

RESEARCH ARTICLE

# Co-Regulations of *Spartina alterniflora* Invasion and Exogenous Nitrogen Loading on Soil N<sub>2</sub>O Efflux in Subtropical Mangrove Mesocosms

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## Abstract

Both plant invasion and nitrogen (N) enrichment should have significant impact on mangrove ecosystems in coastal regions around the world. However, how N<sub>2</sub>O efflux in mangrove wetlands responds to these environmental changes has not been well studied. Here, we conducted a mesocosm experiment with native mangrove species *Kandelia obovata*, invasive salt marsh species *Spartina alterniflora*, and their mixture in a simulated tide rotation system with or without nitrogen addition. In the treatments without N addition, the N<sub>2</sub>O effluxes were relatively low and there were no significant variations among the three vegetation types. A pulse loading of exogenous ammonium nitrogen increased N<sub>2</sub>O effluxes from soils but the stimulatory effect gradually diminished over time, suggesting that frequent measurements are necessary to accurately understand the behavior of N-induced response of N<sub>2</sub>O emissions. With the N addition, the N<sub>2</sub>O effluxes from the invasive *S. alterniflora* were lower than that from native *K. obovata* mesocosms. This result may be attributed to higher growth of *S. alterniflora* consuming most of the available nitrogen in soils, and thus inhibiting N<sub>2</sub>O production. We concluded that N loading significantly increased N<sub>2</sub>O effluxes, while the invasion of *S. alterniflora* reduced N<sub>2</sub>O effluxes response to N loading in this simulated mangrove ecosystem. Thus, both plant invasion and excessive N loading can co-regulate soil N<sub>2</sub>O emissions from mangrove wetlands, which should be considered when projecting future N<sub>2</sub>O effluxes from this type of coastal wetland.

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## Introduction

Although nitrous oxide (N<sub>2</sub>O) contributes only 5% to estimated global warming potentials [1], its global warming strength is 265 times more powerful than CO<sub>2</sub> over a 100-year time frame [2]. N<sub>2</sub>O has also contributed to destruction of ozone in the stratosphere [3]. During the past few decades, the global mean atmospheric concentration of N<sub>2</sub>O increased from 270 ppb in pre-industrial times to 324 ppb in 2011 [2]. The rapid increase in atmospheric N<sub>2</sub>O concentrations has gained much attention in quantifying N<sub>2</sub>O effluxes from various sources.

Coastal wetlands, such as mangroves in tropical and subtropical regions have been recognized as a major marine source of atmospheric N<sub>2</sub>O [4, 5]. In general, N<sub>2</sub>O effluxes from wetlands are related to several biological processes, mainly including nitrification (ammonium oxidation and nitrifier denitrification) [6], denitrification and nitrate-ammonification [7]. These processes can be affected by many abiotic and biotic factors, such as soil temperature, oxygen level, and substrate availability (bioavailable carbon, ammonium and nitrate) in the soil [8, 9]. In addition, it is widely recognized that vegetation type can significantly affect N<sub>2</sub>O effluxes [10]. Plants living in wetland environments can supply oxygen to the rhizosphere via aerenchymous tissue of plants, which creates oxidized microzones surrounding roots and rhizomes and favors nitrification and nitrification-denitrification reactions, facilitating N<sub>2</sub>O production [11–15]. Meanwhile, root exudates and debris can be used as an organic carbon source for microbes. The high activity of microbes could increase soil N mineralization [16] and promote N<sub>2</sub>O production. Therefore, the change in vegetation type, such as plant invasion, may have a potential effect on N<sub>2</sub>O effluxes.

*Spartina alterniflora*, a C<sub>4</sub> salt marsh grass native to the east coast of the USA, was introduced to China in 1979. It has grown vigorously in China and spread over coastal wetlands since that time. During the last two decades, it has aggressively invaded habitats of native mangroves on the southern coast of China, especially in mangrove seedlings established areas [17–19], influencing the native species composition of mangrove ecosystems. As a result, it would alter soil available inorganic nitrogen (N) content and microbial activity [20, 21], which would further change N<sub>2</sub>O production and effluxes. So, it is essential to understand the change of N<sub>2</sub>O effluxes from mangrove wetlands following *S. alterniflora* invasion.

Globally, many mangrove ecosystems are also under significant threat from N pollution because of human activities, such as sewage discharge and surrounding aquaculture operation [22]. Exogenous N loading has greatly changed functions of many mangrove wetlands in tropical and subtropical regions, from pristinely oligotrophic [23] to eutrophic [24]. Usually, pristine mangrove soils, in N-limited environments, show low or negative N<sub>2</sub>O effluxes [25, 26]. Exogenous N loading often stimulate N<sub>2</sub>O effluxes by directly stimulating nitrification and denitrification in the soil or sediment [27, 28]. For example, the Futian mangrove swamp in China, an area with intense human activities, had the highest N<sub>2</sub>O effluxes compared to three other mangrove swamps with slightly anthropogenic disturbance [27]. Similarly, the effluxes of N<sub>2</sub>O from the Puerto Rico mangrove swamp in the northeastern Caribbean Sea, where anthropogenic activities increase nitrogen loading to mangrove sediments, was much higher than those reported previously for intertidal estuarine sediments [29].

In the coastal wetlands, N loading from surrounding aquaculture ponds are always periodic. For example, during the common process of complete pond sediment cleaning, large quantities of sediment with high nutrient levels are washed out and discharged into the adjacent mangrove swamps in a very short period of time [30]. N<sub>2</sub>O effluxes may vary greatly over time because of dynamic changes of soil inorganic N concentration after such exogenous N loading. In order to accurately estimate N loading effect on N<sub>2</sub>O effluxes, attention should be paid to dynamic changes of N<sub>2</sub>O effluxes after a pulse of exogenous N loading. However, to our

knowledge, there has been limited research focusing on the dynamic change of N<sub>2</sub>O effluxes after an N-pulse loading in a wetland (but see [31]).

Although the previous research has pointed out that N<sub>2</sub>O effluxes in mangroves have positive response to excessive N loading, the response ability would be different between vegetation types [32]. After N loading, the divergent growth rate between various species meant dissimilar uptake capabilities of ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) and nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N), which are the important substrates participating in nitrification and denitrification processes. The difference in the competition between microorganisms and plants for N may lead to a differential in soil N<sub>2</sub>O production [33, 34]. Thus, to gain an insight into the relationship between excessive N loading and N<sub>2</sub>O effluxes from mangrove wetlands after *S. alterniflora* invasion, the varying physiological responses of various plant species should also be considered.

In order to understand individual and possible interactive effects of *S. alterniflora* invasion and exogenous N loading on N<sub>2</sub>O effluxes from mangrove ecosystems, we conducted a mesocosm experiment with three vegetation types including the monocultures of *K. obovata* (a common native mangrove species in China) and *S. alterniflora* and their mixture and two N loading treatments (with or without N loading). The aims of the study were (1) to determine the dynamic change of N<sub>2</sub>O effluxes after a pulse N loading, (2) to test whether and how the invasion of *S. alterniflora* alters N<sub>2</sub>O effluxes, and (3) to investigate the interactive effect of plant invasion and N loading on mangrove N<sub>2</sub>O effluxes. We hypothesized that N loading will increase N<sub>2</sub>O effluxes from both mangrove and salt marsh mesocosms, but the invasive *S. alterniflora* mesocosms would have a lower increment of N<sub>2</sub>O effluxes after N loading due to larger N uptake compared with the native mangrove mesocosms.

