

## Effect of *Scirpus mariqueter* on nitrous oxide emissions from a subtropical monsoon estuarine wetland

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[1] The effects of the wetland plant *Scirpus mariqueter* on nitrous oxide (N<sub>2</sub>O) emissions in Yangtze estuary, China, were investigated using an in situ static chamber technique. Field measurements spanned the entire growing season (May to October) and encompassed a wide range of weather conditions typical of the subtropical monsoon climate of this region. Simultaneous measurement of carbon dioxide (CO<sub>2</sub>) and anatomical measurements were conducted to experimentally determine the gas transport mechanisms of *S. mariqueter* on N<sub>2</sub>O flux. *S. mariqueter* had a significant effect on N<sub>2</sub>O flux. Based on the comparison of light-dark and clipped-unclipped gas flux, N<sub>2</sub>O flux was negatively correlated with NEE ( $p < 0.0001$ ) and NPP ( $p < 0.001$ ) under light conditions when *S. mariqueter* was present but positively with temperatures in the dark condition or when *S. mariqueter* was clipped. Besides the plant uptake corresponding to the N<sub>2</sub>O negative flux in light chamber, it is reasonable to assume that because of the limitation of nitrate in sediment, coupled nitrification-denitrification is the main process of N<sub>2</sub>O producing. O<sub>2</sub> transported into the *S. mariqueter* rhizosphere during photosynthesis stimulated denitrifier also would consume the N<sub>2</sub>O and would be induced to the N<sub>2</sub>O diffusing from atmosphere into sediment. Although photosynthetic activity of *S. mariqueter* attenuated N<sub>2</sub>O flux significantly over the course of the entire study period, creating a net sink for atmospheric N<sub>2</sub>O under light condition, the marsh of Chongming Island Dongtan wetland was a net source of atmospheric N<sub>2</sub>O during the active *S. mariqueter* growth phase (averaged flux was 98.3 μg N<sub>2</sub>O m<sup>-2</sup> h<sup>-1</sup>).

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### 1. Introduction

[2] Wetlands receiving increased nitrogen loading are considered as net source of N<sub>2</sub>O [Moseman-Valtierra *et al.*, 2011]. Previous studies showed that uncertainties in the estimate of wetland greenhouse gas fluxes is partially owing to the spatial and temporal variability of measured rates within and across wetland types [e.g., Kammann *et al.*, 1998; Bergström *et al.*, 2007]. Many wetland plants develop an extensive system of internal gas spaces or lacunae to adapt to waterlogged conditions [Schuette *et al.*, 1994], by supporting

aerobic soil microbial processes and gas exchange [Jackson and Armstrong, 1999]. It has been confirmed by numerous studies that wetland plants can play an important role on CH<sub>4</sub> transport, oxidation and production by serving as a conduit in facilitating the CH<sub>4</sub> flux [Van der Nat *et al.*, 1998], releasing O<sub>2</sub> into rhizosphere through radial oxygen loss [Armstrong and Armstrong, 1990] and providing substrates for methanogenesis as labile carbon in root exudates [Joabsson *et al.*, 1999].

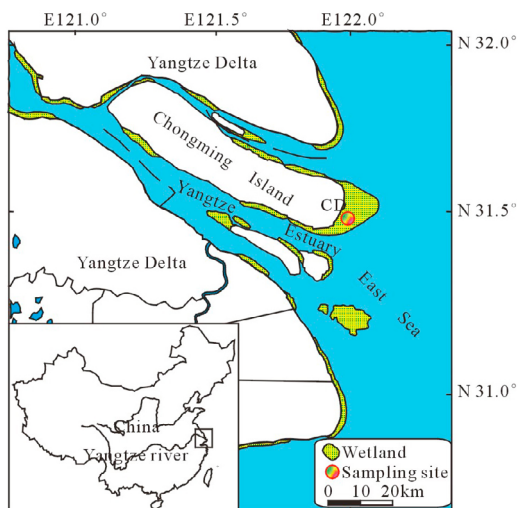
[3] Whereas numerous studies have been conducted to investigate the effects of plants on CH<sub>4</sub> emission from wetland ecosystems, similar studies of N<sub>2</sub>O emission have not been widely or systematically conducted [Chen *et al.*, 1997; Rusch and Rennenberg, 1998; Pihlatie *et al.*, 2005]. In wetland environments, the flux of N<sub>2</sub>O from soil/sediment to the atmosphere is the net result of N<sub>2</sub>O production, further reduction to N<sub>2</sub>, and transport interception [Chen *et al.*, 1997]. Some wetland plants, such as rice (*Oryza sativa* L.) [Mosier *et al.*, 1990], *Pontederia cordata* L., and *Juncus effusus* L. [Reddy *et al.*, 1989], functioned as conduits for N<sub>2</sub>O transport. The efflux of O<sub>2</sub> from plant roots may promote nitrification of NH<sub>4</sub><sup>+</sup>, with the NO<sub>3</sub><sup>-</sup> formed serving as substrate for denitrification [Bodelier *et al.*, 1996], and at

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\* CD: Chongming Dongtan wetland of Chongming island in Yangtze estuary.

