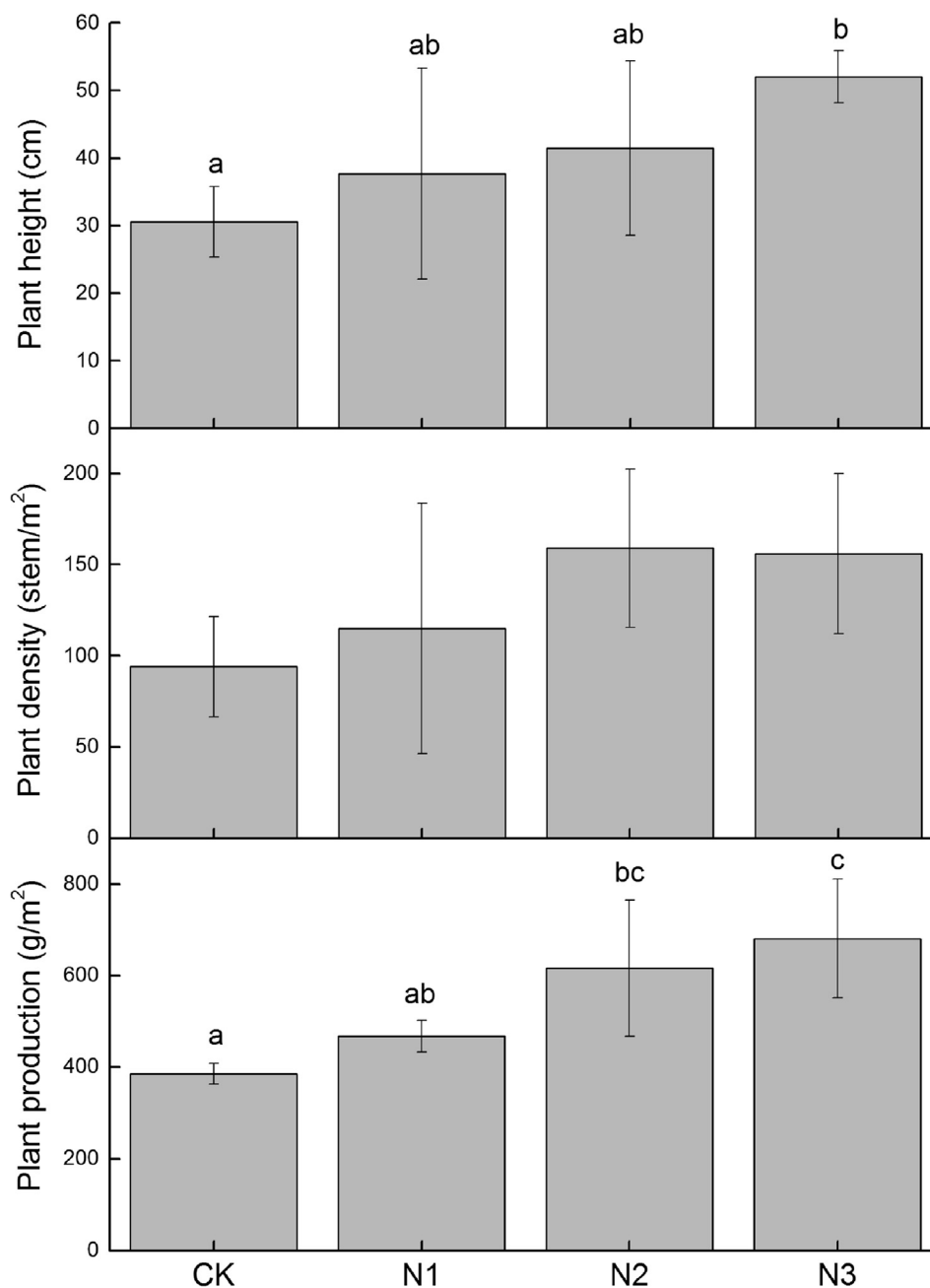


Fig. 2. Effects of nitrogen addition on the plant height, density and aboveground biomass of *P. australis* in a salinized reed wetland in the Yellow River Delta. (In each column, the data markers identified with the same letters are not significantly different ( $P > 0.05$ ) between treatments according to a least significant difference test. Bars without letters mean no significant differences. CK: control plots without N addition; N1: plots with  $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  addition amount; N2: plots with  $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  addition amount; N3: plots with  $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  addition amount.)

