



## Research paper

Effects of invasion of *Spartina alterniflora* and exogenous N deposition on N<sub>2</sub>O emissions in a coastal salt marshYaohong Zhang<sup>a,b,c,\*</sup>, Lin Wang<sup>a,b</sup>, Xiaojin Xie<sup>a,b</sup>, Lidong Huang<sup>a,b</sup>, Yihua Wu<sup>b,d</sup><sup>a</sup> Jiangsu Key Laboratory of Agricultural Meteorology, Nanjing University of Information Science and Technology, Nanjing 210044, China<sup>b</sup> College of Applied Meteorology, Nanjing University of Information Science and Technology, Nanjing 210044, China<sup>c</sup> State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China<sup>d</sup> IMSG at NOAA/NCEP/EMC, 5830 University Research Court, College Park, MD 20740, USA

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## ABSTRACT

Vegetation alteration and nitrogen inputs (via run-off, atmospheric deposition, and wastewater) as a result of anthropogenic activities strongly affected the emission of N<sub>2</sub>O from coastal marshes. To gain insight into impacts of the invasion of *Spartina alterniflora* and N deposition on N<sub>2</sub>O fluxes, mesocosms experiments were conducted to measure N<sub>2</sub>O emissions from marshes vegetated with *S. alterniflora* and a native *Phragmites australis*, with or without exogenous N at the rates of 2.7 g N m<sup>-2</sup>, respectively. Mean N<sub>2</sub>O fluxes during the growing season in *S. alterniflora* mesocosms without N addition was 9.36 μg m<sup>-2</sup> h<sup>-1</sup>, significantly higher than 6.79 μg m<sup>-2</sup> h<sup>-1</sup> in *P. australis* mesocosms. The stimulatory effects could be attributed to higher plant biomass of *S. alterniflora* providing more labile organic C to the rhizosphere for nitrobacteria and denitrifying bacteria, and to more oxygen transported to the rhizosphere facilitating coupled nitrification and denitrification. N deposition increased N<sub>2</sub>O fluxes in *S. alterniflora* and *P. australis* mesocosms by 13.5% and 48.2%, respectively, suggesting that exogenous N significantly promoted N<sub>2</sub>O emissions from coastal marshes. Compared to mesocosms without N fertilization, the increase rate of total N accumulation and above-ground biomass under N addition were 63.1% and 28.0% in the *S. alterniflora* mesocosms, whereas 26.7% and 15.3% in the *P. australis* mesocosms, respectively. This meant stronger competition of *S. alterniflora* with soil microorganisms for the available N, leading to lower increment of N<sub>2</sub>O fluxes in the *S. alterniflora* mesocosms under N addition. Thus, it could be concluded that both the invasion of *S. alterniflora* and atmospheric N deposition dramatically stimulated N<sub>2</sub>O emissions from coastal marshes, and that N<sub>2</sub>O fluxes in the *S. alterniflora* marshes weakly responded to N addition.

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## 1. Introduction

Nitrous oxide (N<sub>2</sub>O) has been recognized as one of the most important trace gases that cause global warming and the destruction of stratospheric ozone (Hansen and Sato, 2004), having a global warming potential of 298 times on the 100-year time horizon compared to carbon dioxide (CO<sub>2</sub>) (Ravishankara et al., 2009). Due to human activities, the atmospheric N<sub>2</sub>O concentration is increasing at a rate of 0.2–0.3% per year (IPCC, 2001), having risen dramatically from pre-industrial value of approximately 270 ppbv to present 319 ppbv (IPCC, 2007). Therefore, more and more attention has been paid to the emission of N<sub>2</sub>O from terrestrial ecosystems.

The major processes leading to N<sub>2</sub>O evolution in soils are (1) aerobic autotrophic nitrification, the stepwise oxidation of ammonia (NH<sub>3</sub>) to nitrite (NO<sub>2</sub><sup>-</sup>) and to nitrate (NO<sub>3</sub><sup>-</sup>) (Kowalchuk and Stephen, 2001); (2) anaerobic heterotrophic denitrification, the stepwise reduction of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup>, nitric oxide (NO), N<sub>2</sub>O and ultimately molecular nitrogen (N<sub>2</sub>) (Knowles et al., 1982; Davidson, 1991). Additionally, in coastal sediments, there may be an important process of N<sub>2</sub>O production, dissimilatory nitrate reduction to ammonium (DNRA) (Baggs, 2008). All above-mentioned processes are carried out by bacteria in soils and sediments. The emission of N<sub>2</sub>O vary spatially (Allen et al., 2007; Wang et al., 2007) and can be affected by several variables including temperature (Davis et al., 2004), water table (Allen et al., 2007), and the chemical and physical characteristics of the soil (Cui et al., 2012). More importantly, it is widely recognized that vegetation type, and species composition profoundly affect N<sub>2</sub>O emissions in coastal marshes (Cheng et al., 2007). Wetland plants can regulate N<sub>2</sub>O production since the aerenchyma supply oxygen to the rhizosphere resulting in oxidized microzones surrounding roots and rhizomes (Rolletschek