**Figure 1.** Map of sampling site.

the same time more N<sub>2</sub>O would be produced in promoted nitrification [Wrage *et al.*, 2001]. Based on the treatments of elevated CO<sub>2</sub> concentration and nitrogen fertilization, Kettunen *et al.* [2005] reported the potential of *Phleum pratense* to increase the N<sub>2</sub>O production via easily decomposable root exudates [Kettunen *et al.*, 2005]. However, only a few long-term studies have been conducted on the dynamics of plant-dependent N<sub>2</sub>O flux under field conditions, many of the controlling factors have not been identified, including the relationship between N<sub>2</sub>O emission and plant productivity.

[4] *Scirpus mariqueter*, an endemic species in the subtropical monsoon estuarine and coastal zone of China, is a long-lived rhizomatous, corm-forming herb growing predominantly in intertidal marshes (mudflats) of the Yangtze estuary [Sun *et al.*, 2001]. The role of *S. mariqueter* in the regulation of N<sub>2</sub>O fluxes, which it is important for determining the function of wetlands as sources and sinks of N<sub>2</sub>O in this area, has not been determined. Our objective for the study was to investigate how *S. mariqueter* affects the variability and magnitude of N<sub>2</sub>O fluxes from the Yangtze estuarine wetland and to explore the relationship between the N<sub>2</sub>O fluxes and plant productivity (or the photosynthetic activity). This was accomplished through examination of *S. mariqueter* structural features combined with measurement of N<sub>2</sub>O fluxes in dark and light enclosures in clipped or unclipped vegetation plots. Our approach was designed to provide a comprehensive means for determining the mechanism of gas transport through *S. mariqueter* and the relationship between plant productivity and N<sub>2</sub>O fluxes.

## 2. Methods and Materials

### 2.1. Physical Setting of Study Area

[5] The Yangtze estuary is located in a typical subtropical monsoon area characterized by four distinct seasons (spring, summer, autumn and winter). Because of the abundant sediment supply from the Yangtze River [Chen and Zhong, 1998], the delta front continues to extend seaward rapidly.

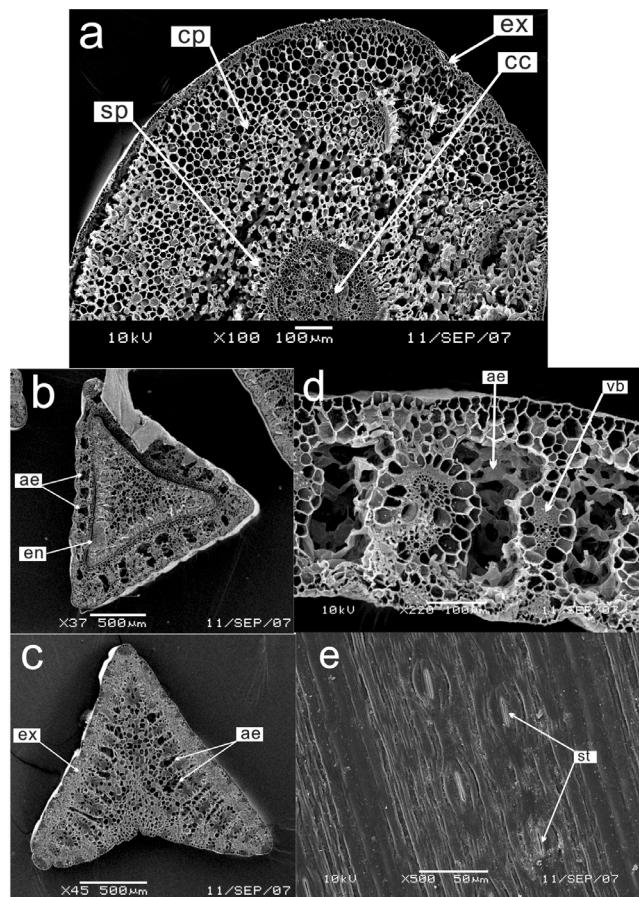
Dongtan wetland of Chongming Island (CD) is the largest and most completely developed wetland in the Yangtze estuary, which has about 100 km<sup>2</sup> of tidelands (as shown in Figure 1), composed of natural high, middle, and low tidal flats. *S. mariqueter* is the dominant native plant in middle tidal flat (marsh). Sampling location, the marsh, is not submerged during the neap tide and submerged for several hours during the spring tide [Wang *et al.*, 2009]. The growing season for *S. mariqueter* generally occurs from late April through early November with the most active growth occurring during the summer months. All the aboveground shoots die off at the end of the growing season, whereas underground parts (i.e., corm and rhizome) persist for several years [Sun *et al.*, 2001].