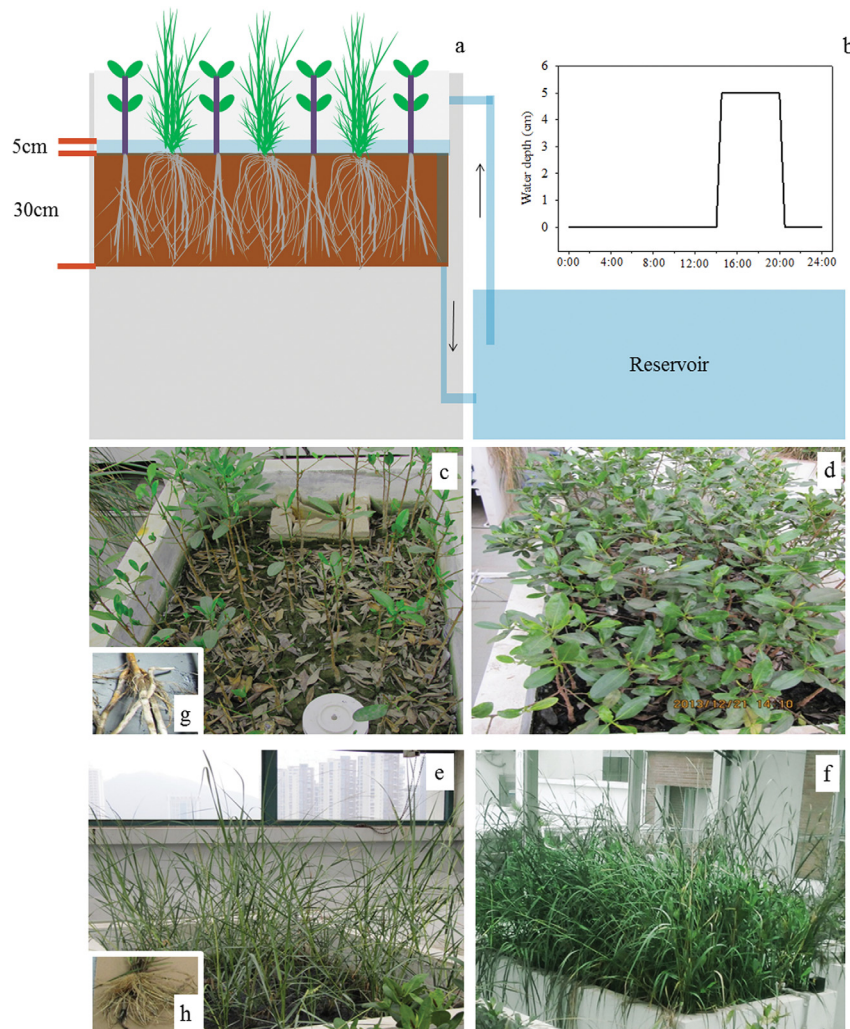
## Materials and Methods

### Experimental design

The tide-system mesocosm experiment was conducted in a roof greenhouse at the Graduate School at Shenzhen, Tsinghua University, China (22°59'N, 113°97'E). The mesocosm system consisted of 18 cement tanks (2.40 m × 1.10 m × 0.50 m in volume) as experimental mesocosms and 2 cement tanks (7.50 m × 1.00 m × 0.70 m in volume) as seawater reservoirs (Fig 1). One reservoir was connected to all the mesocosms without N addition treatment and the other was connected to all the mesocosms with N addition treatment by pipelines of water inputting (used during tide flooding periods) and water outputting (used in tide falling periods). The artificial seawater at a salinity of 14 g l<sup>-1</sup>, representing an average salinity in a typical mangrove environment of southern China, was prepared by dissolving natural sea salts in tap water. Submersible pumps and timers were installed to simulate diurnal tides for all the mesocosms. For the transplanted plants to establish and acclimate, each mesocosm was conditioned under the tidal regime with one tidal cycle a day, 12 h high tide and 12 h low tide for 2 months before starting the experiment on August 31, 2012. After the formal start of the system operation, artificial seawater was pumped from the reservoir to each mesocosm to a depth of 5 cm between 14:00 and 20:00 (local time) to simulate high tide, then seawater was drained back by gravitational force to the reservoir during the low tide period. Tidal flux took about 30 minutes from the initiation of the ebb or flood tide. Tidal water in each reservoir was rotated for about 15 days as a water cycle, then drained and replenished by freshly prepared artificial seawater. In this way, we mimicked field conditions because daily tidal dynamic change might greatly change nitrification and denitrification potential, allowing us to determine whether the effects were robust with respect to natural perturbations.

Each mesocosm was filled with fresh mangrove soils. On June 5, 2012, the mangrove soils were collected from the mudflat connected to the Futian Mangrove Natural Reserve (22°51'N,

113°96'E), a typical mangrove swamp along the southern coast of Shenzhen. We collected the soils after receiving the permission of the Futian Mangrove Nature Reserve in this research. The substrate was sandy, consisting of 73.7% sand, 14.8% silt and 11.6% clay. After stones, benthic animals and plant residues in the soil were carefully removed, the soils was then used to fill each mesocosm to a depth of 30 cm (Fig 1). In the present study, *K. obovata* was chosen because it was the most dominant species among the eight true mangrove species in South China [35]. The mature propagules of *K. obovata* and ramets of *S. alterniflora* were collected from the mangrove forest in Zhangjiangkou Mangrove Nature Reserve in Yunxiao, Fujian (23°56'N, 117°25'E). We collected the plants after receiving the permission of the Zhanjiang Mangrove National Nature Reserve in this research. The propagules of *K. obovata* were cultivated in the sand for 2 months before transplantation to the mesocosms. Then healthy *K. obovata* seedlings with approximately equal height of 15 cm (usually with 3–5 green leaves) were selected for this study. Meanwhile, the young ramets of *S. alterniflora* of approximately equal size with four true leaves were selected for this study. Each *K. obovata* seedling and ramet of



**Fig 1. Section view of the experimental mesocosms used in the experiment (a) and the changes of water depth in the experimental mesocosms with time (b). See Section 2.1 for details; the picture of mesocosms (c: *K. obovata* (KO) without N addition, d: KO with N addition, e: *S. alterniflora* (SA) without N addition, f: SA with N addition); the root of KO (g) and SA (h).**

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*S. alterniflora* covered an area of 0.15 m × 0.15 m and 0.25 m × 0.25 m, respectively. This mimics typical seedling densities of these two species according to our observations in the field.

In this experiment, three vegetation types represented three stages of *S. alterniflora* invasion to *K. obovata*: (i) monoculture of *K. obovata* (no invasion), (ii) mixture of *K. obovata* and *S. alterniflora* (partial invasion), and (iii) monoculture of *S. alterniflora* (complete invasion) (Fig 1). All vegetation types were tested in the absence and presence of nitrogen addition. Ammonium chloride (NH<sub>4</sub>Cl) of 450 mg was added to synthetic seawater of 3000 ml in the reservoir connected to mesocosms with N addition treatment one day before the start of water cycle (i.e. one day before tide rising of the first day) in order to make NH<sub>4</sub>Cl dissolve thoroughly. During a typical 15 day water cycle period, no more NH<sub>4</sub>Cl was added into the reservoir. Because the N of water may be lost by transformation by microbes and volatilization in form of ammonia gas in the reservoir during low tide periods, we measured the actual rates of N loading, which were shown in Fig 2. The N levels we applied are within the range of sewage and wastewater and sludge from aquaculture ponds [36, 37].