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et al., 1998; Sasikala et al., 2009). The formation of the oxidized and anoxic zones in the rhizosphere provided a favorable microenvironment for coupled nitrification and denitrification (Mathieu et al., 2006), which converted  $\text{NH}_4^+$  or  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$ . Meanwhile, emission of  $\text{N}_2\text{O}$  transported by vascular plants such as rice (*Oryza sativa* L.) has been reported (Reddy et al., 1989; Mosier et al., 1990). On the other hand, plants could not only provide root exudates and debris for nitrifiers and denitrifiers in the rhizosphere facilitating  $\text{N}_2\text{O}$  production, but also compete for limited N with microorganisms in pristine coastal ecosystems inhibiting  $\text{N}_2\text{O}$  production (Yang et al., 2012). Therefore, the change in vegetation type as a result of plant invasions may potentially affect  $\text{N}_2\text{O}$  gas transport and production in wetlands ecosystems.

Exogenous nitrogen, from fertilizer-enriched run-off, groundwater, and atmospheric deposition, may have a particularly strong effect on  $\text{N}_2\text{O}$  emissions from wetland ecosystems. Many researchers have found that N addition remarkably stimulated  $\text{N}_2\text{O}$  emission from wetlands since it could be directly metabolized as a source of substrate by nitrifiers and denitrifiers in the soil or sediment (Zhang et al., 2007; Moseman-Valtierra et al., 2011). For example, Liu and Greaver (2009) reported that  $\text{N}_2\text{O}$  emissions were found to increase by 216% in several terrestrial ecosystems compared to un-enriched controls. However, soil respiration rates in coastal marshes have been found to significantly increase under nitrogen loading (Wigand et al., 2009), which could decrease redox potential in the soil and consequently facilitate complete denitrification, the microbial conversion of nitrate to predominantly  $\text{N}_2$  rather than  $\text{N}_2\text{O}$ . Moreover, DNRA, the microbial conversion of nitrate to ammonium, may out-compete denitrification, particularly at low  $\text{NO}_3^-$  concentrations (Ma and Aelion, 2005) and at high sulfide concentrations (An and Gardner, 2002) that characterize coastal ecosystems exposed to terrestrial N loads. In addition, lower redox potential in coastal marshes may favor DNRA process (Yin et al., 2002). A better understanding of how the change in vegetation structure affected  $\text{N}_2\text{O}$  fluxes from coastal ecosystems, particularly in response to N loading, could inform strategies for the sustainable management of coastal lands.

Since 1979, *Spartina alterniflora*, an invasive halophyte, has been intentionally introduced into the coastal marshes of China to stabilize sediments (Qin and Zhong, 1992). Presently, it has rapidly expanded and replaced native Chinese species such as *Phragmites australis* and *Suaeda salsa*, becoming one of the dominant plants in the coastal marsh ecosystem (An et al., 2007). The invasive species, which is characterized by the  $\text{C}_4$  photosynthetic pathway, has much higher rates of net primary productivity than native plants, leading to increased C and N storage in biomass (Qin and Zhong, 1992). On the other hand, atmospheric N deposition in China has been enhancing due to the increase in the use of fertilizers and fossil fuel, with the mean rate of  $12.89 \text{ kg N ha}^{-1} \text{ year}^{-1}$  (Lu and Tian, 2007), much higher than the  $5 \text{ kg N ha}^{-1} \text{ year}^{-1}$  in the world. The nitrogen load from the atmosphere increases the nitrogen availability in wetlands, which could increase the productivity of vegetation and availability of vegetation-derived organic substrates, and consequently affect carbon and nitrogen cycling in the wetland ecosystems (Zhang et al., 2007). Tyler et al. (2007) reported that N loading to coastal marshes enhanced the vulnerability of intertidal habitats, promoting the spread of the *S. alterniflora*. Apparently, N loading, together with rapid spread of the *S. alterniflora*, might interactively affect  $\text{N}_2\text{O}$  fluxes. However, there is little information on the effects of the invasion of *S. alterniflora* and exogenous N on  $\text{N}_2\text{O}$  emissions from coastal marshes.