### 2.2. Experimental Design and Gas Fluxes

[6] Nonsteady state chamber composed by two parts, base and cover chamber was used to investigate N<sub>2</sub>O fluxes and the effects of *S. mariqueter*. The base is 5 cm height and 30 cm diameter and has a 3 cm height and 1.5 cm width U shape groove, is made from 1 mm stainless steel plate. Because of the tide cycling, if the base is previously installed in the marsh, there will be much particles settling down in the base. So we sharpened the blade of the end of base, making it could cut into the sediment with little disturbance. Two kind Cover chambers (50 cm net height × 30 cm ID), dark (opaque) and light (transparent), made from 0.4 mm thickness iron sheet and 3 mm thickness Perspex cylinder were adopted. Dark chamber was covered by an insulating layer and aluminum foil to insulate and reduce heat transmission and reflect light. Sampling port with three way valve, electric fan, thermometer, and pressure port were installed on every chamber. All the connections were made “air tight” and sealed using silicon rubber. During sampling, the bases were installed on the sediment surface about 15 to 1/2 h before sampling, and then chambers were fixed on the base and sealed by water in the U shape groove.

[7] Gas flux samples were collected under both light and dark chambers within the *S. mariqueter* dominated zone, monthly from May to October 2004. In May, samples were collected one time in the morning, and four times in June at the morning and afternoon. From June to September, we took eight time measurement of flux from dawn to dusk every 1 1/2 h. Immediately after installing each chamber and again after 30 min, a 180 ml gas sample was drawn using a syringe with a three-way airtight valve, which was injected into a gas sampling bag, (A plastic bag plated with Aluminum inside, which is inert to the air and has a screw vent port with septum. Air sample could be injected into bag by syringe.). The clipping procedure was conducted monthly from July to October at the study site. *S. mariqueter* plants within six 40 × 40 cm plots were carefully cut and removed, leaving about 1 cm stubble, without disturbing the surface sediment. The sampling procedure was detailed by Wang *et al.* [2009].

[8] Gaseous flux (F) was calculated as the concentration change of the gases in chamber during sampling time ( $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$  or  $\text{mg CO}_2 \text{ m}^{-2} \text{h}^{-1}$ ). By comparing N<sub>2</sub>O fluxes in chambers under light versus dark conditions, the variability in flux resulting from plant respiration and gross photosynthetic activity was assessed. Comparing N<sub>2</sub>O fluxes in chambers with and without the presence of



**Figure 2.** Transverse and longitudinal section of vegetative organs of *S. mariqueter* observed by scanning electron microscopy. (a) Corm, (b) stem, (c) leaf, (d) aerenchymas in stem, (e) and leaf surface showing stomatas arranged in row. Legend: ae, aerenchyma; cc, central cylinder; cp, cortex parenchyma; en, endodermis; ex, exodermis; sp, stellate parenchyma; vb, vascular bundle.

aboveground vegetation allowed us to weigh the contribution of *S. mariqueter* on gas transfer from the soil-plant system. Net ecosystem exchange (NEE) was equivalent to the inverse of CO<sub>2</sub> flux in light unclipped conditions. Positive values of NEE indicated net fixation of atmospheric CO<sub>2</sub> by *S. mariqueter* community. Net primary production (NPP) in light chambers, defined as the difference between gross primary production and autotrophic respiration [Lovett *et al.*, 2006], was calculated by subtracting the dark clipped CO<sub>2</sub> flux (respiration of sediment and microbial) from the light unclipped CO<sub>2</sub> flux (NEE). Gas samples were analyzed by gas chromatography (HP5890II) equipped with ECD [Wang *et al.*, 2009]. Analysis was performed within 3 days of sampling.

### 2.3. Vegetation and Environmental Parameters

[9] Vegetation samples were collected during each sampling events from seven 50 × 50 cm randomly placed quadrats at 3–5 m spacing. In each quadrat, the aboveground portion of *S. mariqueter* was cut, and the height and stem density of the vegetation was recorded. Biomass and

environmental parameters, including the Air temperature, ground temperature, photosynthetically active radiation, the density and height of *S. mariqueter* community, and organic carbon content and median grain size of sediment were measured and presented by Wang *et al.* [2009, Table 1].

### 2.4. Anatomical Studies

[10] For anatomical characterization of *S. mariqueter*, transverse and longitudinal sections of corms, stems, and leaves were dissected by hand using fresh material. The sample pieces were dehumidified in a mixture of 3% glutaraldehyde, 1.5% acrolein and 1.5% paraformaldehyde in phosphate buffer (pH 6.8), dehydrated in acetone and then dried in a critical point dryer. They were examined by scanning electron microscopy.