### Measurements of N<sub>2</sub>O effluxes

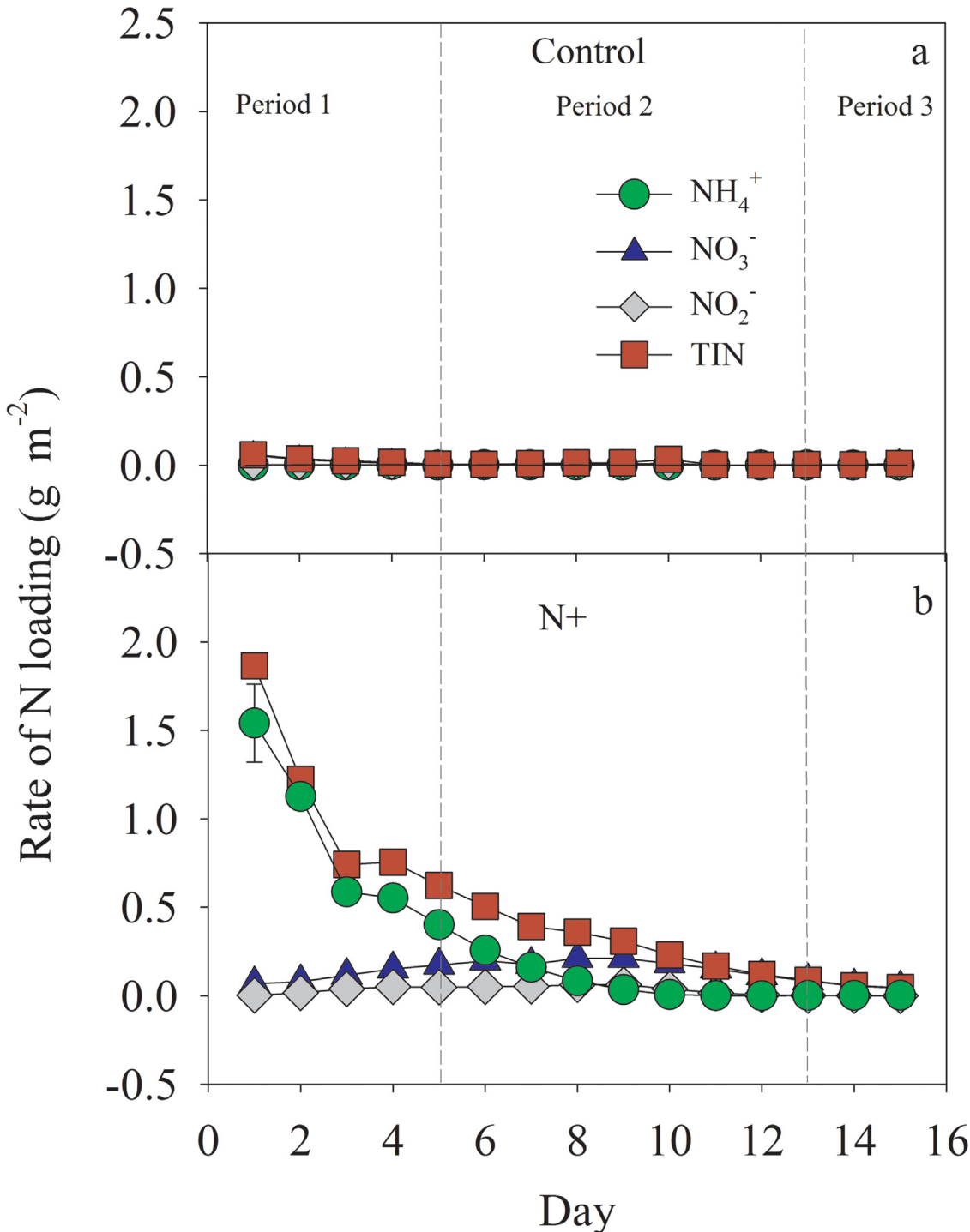
For the results reported in this paper, we selected a typical 15 day water cycle period. Artificial seawater pumping started at 14:00–20:00 on June 18, 2013 (Day 1) from a new pool of tidal water with desired salinities and N levels specified above. We started the collection of gas samples on the day after a new seawater change (i.e. on June 19, 2013) to investigate the effect of N loading on N<sub>2</sub>O effluxes during daytime of tide falling periods. During this water cycle period, the N<sub>2</sub>O effluxes in each mesocosm were quantified using a static chamber-gas chromatograph method on the day 2, 4, 6, 8, 10, 14 and 16. Each static chamber, which was made of PVC, covered a soil area of 0.025 m<sup>2</sup> and had an internal volume of about 4000 ml. The open end of the chamber was inserted 3–5 cm into the soil with the air-tight valve open. Gas sampling was carried out 30 minutes after the chamber was inserted. Generally, five 8 ml gas samples of chamber air were collected by passing a hypodermic needle attached to a 10 ml glass syringe and was injected into pre-evacuated vials for laboratory analysis. Meanwhile, to minimize any effect of diurnal variation in N<sub>2</sub>O effluxes, samplings were conducted at roughly the same time of the day for the same mesocosm between 9:00 and 11:00. The deployment time was set to 45–90 min with sampling at 15–30 min intervals depending on the N<sub>2</sub>O efflux rates.

The N<sub>2</sub>O concentration of gas samples collected was analyzed within 24 h after sampling using a gas chromatograph (GC, Agilent 6820, Agilent Technologies, USA) equipped with an electron capture detector (mECD) and a HP-Plot/column (J&W GC Columns, Agilent Technologies, USA). The temperatures of the injector, column and detector were 50°C, 50°C and 300°C, respectively, with a carrier gas (N<sub>2</sub>) flow rate of 15 ml min<sup>-1</sup>. The relative standard deviation (RSD) of replicate standard measurements was 0.8%. During gas measurements, standard samples were analyzed with every 10 samples of determination to ensure that each sample run maintained a RSD below 3% in 12 h. N<sub>2</sub>O concentrations were quantified by comparing the peak areas of samples against the standard curves. N<sub>2</sub>O efflux rates were calculated from the linear change of the measured gas concentrations in the chamber headspace with known headspace volume and sampling time. Estimates of cumulative N<sub>2</sub>O emissions for each mesocosm were based on linear interpolation, with the sum of cumulative experiment period emissions representing one water cycle.

### Sampling and analyses of water and soil

Everyday input water samples from each reservoir were collected before the start of tide flooding and output water samples from each mesocosm were collected before the start of tide falling during the experiment water cycle period. The water samples were transported to the

laboratory and analyzed immediately. The samples were filtered through a 0.45 μm filter and analyzed for NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, nitrite nitrogen (NO<sub>2</sub><sup>-</sup>-N). We used standard procedures and methods for the determination, including the Sodium salicylate-sodium hypochlorite Method for NH<sub>4</sub><sup>+</sup>-N, the UV Spectrophotometer Method for NO<sub>3</sub><sup>-</sup>-N and the α-Naphthylamine



**Fig 2. The dynamics of rates of ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N), nitrite nitrogen (NO<sub>2</sub><sup>-</sup>-N) and total inorganic nitrogen (TIN-N) loading with (b) and without (Control: a) N addition during the whole experiment period.**

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Method for NO<sub>2</sub><sup>-</sup>-N. Total inorganic nitrogen (TIN-N) was calculated by summing NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N [38]. Rates of N loading were calculated by multiplying N concentration of input water by the area and the flooding depth (5cm) of each mesocosm.

Three soil cores (0–15 cm) were collected around the chamber using hand-held PVC corers from each mesocosm at day 4 after gas sampling. Soil total carbon and nitrogen contents were measured by the Elementar (Vario ELIII, German). The NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N contents in the KCl (2 M) extracts were also determined by the methods above. Both soil nitrification potential activity (PNA) and denitrification potential activity (PDA) were measured according to the method of Chen et al. (2012) [39].

## Measurements of plant biomass

*K. obovata* biomass, measured on July 5, 2013, was evaluated by a non-destructive allometric technique [40]. The relationships between biomass (dry weight) of each plant part, namely leaf, stem and plumular axis, and their leaf number (LN), and stem height (SH), propagule height (PH) and basal diameter (D) were obtained by harvesting 12 individuals of *K. obovata* from each mesocosm randomly. The best-fit equations for estimating biomass of leaves (LB), stems (SB) and propagules (PB) were  $LB = 0.32 LN - 0.89$  ( $r^2 = 0.89$ ),  $SB = 0.23 \times SH \times D \times D + 0.40$  ( $r^2 = 0.80$ ), and  $PB = 0.52 \times PH \times D \times D + 2.53$  ( $r^2 = 0.85$ ), respectively. The total aboveground biomass was calculated by summing LB, SB and PB. The best-fit equations for estimating the total belowground biomass was  $BGB = 0.31 \times AGB + 1.16$  ( $r^2 = 0.85$ ).

*S. alterniflora* biomass was measured also on July 5, 2013 but was evaluated by a non-destructive height–weight method [41]. The relationships between aboveground biomass (B) of plant and their height (H) were obtained by harvesting 200 individuals of *S. alterniflora* from each mesocosm randomly. The best-fit equations for estimating aboveground biomass of was  $\ln B = 1.44 \ln H - 5.46$  ( $r^2 = 0.85$ ). The best-fit equations for estimating BGB of were  $\ln BGB = 0.61 \times AGB + 34.88$  ( $r^2 = 0.86$ ).

Plant samples were analyzed for N concentration with a Elementar elemental analyzer (Vario ELIII, German) and the total N accumulation was calculated by multiplying the N concentration by dry weight.