et al., 1982; Song et al., 2011). Our result, however, was not in line with the previous studies. The soil TC content in our study site was significantly increased by N addition, which is supported by the results of Liu et al. (2016). Based on a meta-analysis, the authors reported that N addition could increase soil lignin content in various ecosystems, and the lignin content of decomposed herbaceous litter increased significantly under N addition. This might be attributed to the increased plant growth with N addition and the increased contribution of litter decomposition to soil C pool.

Study of enzyme activities can provide information about ongoing biochemical processes in soil. There is growing evidence that soil enzyme activities associated with nutrient acquisition show a considerable sensitivity to increasing N concentration (Guan et al., 2013; López-Aizpún et al., 2018). Different environmental conditions can present

different responses. Studies have shown that N addition may have positive, neutral or negative effects on the activities of soil enzymes in forests, grasslands or freshwater marshes (Keeler et al., 2009; Jing et al., 2017; Song et al., 2017). As predicted, urease activity increased with increasing N addition in this study. Similar results were also reported by Guan et al. (2011) and Wang et al. (2013). However, other studies have shown that the activity of N-acquiring enzyme (urease) decreased with N addition (Song et al., 2017). The reason could be that the abundance of microorganisms who secrete enzymes in soils varies in different ecosystems/conditions and may respond differently to N addition. The increase of urease activity in our study may have been attributed to the alleviation of N limitation in soil microorganisms. Nevertheless, it is worth noting that for a N limited ecosystem, the positive effects of N deposition on soil enzyme activities may become

negative when the level of soil N exceeds a critical threshold (Johnson et al., 1998; Wang et al., 2013). Phosphatase is also an important enzyme, which is involved in the hydrolysis of organic phosphorus into different forms of inorganic phosphorus, which are assimilable by plants (Amador et al., 1997). Many previous studies have proved that N addition can stimulate the activity of phosphatase (Kizilkaya and Bayraklı, 2005; Marklein and Houlton, 2012). The results of our study was in agreement, suggesting that in N-limited ecosystems, the addition of N stimulates microbial activity and increases the demand of P, leading to increases in P acquiring enzymes. Invertase activity plays a critical role in releasing low molecular weight sugars that are important energy sources of microorganisms. Some researchers observed increased invertase activity under N addition (Song et al., 2013), but others found no changes in soil invertase activity when N was added (Yang et al., 2008). Soil invertase activity has also been reported to correlate with C:N ratio in soil or litter (Wang et al., 2013). Our study found a negative correlation between soil C:N ratio and invertase activity, and N addition did not change invertase activity. The results suggest that factors other than N addition, such as C:N ratio, soil organic matter, and salt content, might influence the activity of this enzyme.

Nitrogen addition not only affects the activity of certain soil enzymes through changing soil microbial biomass (Cusack et al., 2011) or soil environment (such as soil pH, soil C, N and P concentration) (Sinsabaugh et al., 2008; Song et al., 2017), but also affects plant growth (Bai et al., 2010). The aboveground biomass in our study increased in response to N addition in N2 and N3 treatments, implying that as a N limited wetland ecosystem, its soil quality has improved after five years of N addition. Furthermore, with the increasing of plant height and aboveground biomass, the plant coverage increased simultaneously, thereby reducing the rate of soil evaporation (Shaygan et al., 2018). It is clear that soil evaporation generally correlates positively with soil salt content in saline ecosystems (Wang et al., 2016; Shaygan et al., 2018). Therefore, N addition can indirectly decrease the soil salt content of soil surface layer via stimulating plant productivity.

## Acknowledgments

We are grateful for the support from the Strategic Priority Research Program of the Chinese Academy of Sciences, Grant No. XDA23050202, and the National Natural Science Foundation of China (41871091 and U1806218). We thank the Yellow River Delta Ecology Research Station of Coastal Wetland, CAS, with the help of field work. We also thank the two reviewers for their helpful and constructive reviews of this paper.

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