### 2.5. Data Analysis

[11] N<sub>2</sub>O flux from each treatment was calculated by averaging the three replicates for each sampling time. The data of environmental parameter used in correlation analysis and regression analysis with N<sub>2</sub>O fluxes are in Table 1 of Wang *et al.* [2009], the simultaneous research focusing on methane emission.

## 3. Results

### 3.1. Anatomical Characterization of *S. mariqueter*

[12] By using scanning electron microscopy, intercellular gas spaces were observed in all vegetative parts of *S. mariqueter* (see Figure 2). In the transverse section of the corm, intercellular gas spaces were found to be well developed in the proportionally large cortex and central cylinder, with hexagonal packing arrangements. Stellate parenchyma surrounding the central cylinder showed high porosity, with no aerenchyma. In the transverse section of the aerial stem, large aerenchyma was visible, embedded regularly in the outer cortex, while the complete inner cortex was formed by cortical gas spaces. In the leaves, lysigenous-like aerenchyma were observed in the cortex parenchyma. The structure was narrow, symmetrically arranged and not as extensive as those in the stem. In addition, stomata were located primarily on the longitudinal section of leaf epidermis.

### 3.2. Seasonal Variation of Nitrous Oxide Fluxes

[13] N<sub>2</sub>O concentration in the headspace of chamber is ambient, about from 300 to 360 ppbv. During the study period, monthly averaged N<sub>2</sub>O flux under light conditions ranged from  $-76.3 \pm 57.4 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$  to  $36.5 \pm 65.4 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ . Except for in June, CD was a sink for atmospheric N<sub>2</sub>O with a maximum absorption flux of  $-76.3 \pm 57.4 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$  measured in August (Table 1). There was no significant correlation between temperatures and N<sub>2</sub>O flux in the light enclosures. However, PAR was significantly correlated with light N<sub>2</sub>O flux ( $p < 0.01$ ), indicating that the photosynthetic activity of *S. mariqueter*, which was closely correlated with PAR, apparently affected the seasonal variation of N<sub>2</sub>O flux. During June, CD became a source of atmospheric N<sub>2</sub>O, corresponding to low PAR levels measured during that month. Overall, monthly N<sub>2</sub>O absorption (negative flux) rate increased along with the growth of *S. mariqueter*, and decreased when plants senesced. On a monthly basis, the

**Table 1.** Seasonal Variation in N<sub>2</sub>O Flux ( $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ ) in Clipped and Nonclipped Treatments ( $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ )<sup>a</sup>

	Light	Dark	Light (Clipped)	Dark (Clipped)
May	-67.4 ± 14.6	730 ± 19.2	-	-
Jun.	36.5 ± 65.4	107 ± 50.0	-	-
Jul.	-42.6 ± 52.0	246 ± 36.8	80.9 ± 68.2	110 ± 69.3
Aug.	-76.3 ± 57.4	152 ± 25.1	130 ± 41.6	129 ± 18.6
Sep.	-75.1 ± 65.9	139 ± 23.1	135 ± 41.6	125 ± 30.1
Oct.	-16.2 ± 24.4	47.2 ± 2.35	35.8 ± 9.76	43.1 ± 9.33

<sup>a</sup>Values are means and standard deviation of sequential measurements performed during monthly sampling events. Standard deviation represents variation during each sampling.

differences in N<sub>2</sub>O flux between light and dark chambers were significant ( $p < 0.05$  in May and June,  $p < 0.001$  in July, August, September, and October). In contrast to the N<sub>2</sub>O fluxes under light conditions, N<sub>2</sub>O flux in the dark chambers was a source of atmospheric N<sub>2</sub>O during the whole growing season, highlighting the relationship between photosynthetic activity of *S. mariqueter* and inhibition of N<sub>2</sub>O flux. Furthermore, significant positive correlations were found between N<sub>2</sub>O flux and temperatures (AT and SGT,  $p < 0.01$ ) in dark chambers.

### 3.4. Nitrous Oxide Fluxes of Clipping Treatment

[14] Compared with N<sub>2</sub>O fluxes in unclipped light chambers, cutting the aboveground part of *S. mariqueter* under light conditions enhanced N<sub>2</sub>O emission in all months ( $p < 0.001$ ). Cutting the aboveground part of *S. mariqueter* in the dark chambers significantly attenuated the N<sub>2</sub>O fluxes in July ( $p < 0.001$ ) and August ( $p < 0.05$ ). However, substantial attenuation of N<sub>2</sub>O flux occurred only in July, when flux decreased by about 55% in the clipped dark chambers compared to the nonclipped dark chambers. N<sub>2</sub>O flux in both light and dark clipped chambers exhibited a similar temperature-induced diurnal pattern during the four month period, with peak emission observed during late afternoon when the 10 cm GT were usually highest (see Figure 3).