## Statistical analyses

The main and interactive effects of sampling time, vegetation type and nitrogen addition treatment on N<sub>2</sub>O effluxes were tested with a parametric three-way analysis of variance (ANOVA). A one-way analysis of variance (ANOVA) performed to examine the significant differences of N<sub>2</sub>O effluxes between vegetation types for each period. One-way ANOVA was also used to analyze the differences in parameters of soil properties and plant traits between the treatments, followed by the least significant difference (Duncan) test at  $P < 0.05$ . Linear regression analyses were performed to evaluate the relationships of NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N and TIN-N loading rates and N<sub>2</sub>O effluxes. The student's t-test was used to examine the significant difference of slopes and intercepts of linear regression lines of TIN-N and N<sub>2</sub>O effluxes, and NH<sub>4</sub><sup>+</sup>-N and N<sub>2</sub>O effluxes, NO<sub>3</sub><sup>-</sup>-N and N<sub>2</sub>O effluxes and NO<sub>2</sub><sup>-</sup>-N and N<sub>2</sub>O effluxes. All statistical analyses were performed using SPSS 19.0 for Windows (SPSS Inc. USA).

## Results

### Rate of N loading

In the treatment without N addition, NO<sub>3</sub><sup>-</sup>-N was the dominant dissolved N form of the input water and contributed 70.08% of TIN-N load. The rate of NO<sub>3</sub><sup>-</sup>-N loading declined gradually with

time. With N addition, after the refreshment of tidal water, the rate of NH<sub>4</sub><sup>+</sup>-N loading in the mesocosms decreased with time from 1.54 to 0.002 g m<sup>-2</sup>, However, the rate of NO<sub>3</sub><sup>-</sup>-N loading in the mesocosms increased to a peak by the day 8 (0.21 mg m<sup>-2</sup>), and then decreased gradually to 0.04 mg m<sup>-2</sup>. A similar variation pattern was observed for nitrite (NO<sub>2</sub><sup>-</sup>-N), with the highest rate (0.06 mg m<sup>-2</sup>) also on day 8. The rate of TIN-N loading generally decreased with time (Fig 2).

### Soil properties and plant traits

In treatments without N addition, concentrations of soil total carbon, nitrogen, NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were not significantly affected by plant community composition, except that the NH<sub>4</sub><sup>+</sup>-N concentration in mixture mesocosms was significantly lower than that in *S. alterniflora* mesocosms and *K. obovata* mesocosms. In treatments with N addition, NH<sub>4</sub><sup>+</sup>-N concentrations differed among the three plant communities, ranging from 2.25 mg kg<sup>-1</sup> in the *S. alterniflora* mesocosms, and 3.02 mg kg<sup>-1</sup> in the mixture mesocosms to 3.27 mg kg<sup>-1</sup> in the *K. obovata* mesocosms. PNA also varied with plant species. N loading increased the soil total carbon, NH<sub>4</sub><sup>+</sup>-N and PNA significantly. However, soil total N, NO<sub>3</sub><sup>-</sup>-N and PDA were not significantly different between the N addition and without N addition mesocosms (Table 1).

Without N addition, no significant differences in aboveground biomass were found among vegetation types, while the belowground biomass in *S. alterniflora* mesocosms was 1.02 kg m<sup>-2</sup> significantly higher than that in *K. obovata* mesocosms. N addition increased total biomass in the *S. alterniflora*, *K. obovata* and their mixture mesocosms by 55.26%, 176.76% and 148.78%, respectively. Under N addition condition, above- and below-ground biomass of *S. alterniflora* were evidently higher than that of *K. obovata* mesocosms. However, the ratio of aboveground to below-ground biomass was 3.21 in *K. obovata* mesocosms, significantly higher than 0.99 in *S. alterniflora* mesocosms. Therefore, *S. alterniflora* was more productive compared to *K. obovata* (Table 1).

### N<sub>2</sub>O effluxes

Without N addition, the N<sub>2</sub>O effluxes from *K. obovata*, the mixture and the *S. alterniflora* mesocosms ranged from 0.27 to 0.93, from 0.52 to 1.11 and from 0.32 to 0.70 μmol m<sup>-2</sup> h<sup>-1</sup> in

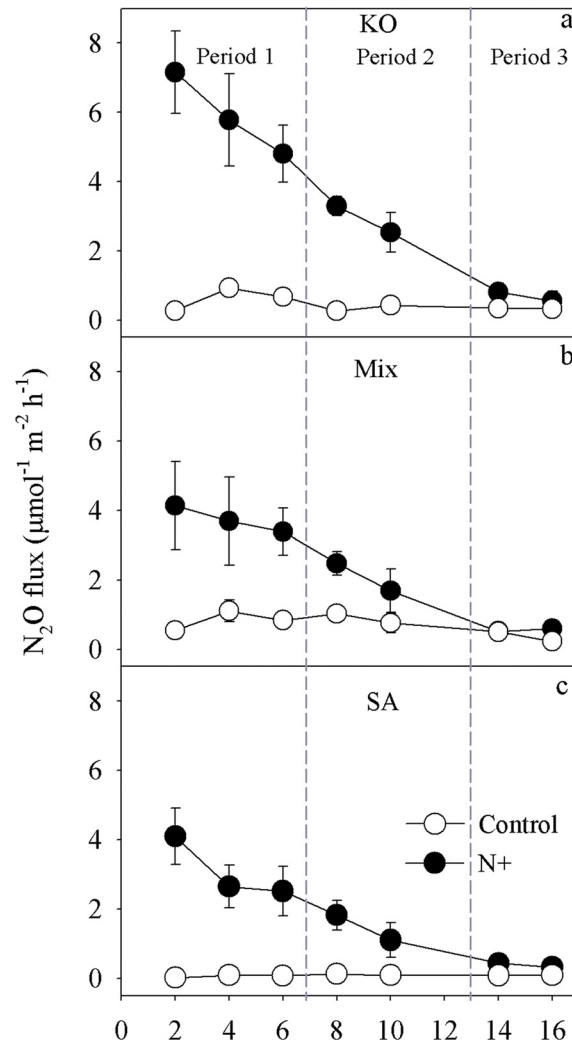
**Table 1. Soil and plant properties for the *K. obovata* (KO), *S. alterniflora* (SA) and their mixture mesocosms with and without (Control) exogenous N addition.**

	Control			N addition		
	KO	Mix	SA	KO	Mix	SA
Soil total carbon (g C kg <sup>-1</sup> )	8.58±1.18ab	7.15±0.47a	7.51±0.34a	8.77±0.77b	8.20±0.21b	8.81±1.11b
Soil total nitrogen(g N kg <sup>-1</sup> )	0.33±0.01a	0.30±0.03a	0.32±0.02a	0.30±0.02a	0.34±0.02a	0.32±0.02a
NH <sub>4</sub> <sup>+</sup> -N(mg N kg <sup>-1</sup> )	1.83±0.62bc	0.55±0.03a	1.06±0.15b	3.27±0.29d	3.02±0.97cd	2.25±0.36c
NO <sub>3</sub> <sup>-</sup> -N (mg N kg <sup>-1</sup> )	1.72±0.27a	1.72±0.16a	1.8±0.28a	2.45±0.42a	1.77±0.30a	2.38±0.44a
PNA (mg NO <sub>2</sub> <sup>-</sup> -N g <sup>-1</sup> DW d <sup>-1</sup> )	0.34±0.01a	0.49±0.01b	0.39±0.01a	1.89±0.02d	1.61±0.08d	0.83±0.04c
PDA (μmol N <sub>2</sub> O-N g <sup>-1</sup> DW d <sup>-1</sup> )	0.11±0.01a	0.19±0.04a	0.16±0.02a	0.11±0.01a	0.13±0.01a	0.14±0.01a
Water content (%)	0.52±0.02a	0.47±0.03a	0.52±0.10a	0.51±0.06a	0.46±0.03a	0.47±0.05a
Initial AGB (kg m <sup>-2</sup> )	0.22±0.003c	0.14±0.007b	0.09±0.005a	0.22±0.003c	0.15±0.006b	0.08±0.007a
AGB (kg m <sup>-2</sup> )	0.58±0.06a	0.65±0.04a	0.62±0.09a	0.90±0.13b	1.92±0.19c	2.03±0.19c
Initial BGB (kg m <sup>-2</sup> )	0.07±0.001b	0.09±0.003a	0.09±0.006a	0.07±0.001c	0.1±0.007a	0.08±0.006a
BGB (kg m <sup>-2</sup> )	0.18±0.02a	0.77±0.11c	1.02±0.18c	0.28±0.04b	2.01±0.82d	2.05±0.07d
Total N accumulation of plant (g m <sup>-2</sup> )	6.29±0.85a	14.94±0.48b	21.91±3.41c	11.69±2.24b	45.8±5.69d	53.65±8.28d

Different letters within the same row indicate significant difference at *P* < 0.05. AGB: aboveground biomass; BGB: belowground biomass; Initial AGB and BGB: AGB and BGB measured at the 1st water cycle in September, 2012; PNA: nitrification potential activity; PDA: denitrification potential activity.