In this study, we designed mesocosm experiments to examine how the *S. alterniflora* invasion affected  $\text{N}_2\text{O}$  emissions in the tidal marshes. Specifically, the objectives of this study are to: (1) investigate whether and how the invasion of *S. alterniflora* affected

**Table 1**  
Soil properties of the mesocosms.

Soil properties	<i>S. alterniflora</i> mesocosm	<i>P. australis</i> mesocosm
Soil organic C ( $\text{g C kg}^{-1}$ )	4.67 ± 1.12a	3.48 ± 1.23b
Soil N ( $\text{g N kg}^{-1}$ )	0.378 ± 0.108a	0.312 ± 0.089b
Soil water-soluble sulfur ( $\text{mg S kg}^{-1}$ )	25.42 ± 2.31a	12.18 ± 1.86b
Bulk density ( $\text{g cm}^{-3}$ )	1.39 ± 0.09b	1.43 ± 0.10a
C/N ratio	12.9 ± 0.6a	11.3 ± 0.3b

Means with the standard errors ( $n = 6$ ). Different letters within the same row indicate significant differences at  $P < 0.05$ .

$\text{N}_2\text{O}$  emissions; (2) evaluate the effect of exogenous N input on  $\text{N}_2\text{O}$  emissions from coastal marshes; (3) explore the relationships between  $\text{N}_2\text{O}$  emissions and plant characteristics.

## 2. Materials and methods

### 2.1. Experimental setup

The outdoor mesocosm experiment was conducted at the Station of Agricultural Meteorology ( $32^\circ 14' \text{ N}$ ,  $118^\circ 42' \text{ E}$ ), Nanjing University of Information Science and Technology, Nanjing, China. The circular mesocosm with a 40-cm height and 25-cm inner diameter was constructed using PVC (Zhang et al., 2010a). At the upper end of the mesocosm was a water trough for sealing the flange of the gas sampling chamber for measurement of  $\text{N}_2\text{O}$  emission. In May 2010, soil and plants were collected from the coastal salt marsh in Wanggang, Dafeng city, Jiangsu province, China ( $33^\circ 12' \text{ N}$ ,  $120^\circ 47' \text{ E}$ ). The intact soil columns (30 cm depth and 25 cm diameter) taken by a stainless steel cylindrical sampler were transferred into the mesocosms with as little disturbance as possible. Plants of approximately equal size with five true leaves were selected for this study. A single young ramet of either *S. alterniflora* or *P. australis* was planted in each mesocosm. All mesocosms were immediately transported to the experimental site in Nanjing, and then buried in the field to a depth of 30 cm in a randomized block design. A 5-cm depth of saltwater in each mesocosm was maintained over the growing season by adding saltwater sampled from Wanggang estuary. During the experimental period, water salinity was maintained at approximately 5‰. Soil properties are shown in Table 1. A rain-diversion system was constructed over the experiment site and was opened on sunny days and closed on rainy days.

The experiment included four treatments: (1) *S. alterniflora* fertilized without N (S); (2) *S. alterniflora* fertilized with  $2.7 \text{ g N m}^{-2}$  (S-N); (3) *P. australis* fertilized without N (P); (4) *P. australis* fertilized with  $2.7 \text{ g N m}^{-2}$  (P-N). Each treatment was replicated three times. The rate of N application in this study was based on the mean yearly amount of N deposition measured in Jiangsu province (Wang et al., 2004). N fertilizer as  $\text{NH}_4\text{NO}_3$  was evenly divided into 12 applications from May to October and applied biweekly during the growing season.

### 2.2. $\text{N}_2\text{O}$ flux measurement

The  $\text{N}_2\text{O}$  flux in the experimental mesocosms was measured using a static chamber-gas chromatograph method. A transparent, open-bottom cylindrical chamber (100 cm height and 27 cm diameter) was constructed using Plexiglass, on the roof of which one internal battery-operated fan was installed to mix air within the chamber. While collecting gas samples, the flange of the chamber was inserted into the water trough at the upper end of the mesocosm. Four samples of the chamber air were manually extracted into 50 ml syringes at 0, 10, 20 and 30 min after closure, and then injected into pre-evacuated vials for analysis. The air temperature

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