### 3.5. Nitrous Oxide Flux at Wetlands

[15] N<sub>2</sub>O emission from wetland research was taken on at many kinds of typical wetland around the world (see Figure 4). Reported data show that N<sub>2</sub>O flux at wetland has a large range from negative value to positive (from about -32.4 to 1292  $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ ), while wetland is the source of the atmosphere on the whole (see Table 2). In our research, although in light chamber marsh was a sink of atmospheric N<sub>2</sub>O, the *S. mariqueter* marsh was a net source of atmospheric N<sub>2</sub>O, with an average N<sub>2</sub>O emission rate of 98.3  $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$  during the principal growing season from May to October. It is interesting that N<sub>2</sub>O flux in Arctic and Antarctic area is higher. The budget of global N<sub>2</sub>O emission from wetland need a detail spatial data, and the data also need to be considered the effect of radiation.

## 4. Discussion

### 4.1. Gas Transport Mechanism in *S. mariqueter*

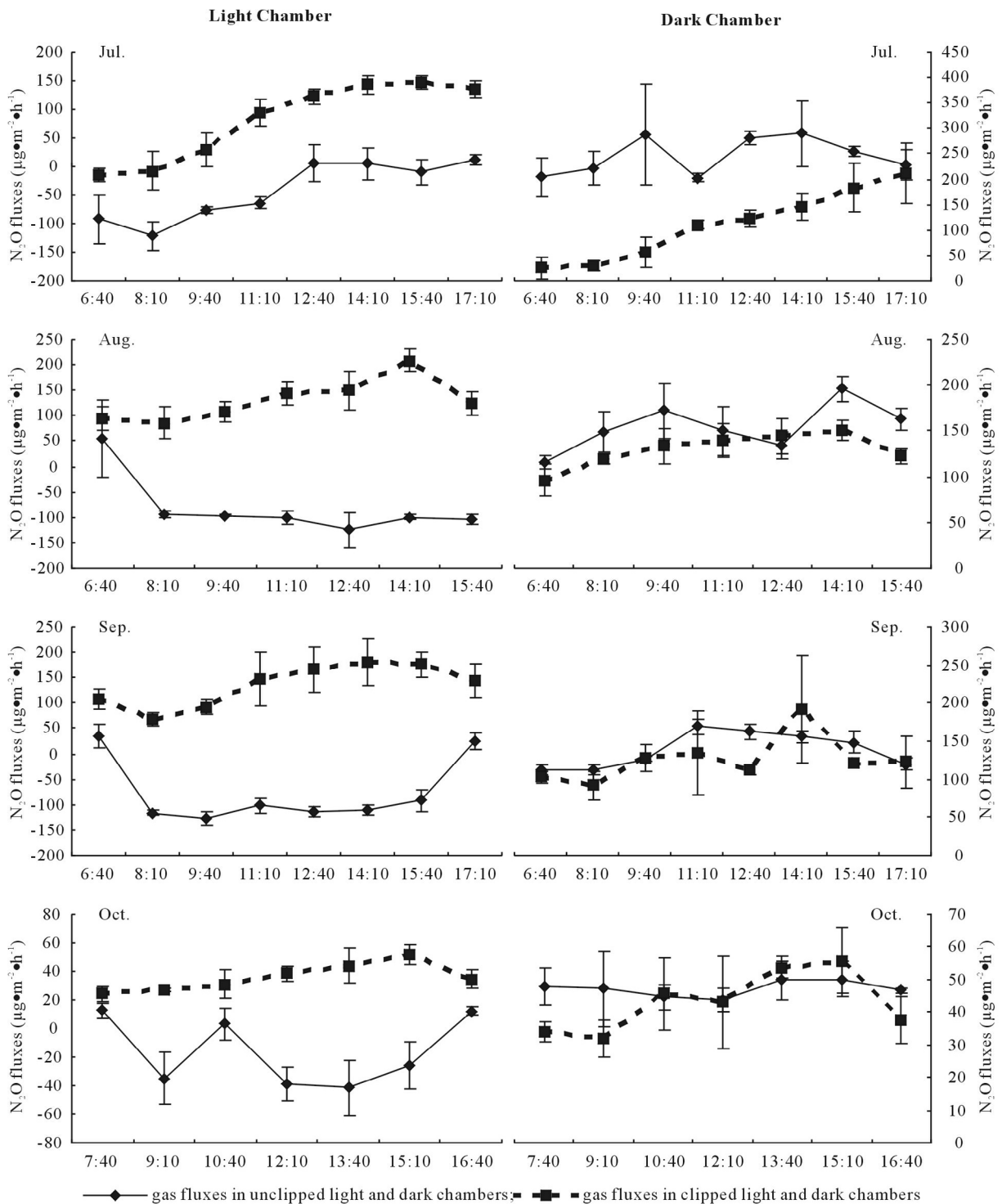
[16] Researchers had demonstrated that mechanisms of gas transport commonly employed by wetland plants include molecular diffusion, characterized by migration of gas