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**Fig 3. Variation of N<sub>2</sub>O effluxes in different vegetation types (a: KO, b: mixture and c: SA) with and without N addition (Control) during the entire experiment period.** See Fig 1 for abbreviations.

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the, respectively (Fig 3). N addition significantly increased mean N<sub>2</sub>O effluxes (Table 2). With N addition, the N<sub>2</sub>O effluxes ranged from 0.56 to 7.17, from 0.23 to 4.14 and from 0.19 to 4.10 µmol m<sup>-2</sup> h<sup>-1</sup> in the *K. obovata*, the mixture and the *S. alterniflora* mesocosms, respectively (Fig 3). N<sub>2</sub>O effluxes for all three vegetation types generally decreased with sampling time. In addition, there was a significant interaction of plant species × N fertilization on N<sub>2</sub>O effluxes (Table 2).

We further analyzed N<sub>2</sub>O effluxes over the 15-day period by dividing the sampling time into three periods according to the N loading rate and main species of N in the input water with N enrichment as shown in Fig 3. NH<sub>4</sub><sup>+</sup>-N was the major species of N in the first period during day 1 to 6 with high N loading (TIN-N loading rates ranging from 0.51 to 1.87 g m<sup>-2</sup>); NO<sub>3</sub><sup>-</sup>-N was the major species of N in the second period during day 7 to day 13 with modest N loading (TIN-N loading rates ranging from 0.12 to 0.39 g m<sup>-2</sup>) and in the last period total N loading rates have decreased to background level during day 14 to day 16 (TIN-N loading rates ranging from 0.047 to 0.057 g m<sup>-2</sup>) (Fig 2). Without N addition, the N<sub>2</sub>O effluxes from *K. obovata*, the mixture and the *S. alterniflora* mesocosms did not vary significantly between

**Table 2. Results of three-way ANOVA on the effects of vegetation type (V), sampling time (individual day, D), N addition treatment (N) and their interactions on N<sub>2</sub>O effluxes during the entire experimental period, and repeated measures ANOVA on the effects of V, N and their interactions on N<sub>2</sub>O effluxes for the three periods.**

		df	P
	V	2	<0.001
	N	1	<0.001
	D	6	<0.001
Whole experiment period	V × N	2	<0.001
	V × D	12	0.393
	N × D	6	<0.001
	V × N × D	12	0.841
Period 1 (Day 1–6)	V	2	0.003
	N	1	<0.001
	V × N	2	0.003
Period 2 (Day 7–13)	V	2	0.019
	N	1	<0.001
	V × N	2	0.01
Period 3 (Day 14–16)	V	2	0.227
	N	1	0.709
	V × N	2	0.27

df: degree of freedom

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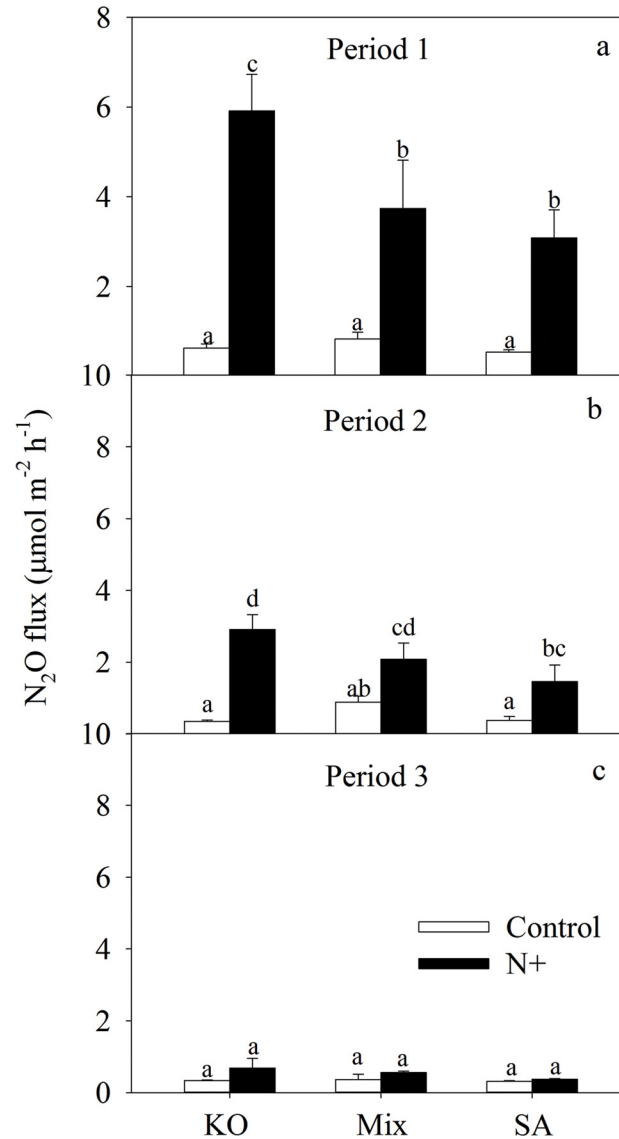
vegetation types during all three periods, while there were trends that N<sub>2</sub>O effluxes were higher from mixture mesocosms than that from *K. obovata* and *S. alterniflora* mesocosms during all three periods. With N addition, compared to that from *K. obovata* mesocosms, the mean N<sub>2</sub>O effluxes were significantly lower from the *S. alterniflora* mesocosms in period 1 and 2 (Fig 4). However, in period 3, there were no significant difference among the vegetation types.

### Relationship between N<sub>2</sub>O effluxes and N loading

When integrating all data either with or without N addition for analysis in each vegetation type respectively, N<sub>2</sub>O effluxes were found to significantly correlate to the rate of TIN-N, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N loading ( $P < 0.05$ ), except for the relationship between N<sub>2</sub>O effluxes and NO<sub>3</sub><sup>-</sup>-N & NO<sub>2</sub><sup>-</sup>-N loading rate in the *S. alterniflora* mesocosms. The slopes of the linear relationship between rate of TIN-N loading and N<sub>2</sub>O effluxes for *S. alterniflora* and mixture mesocosms were significantly lower than that for *K. obovata* mesocosms by student's t-test ( $P < 0.05$ ) (Fig 5). Thus, the increase rate of N<sub>2</sub>O effluxes by N addition was much lower in the *S. alterniflora* and mixture than native *K. obovata* mesocosms.

### Discussion

Our results from this mesocosm experimental study showed that a pulse loading of exogenous ammonium nitrogen resulted in a stimulatory effect on N<sub>2</sub>O effluxes but the effect gradually diminished over time and that the invasion of *S. alterniflora* could mitigate N<sub>2</sub>O effluxes following N loading. In addition, there was a significant antagonism effect between plant invasion and exogenous N loading on mangrove N<sub>2</sub>O effluxes. Our findings offer new insights into how plant invasion and N loading modulate N<sub>2</sub>O effluxes from mangrove wetlands.



**Fig 4.** Mean rates of N<sub>2</sub>O effluxes from mesocosms with different vegetation types (a: KO, b: mix and c: SA) with and without N addition (Control) for the three periods. See Fig 1 for abbreviations.