molecules along a concentration gradient, and convective flow in response to a pressure differential [Armstrong et al., 1992; Brix et al., 1992]. Previous studies have demonstrated significant differences in plant-mediated CH<sub>4</sub> fluxes under dark versus light conditions [Whiting and Chanton, 1996; Van der Nat and Middelburg, 2000], when wetland plants were the primary conduits facilitating CH<sub>4</sub> emission via convective throughflow and stomata-controlled transport [Van der Nat and Middelburg, 1998]. Significantly attenuated CH<sub>4</sub> emission rates have been observed when aboveground portions of the vegetation were clipped under light conditions, because the capacity for pressurized transport was eliminated [Van der Nat and Middelburg, 2000]. However, there can be significant increase in CH<sub>4</sub> flux under dark conditions when the stem or the leaf is cut, if diffusion is the primary transport mechanism in the wetland plant, and especially diffusion had been limited by the resistance of the aboveground portion of the plant [Schimel, 1995].

[17] Although many studies focused on CH<sub>4</sub> transport by wetland plants, the effects of plant on N<sub>2</sub>O emission have not been extensively studied, both of these gases, are soluble in water, so they can be transported concurrently within wetland plants by convective throughflow or diffusion. Some studies have reported that wetland plants can affect N<sub>2</sub>O fluxes by acting as a transport conduit [Reddy et al., 1989; Mosier et al., 1990], influencing nitrification-denitrification processes in the rhizosphere [Reddy et al., 1989; Bodelier et al., 1996; Kettunen et al., 2005], and/or producing N<sub>2</sub>O by photosynthesis of NO<sub>2</sub><sup>-</sup> in the leaves [Smart and Bloom, 2001].

[18] Same as many other wetland plants, *S. mariqueter* possesses abundant intercellular gas space in the stem and leaf tissues (see Figures 2b–2d), indicating that convective throughflow is likely, particularly when an intensive pressure differential exists. In the previous research on CH<sub>4</sub> emission, when the transpiration was higher, molecular diffusion and convective gas flow were the two main mechanisms of CH<sub>4</sub> transport in *S. mariqueter* plants [Wang et al., 2009]. In July, when *S. mariqueter* was exuberant and PAR and temperature were relatively high, higher gas transport efficiency via convective throughflow was expected. Cutting the aboveground portion of *S. mariqueter* significantly decreased the CH<sub>4</sub> fluxes in light chambers [Wang et al., 2009], because the capacity for pressurized transport has been eliminated [Van der Nat and Middelburg, 2000]. But in light chambers, clipping the aboveground portion of *S. mariqueter* significantly enhanced N<sub>2</sub>O flux compared with the unclipped chambers ( $p < 0.001$ ) (see Figure 3), there was no observable of the transportation of N<sub>2</sub>O by convective throughflow. On the other hand, N<sub>2</sub>O fluxes in the unclipped dark were also significantly higher than those in light chambers ( $p < 0.001$ ), suggesting that *S. mariqueter* photosynthetic activity significantly decreased the N<sub>2</sub>O emission.

[19] A microscopic anatomical evaluation of *S. mariqueter* revealed that cortical gas spaces occupied the full cortex (see Figure 2a), suggesting low resistance on transporting gas from root to stem and leaf. Such a configuration generally has little effect on gas diffusion [Schuette et al., 1994; Sorrell et al., 1997], although the potential diffusion resistance at the transition between the rhizosphere and the root aerenchyma was unknown. Based on the previous research in which molecular diffusion became the primary transport



**Figure 3.** Diurnal change of N<sub>2</sub>O fluxes in light and dark chambers in the unclipped and clipped treatments. Bars represent standard deviation of the triple duplicates.

mechanism when *S. mariqueter* began to senesce, clipping the aboveground portion of *S. mariqueter* enhanced CH<sub>4</sub> emission indicating the resistance of stems and leaves [Wang *et al.*, 2009], suggesting that aboveground portion of vegetation was a factor regulating CH<sub>4</sub> diffusion [Schimel, 1995]. While cutting of the aboveground portion of *S. mariqueter*

significantly decreased the N<sub>2</sub>O flux in dark chamber ( $p < 0.001$ ) especially in July and August, It was clear that gas transport by *S. mariqueter* was not the primary factor governing N<sub>2</sub>O emission in wetland. Therefore, the *S. mariqueter* transport function was to a degree influenced by its other



**Table 2.** N<sub>2</sub>O Emission Flux at Some Typical Areas Around the World<sup>a</sup>

Number	Location character	Flux ( $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ )	Reference
1	Subarctic tundra	79.2–1292	[Repo et al., 2009]
2	Peatland	−1.25–8.33	[Regina et al., 1996]
3	Intertidal mud flat	1.79	[Middelburg et al., 1995]
4	Intertidal saltmarsh and mudflats	14	[Kenny et al., 2004]
5	Intertidal saltmarsh and mudflats	11.5	[Kenny et al., 2004]
6	Coastal marsh	35.5	[Schiller and Hastie, 1994]
	Coastal fen	121.0	
7	Riparian Forest	11.2 ± 1.77	[Ullah and Moore, 2011]
	Wetland	17.8 ± 3.01–48.3 ± 18.6	
8	Intertidal salt marsh	−1.33 ± 0.88	[Moseman-Valtierra et al., 2011]
9	Salt marsh	5.56	[Smith et al., 1983]
	Brackish marsh	8.61	
	Fresh marsh	9.87	
10	Mangrove	3.36–218	[Corredor et al., 1999]
11	Freshwater marsh	65 ± 37	[Zhang et al., 2007]
12	Mangrove	17.1–33.3	[Krihika et al., 2008]
13	Subtropical mangrove	−2–14	[Kreuzwieser et al., 2003]
14	Subtropical mangrove	−4–65	[Allen et al., 2007]
15	Temperate mangrove and salt marsh	15.7	[Livesley and Andrusiak, 2012]
16	Antarctic tundra	−32.4–135	[Zhu et al., 2008]
17	Antarctic tundra	0.6 ± 1.7–1.1 ± 2.2	[Zhu et al., 2005]
18	Antarctic lakeshore soils	52.5–132	[Gregoricha et al., 2006]
19	Subtropical intertidal salt marsh	98.3	This study

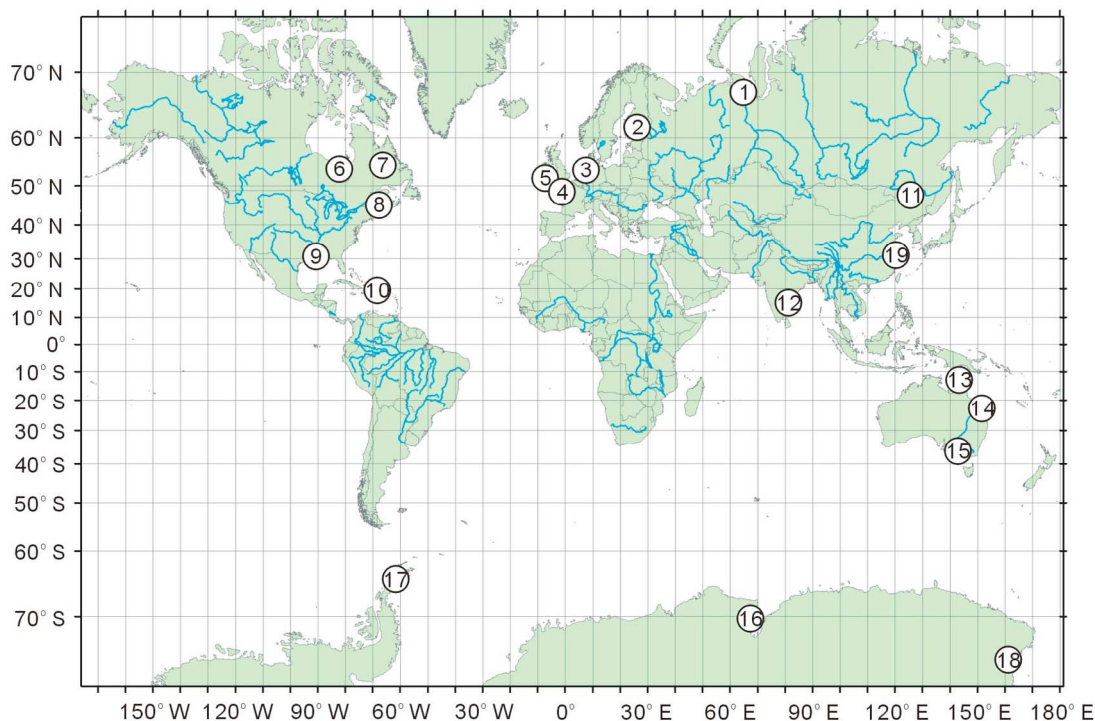
<sup>a</sup>The data were converted with same unit according the data in every research.

physiological activities, such as production of root exudates and oxygenation of the rhizosphere.