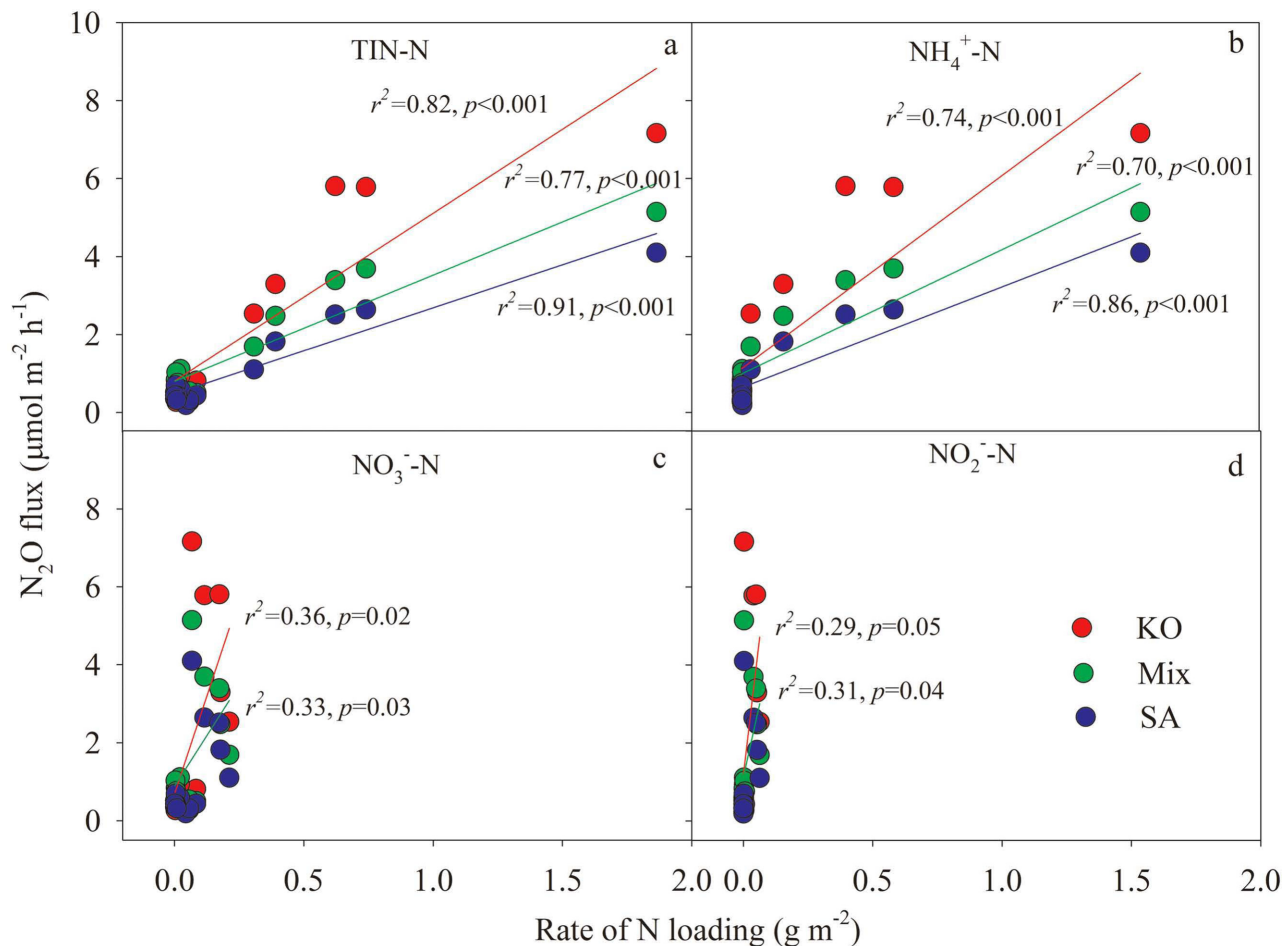
doi:10.1371/journal.pone.0146199.g004

### Pulse effect of N addition on N<sub>2</sub>O effluxes

Compared to the treatment with N addition, we found that the N<sub>2</sub>O effluxes were relatively low (<1.50 µmol m<sup>-2</sup> h<sup>-1</sup>) in the treatment without N addition in the *K. obovata*, *S. alterniflora* and their mixture mesocosms, which was consistent with the conclusions from the previous studies that natural coastal wetlands were weak sources for atmospheric N<sub>2</sub>O, such as undisturbed mangrove and salt marshes ecosystems [26, 27]. However, we did not observe any N<sub>2</sub>O sink in either pure *S. alterniflora* stand or the mixture of *S. alterniflora* and native mangrove seedlings, which was different from the results of Yuan, et al. (2014) [26]. A likely explanation for this difference is that in our study soil nitrate levels never reached the very low level (< 1 mg N kg<sup>-1</sup>) [42].

N<sub>2</sub>O effluxes were significantly increased by the N loading in both mangrove and *Spartina* mesocosms, which was also found in salt marshes [34]. Although N<sub>2</sub>O emissions accounted for

a very low proportion of total N loading, ranging from 0.23–0.47% for the mesocosms with N addition treatment and from 2.22 to 3.55% for those without N loading (S1 Table), we could not neglect N loading effect on N<sub>2</sub>O emission, considering 265 times higher potential warming strength than CO<sub>2</sub>. Previous research also found relative lower proportions (ranging from 0.05–0.48% at N loading rate of 1.4 g m<sup>-2</sup> in field experiment of salt marsh coastal wetland [31]. The stimulating effect of N loading on N<sub>2</sub>O effluxes results might be attributed to the fact that enzyme activities and the microbial population sizes including nitrifiers and denitrifiers in surface sediment had a dramatic increase after N addition [43, 44] and N loading supply the source of substrate for nitrification and denitrification in the soil or sediment. However, for the exogenous N loading, N<sub>2</sub>O effluxes may be underestimated or overestimated if they were not measured continuously after the N loading, as shown in this study that the stimulatory effect of N loading on N<sub>2</sub>O effluxes decreased gradually over time. A contributing factor to this result is that N<sub>2</sub>O fluxes were significantly related to N loading rates and these N loading rates usually decreased gradually after a pulse of nitrogen loading due partly to assimilation by wetland plants, transformation by microbes, and adsorption to sediments. So, N addition significantly increased the mean N<sub>2</sub>O effluxes in the first (Day 0–5) and second period (Day 6–10) in *S. alterniflora* mesocosms, *K. obovata* mesocosms and their mixture, but had no effect in the third period (Day 11–15). This result suggests that discretely or infrequently measuring is inadequate to accurately predict the behavior of exotic N-induced response of N<sub>2</sub>O effluxes.



**Fig 5. Relationships between N<sub>2</sub>O effluxes and rate of TIN-N (a), NH<sub>4</sub><sup>+</sup>-N (b), NO<sub>3</sub><sup>-</sup>-N (c) and NO<sub>2</sub><sup>-</sup>-N (d) loading for different vegetation types (n = 14).**

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Meanwhile, with N addition, we found the mean N<sub>2</sub>O effluxes in the first period were higher than in the second period. We inferred that, except for the higher N loading rates in the first period, the higher N<sub>2</sub>O effluxes may also be attributed to varying responses of N<sub>2</sub>O effluxes to different N species. Previous study pointed out that N<sub>2</sub>O effluxes were larger when NH<sub>4</sub><sup>+</sup>-N was the dominant substrate and nitrification was the main source of nitrous oxide compared to the condition when nitrate and nitrite predominate [29]. In our study, major nitrogen species of input water were transformed from ammonium in the first period to nitrate in the second period with time, which could partly explain the reason for the different rates of N<sub>2</sub>O effluxes between the two periods.

Therefore, in order to get more accurate results especially in coastal wetlands which are frequently affected by tide, we should pay more attention to sampling time, daily N loading rates and N species of input water after N pulse addition. In addition, we added ammonium into seawater as the initial nitrogen species to assess the anthropogenic effects on N<sub>2</sub>O effluxes from coastal wetland, as it constitutes a major form of human inputs such as sewage and wastewater and sludge from aquaculture ponds. Future studies should also investigate the effects of other N species such as NO<sub>3</sub><sup>-</sup>-N on N<sub>2</sub>O effluxes.

### Effects of plant invasion on N<sub>2</sub>O effluxes

Without N addition, there were no significant difference of N<sub>2</sub>O effluxes between the invasive *S. alterniflora* and native *K. obovata* mesocosms and more productive *S. alterniflora* did not result in higher N<sub>2</sub>O effluxes. This finding implied that the invasion of *S. alterniflora* played a limited role in mediating N<sub>2</sub>O effluxes from mangrove in N-limited condition. Previous research work have shown that fast-growing species such as *S. alterniflora*, a salt marsh species, increased N mineralization of soil to a greater extent than more conservative species such as *K. obovata*, a mangrove forest species [16, 45]. However, it has also been found that the rate of net N uptake was four- to six-fold higher for faster growing species than slower growing plant species (e.g., Poorter et al. (1991) [46]). Considering the high growth and high root uptake capacity of *S. alterniflora* [47], the mineralized N in soil could be largely consumed by *S. alterniflora*, which would result in no buildup of the soil mineral N pool. Therefore, without N addition, no difference in N<sub>2</sub>O effluxes between *S. alterniflora* and *K. obovata* mesocosms could be attributed to the similar concentration of soil N pool.

Meanwhile, we found significant antagonism effects of plant invasion and nitrogen loading on soil N<sub>2</sub>O effluxes. With N addition, the mean N<sub>2</sub>O effluxes from the invasive *S. alterniflora* were lower than that from native *K. obovata* mesocosms. In addition the increase rates of N<sub>2</sub>O effluxes by N addition were also lower in the *S. alterniflora* than native *K. obovata* mesocosms, indicating the invasion of *S. alterniflora* reduced N<sub>2</sub>O effluxes response to N loading in this simulated mangrove ecosystem. This result may be attributed to a crucial effect of vegetation type on the quality of N<sub>2</sub>O effluxes. On one hand, as important substrates participating in the processes of nitrification and denitrification by microbes [48], NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N would be absorbed by vegetation, which may inhibit N<sub>2</sub>O production due to the competition between the plant and microorganism for soil inorganic N. In our study, compared to mangrove mesocosms, higher biomass and higher uptake of N in *S. alterniflora* mesocosms were found, which might mean higher photosynthetic capacity, leading to stronger competition ability for N source and lower soil inorganic N content for N<sub>2</sub>O production by microbes. On the other hand, in wetland, plants with aerenchyma can supply oxygen to the rhizosphere [12, 13], which can form oxidized zone in the rhizosphere that stimulates nitrification and nitrification-denitrification processes [15, 49], therefore facilitating N<sub>2</sub>O effluxes. Previous researches have shown that stronger oxidation activity in the rhizosphere of mangrove species like *K. obovata*



than salt marsh species such as *S. alterniflora* [50, 51]. Indeed, we found significant higher PNA in *K. obovata* mesocosms compared to *S. alterniflora* mesocosms. Therefore, we inferred that the higher N<sub>2</sub>O effluxes in the *K. obovata* mesocosms than in *S. alterniflora* mesocosms could be partly attributed to the stronger oxidation in the rhizosphere of mangrove species.

The mixture mesocosms of *S. alterniflora* and *K. obovata*, reflecting partly invasion of *S. alterniflora*, had higher plant richness compared to the monoculture *K. obovata* or *S. alterniflora*. With N addition, both partly and complete invasion (in monoculture *S. alterniflora* and mixture mesocosms) reduced N<sub>2</sub>O effluxes from mangrove soil in the first period. However, only complete invasion of *S. alterniflora* reduced N<sub>2</sub>O effluxes from mangrove soil in the second period. Moreover, both complete and partly invasion did not reduce N<sub>2</sub>O effluxes from mangrove soil with N addition in the third period and without N addition for all three periods. In conclusion, *S. alterniflora* invasion could reduce N<sub>2</sub>O effluxes regardless of invasion degree under relatively higher nitrogen availability, but no relief effect of *S. alterniflora* invasion on N<sub>2</sub>O effluxes occurred under N-limiting condition. The results indicated that N addition can improve the effect of partly *S. alterniflora* invasion on N<sub>2</sub>O effluxes in mangrove, perhaps because N greatly increased *S. alterniflora* growth and competition with mangrove seedlings [52], leading to exhibition of *S. alterniflora* characteristic in mixture mesocosms with N addition.

Although substrate condition and results of our controlled mesocosm experiment were similar to field experiment [27], cautions must be exercised when extrapolating results from controlled mesocosm studies to field-scale processes.

## Conclusions

N<sub>2</sub>O effluxes were significantly increased by the N loading in the *K. obovata*, the *S. alterniflora* and their mixture mesocosms, but the stimulatory effect of N loading on the N<sub>2</sub>O effluxes decreased gradually with time and disappeared when the N loading rates returned to the background level. The lower N<sub>2</sub>O effluxes and a weaker response to N addition in the *S. alterniflora* mesocosms than the *K. obovata* mesocosms were partly due to significantly higher growth of *S. alterniflora*, which could have led to lower increment of available N in the sediments for N<sub>2</sub>O production; and also due to higher oxidation capacity in the rhizosphere of *K. obovata*, which stimulated nitrification and nitrification-denitrification processes. Thus, *S. alterniflora* invasion into mangrove habitats could reduce N<sub>2</sub>O effluxes in case of amount of exogenous N loading, such as the input of sewage and wastewater and sludge from aquaculture ponds. These findings should be considered when projecting future N<sub>2</sub>O effluxes from global mangrove wetlands under the influences of both biological invasion and increasing exogenous N loading.

## Supporting Information

**S1 Table. Mass balance of nitrogen (g m<sup>-2</sup>) in the mangrove and *Spartina* mesocosms under different N loading during the experiment period from June 5, 2013 to July 5, 2013.**

(DOCX)

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## Author Contributions

Conceived and designed the experiments: DJ YL GL. Performed the experiments: DJ FQ JF HW JG WL RP. Analyzed the data: DJ XX YL GL. Contributed reagents/materials/analysis tools: DJ FQ. Wrote the paper: DJ XX XZ YL GL.

## References

1. Mosier A, Kroeze C, Nevison C, Oenema O, Seitzinger S, van Cleemput O. Closing the global N<sub>2</sub>O budget: nitrous oxide emissions through the agricultural nitrogen cycle—OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. *Nutr Cycl Agroecosys*. 1998; 52(2–3): 225–248.
2. IPCC. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA.: Cambridge University Press; 2013.
3. Crutzen PJ, Ehhalt DH. Effects of nitrogen fertilizers and combustion on the stratospheric ozone layer. *Ambio*. 1977; 6(2–3): 112–117.
4. Bange HW, Rapsomanikis S, Andreae MO. Nitrous oxide in coastal waters. *Global Biogeochem Cycles*. 1996; 10(1): 197–207.
5. Nevison CD, Weiss RF, Erickson DJ. Global Oceanic Emissions of Nitrous-Oxide. *J Geophys Res-Oceans*. 1995; 100(C8): 15809–15820.
6. Kowalchuk GA, Stephen JR. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. *Annu Rev Microbiol*. 2001; 55: 485–529. PMID: [11544365](#)
7. Knowles R. Denitrification. *Microbiol Rev*. 1982; 46(1): 43–70. PMID: [7045624](#)
8. Wrage N, Velthof GL, Van Beusichem ML, Oenema O. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol Biochem*. 2001; 33(12): 1723–1732.
9. Weier KL, Doran JW, Power JF, Walters DT. Denitrification and the dinitrogen nitrous-oxide ratio as affected by soil-water, available carbon, and nitrate. *Soil Sci Soc Am J*. 1993; 57(1): 66–72.
10. Sun H, Zhang C, Song C, Chang SX, Gu B, Chen Z, et al. The effects of plant diversity on nitrous oxide emissions in hydroponic microcosms. *Atmos Environ*. 2013; 77: 544–547.
11. Edwards KR, Čížková H, Zemanová K, Šantrůčková H. Plant growth and microbial processes in a constructed wetland planted with *Phalaris arundinacea*. *Ecol Eng*. 2006; 27(2): 153–165.
12. Rolletschek H, Bumiller A, Henze R, KOHL JG. Implications of missing efflux sites on convective ventilation and amino acid metabolism in *Phragmites australis*. *New Phytol*. 1998; 140(2): 211–217.
13. Sasikala S, Tanaka N, Wah Wah H, Jinadasa K. Effects of water level fluctuation on radial oxygen loss, root porosity, and nitrogen removal in subsurface vertical flow wetland mesocosms. *Ecol Eng*. 2009; 35(3): 410–417.
14. Inamori R, Gui P, Dass P, Matsumura M, Xu KQ, Kondo T, et al. Investigating CH<sub>4</sub> and N<sub>2</sub>O emissions from eco-engineering wastewater treatment processes using constructed wetland microcosms. *Process Biochem*. 2007; 42(3): 363–373.
15. Inamori R, Wang Y, Yamamoto T, Zhang J, Kong H, Xu K, et al. Seasonal effect on N<sub>2</sub>O formation in nitrification in constructed wetlands. *Chemosphere*. 2008; 73(7): 1071–1077. doi: [10.1016/j.chemosphere.2008.07.064](#) PMID: [18782640](#)
16. Van der Krift TAJ, Berendse F. The effect of plant species on soil nitrogen mineralization. *J Ecol*. 2001; 89(4): 555–561.
17. An SQ, Gu BH, Zhou CF, Wang ZS, Deng ZF, Zhi YB, et al. *Spartina* invasion in China: implications for invasive species management and future research. *Weed Res*. 2007; 47(3): 183–191.
18. Chen L, Wang W, Zhang Y, Lin G. Recent progresses in mangrove conservation, restoration and research in China. *J Plant Ecol*. 2009; 2(2): 45–54.
19. Zhang Y, Huang G, Wang W, Chen L, Lin G. Interactions between mangroves and exotic *Spartina* in an anthropogenically disturbed estuary in southern China. *Ecology*. 2012; 93(3): 588–597. PMID: [22624213](#)
20. Hall SJ, Asner GP. Biological invasion alters regional nitrogen-oxide emissions from tropical rainforests. *Glob Change Biol*. 2007; 13(10): 2143–2160.
21. Nagata O, Takakai F, Hatano R. Effect of *Sasa* invasion on global warming potential in *Sphagnum* dominated poor fen in Bibai. *Phyton-Ann Rei Bot A*. 2005; 45(4): 299–307.

22. Vaiphasa C, De Boer WF, Skidmore AK, Panitchart S, Vaiphasa T, Bamrongruga N, et al. Impact of solid shrimp pond waste materials on mangrove growth and mortality: a case study from Pak Phanang, Thailand. *Hydrobiologia*. 2007; 591(1): 47–57.
23. Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, et al. Human alteration of the global nitrogen cycle: sources and consequences. *Ecol Appl*. 1997; 7(3): 737–750.
24. Howarth RW, Sharpley A, Walker D. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries*. 2002; 25(4): 656–676.
25. Allen DE, Dalal RC, Rennenberg H, Meyer RL, Reeves S, Schmidt S. Spatial and temporal variation of nitrous oxide and methane flux between subtropical mangrove sediments and the atmosphere. *Soil Biol Biochem*. 2007; 39(2): 622–631.
26. Yuan J, Ding W, Liu D, Kang H, Freeman C, Xiang J, et al. Exotic *Spartina alterniflora* invasion alters ecosystem–atmosphere exchange of CH<sub>4</sub> and N<sub>2</sub>O and carbon sequestration in a coastal salt marsh in China. *Glob Change Biol*. 2014; 2015(21): 1567–1580.
27. Chen GC, Tam NFY, Ye Y. Summer fluxes of atmospheric greenhouse gases N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> from mangrove soil in South China. *Sci Tot Environ*. 2010; 408(13): 2761–2767.
28. Kreuzwieser J, Buchholz J, Rennenberg H. Emission of methane and nitrous oxide by Australian mangrove ecosystems. *Plant Biol*. 2003; 5(4): 423–431.
29. Corredor JE, Morell JM, Bauza J. Atmospheric nitrous oxide fluxes from mangrove sediments. *Mar Pollut Bull*. 1999; 38(6): 473–478.
30. Wu H, Peng RH, Yang Y, He L, Wang WQ, Zheng TL, et al. Mariculture pond influence on mangrove areas in south China: Significantly larger nitrogen and phosphorus loadings from sediment wash-out than from tidal water exchange. *Aquaculture*. 2014; 426: 204–212.
31. Moseman-Valtierra S, Gonzalez R, Kroeger KD, Tang J, Chao WC, Crusius J, et al. Short-term nitrogen additions can shift a coastal wetland from a sink to a source of N<sub>2</sub>O. *Atmos Environ*. 2011; 45(26): 4390–4397.
32. Abalos D, Deyn GB, Kuyper TW, Groenigen JW. Plant species identity surpasses species richness as a key driver of N<sub>2</sub>O emissions from grassland. *Glob Change Biol*. 2014; 20(1): 265–275.
33. Cheng X, Peng R, Chen J, Luo Y, Zhang Q, An S, et al. CH<sub>4</sub> and N<sub>2</sub>O emissions from *Spartina alterniflora* and *Phragmites australis* in experimental mesocosms. *Chemosphere*. 2007; 68(3): 420–427. PMID: [17316757](#)
34. Zhang YH, Wang L, Xie XJ, Huang LD, Wu YH. Effects of invasion of *Spartina alterniflora* and exogenous N deposition on N<sub>2</sub>O emissions in a coastal salt marsh. *Ecol Eng*. 2013; 58: 77–83.
35. Tam N, Wong YS. Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. *Environ Pollut*. 2000; 110(2): 195–205. PMID: [15092834](#)
36. Fontenot Q, Bonvillain C, Kilgen M, Boopathy R. Effects of temperature, salinity, and carbon: nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater. *Bioresour Technol*. 2007; 98(9): 1700–1703. PMID: [16935499](#)
37. Wu Y, Tam NFY, Wong MH. Effects of salinity on treatment of municipal wastewater by constructed mangrove wetland microcosms. *Mar Pollut Bull*. 2008; 57(6–12): 727–734. doi: [10.1016/j.marpolbul.2008.02.026](#) PMID: [18374366](#)
38. Jin X, Tu Q. Criterion to lake eutrophication survey. Beijing: China Environmental Science Press; 1990.
39. Chen GC, Tam NFY, Ye Y. Spatial and seasonal variations of atmospheric N<sub>2</sub>O and CO<sub>2</sub> fluxes from a subtropical mangrove swamp and their relationships with soil characteristics. *Soil Biol Biochem*. 2012; 48: 175–181.
40. Snedaker S, Snedaker J. *The Mangrove Ecosystem: Research Methods*. UK: United Nations Educational, Scientific and Cultural Organization Published, Richard Clay (The Chaucer Press) Ltd.; 1984.
41. Bonham CD, Ahmed J. *Measurements for terrestrial vegetation*. New York: John Wiley and Sons; 1989. pp. 338.
42. Ryden JC. Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. *J Soil Sci*. 1983; 34(2): 355–365.
43. Tam N, Wong A, Wong MH, Wong YS. Mass balance of nitrogen in constructed mangrove wetlands receiving ammonium-rich wastewater: Effects of tidal regime and carbon supply. *Ecol Eng*. 2009; 35(4): 453–462.
44. Tam N. Effects of wastewater discharge on microbial populations and enzyme activities in mangrove soils. *Environ Pollut*. 1998; 102(2): 233–242.
45. Personeni E, Loiseau P. Species strategy and N fluxes in grassland soil—A question of root litter quality or rhizosphere activity? *Eur J Agron*. 2005; 22(2): 217–229.

46. Poorter H, Vanderwerf A, Atkin OK, Lambers H. Respiratory energy-requirements of roots vary with the potential growth-rate of a plant-species. *Physiol Plantarum*. 1991; 83(3): 469–475.
47. Aerts R, Chapin FS III. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv Ecol Res*. 1999; 30: 1–67.
48. Tauchnitz N, Brumme R, Bernsdorf S, Meissner R. Nitrous oxide and methane fluxes of a pristine slope mire in the German National Park Harz Mountains. *Plant Soil*. 2008; 303(1–2): 131–138.
49. Gui P, Inamori R, Matsumura M, Inamori Y. Evaluation of constructed wetlands by wastewater purification ability and greenhouse gas emissions. *Water Sci Technol*. 2007; 56(3): 49–55. PMID: [17802837](#)
50. Ando T, Yoshida S, Nishiyama I. Nature of oxidizing power of rice roots. *Plant Soil*. 1983; 72(1): 57–71.
51. Chiu CY, Chou CH. Oxidation in the rhizosphere of mangrove *Kandelia candel* seedlings. *Soil Sci Plant Nutr*. 1993; 39(4): 725–731.
52. Mckee KL, Rooth JE. Where temperate meets tropical: multi-factorial effects of elevated CO<sub>2</sub>, nitrogen enrichment, and competition on a mangrove-salt marsh community. *Glob Change Biol*. 2008; 14(5): 971–984.