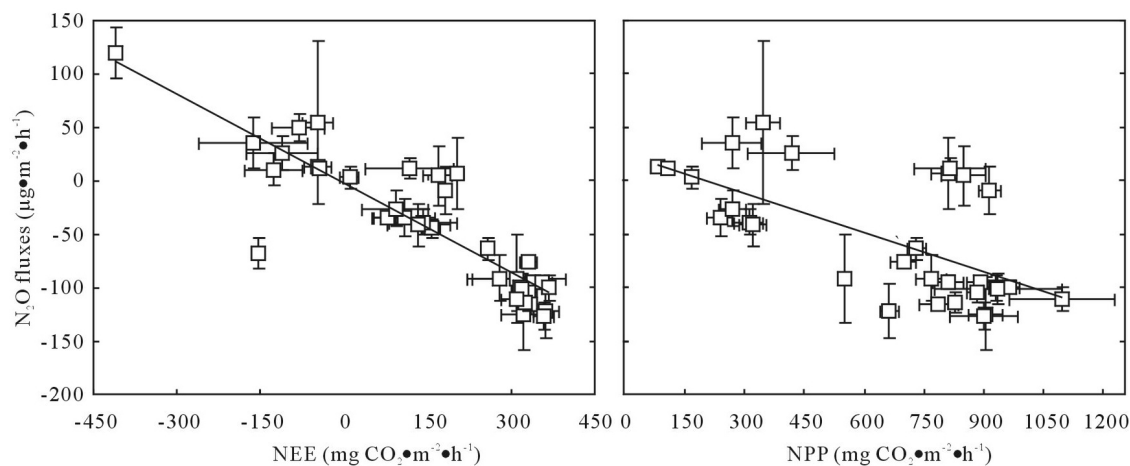
#### 4.2. Effect of *S. mariqueter* on Nitrous Oxide Fluxes

[20] Compared to CH<sub>4</sub>, there is not a large amount of research on plant-dependent N<sub>2</sub>O flux from wetland ecosystems, including information on the relationship between N<sub>2</sub>O flux and plant productivity. In the study of nitrogen loading in a freshwater marsh from Sanjiang plain, North China,

Zhang et al. [2007] proposed that *Deyeuxia angustifolia* had the potential to increase the production of N<sub>2</sub>O by supplying easily decomposable root exudates, which enhance microbial activity in soil. Based on a significant correlation between N<sub>2</sub>O flux and aboveground biomass, they inferred that a large fraction of N<sub>2</sub>O flux was facilitated by *Deyeuxia angustifolia* via the transpiration stream [Zhang et al., 2007]. However, in other studies, there was no correlation between plant characteristics and N<sub>2</sub>O flux [Chen et al., 1997; Müller, 2003].



**Figure 4.** Map of N<sub>2</sub>O fluxes research locations of the world.



**Figure 5.** Diurnal gas fluxes in light chamber in unclipped treatments, plotted against net ecosystem exchange (NEE) and net primary production (NPP). Bars represent standard deviation of the triple duplicates.

Yan *et al.* [2000] further concluded that N<sub>2</sub>O is mainly transported by diffusion through the soil surface rather than through plants [Yan *et al.*, 2000].

[21] In our study, the effect of *S. mariqueter* on N<sub>2</sub>O flux was relatively distinct and straightforward. By comparing N<sub>2</sub>O flux in light and dark chambers, we conclude that the photosynthetic activity of *S. mariqueter* attenuated N<sub>2</sub>O flux significantly during the entire study period (see Table 1 and Figure 3). With the exception of June, monthly averaged N<sub>2</sub>O absorption rate varied with the growth of *S. mariqueter*. Significant positive correlations were found between N<sub>2</sub>O emission in dark chambers and temperature; conversely, there was a significant negative correlation between N<sub>2</sub>O flux and PAR under light conditions ( $p < 0.01$ ). Furthermore, clipping the aboveground part of *S. mariqueter* in light chambers greatly enhanced N<sub>2</sub>O emission during each monthly measurements. On the other hand, there was no significant difference in N<sub>2</sub>O flux between clipped and unclipped dark chambers. We interpret these results as follows: PAR governed the photosynthetic rate and growth of *S. mariqueter*; consequently, a significant inhibitory effect on N<sub>2</sub>O emission was imposed by the *S. mariqueter* community. Scatterplots of diurnal N<sub>2</sub>O flux in light unclipped chambers versus NEE and NPP show the significant negative correlation between N<sub>2</sub>O flux and NEE and NPP (see Figure 5), the regression curve of N<sub>2</sub>O fluxes and NEE, NPP was described by the linear function: N<sub>2</sub>O flux ( $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ ) =  $-0.2790 \times \text{NEE (mg CO}_2 \text{ m}^{-2} \text{h}^{-1}) - 2.0345$  ( $R^2 = 0.7758$ ,  $p < 0.0001$ ), N<sub>2</sub>O flux ( $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ ) =  $-0.1224 \times \text{NPP (mg CO}_2 \text{ m}^{-2} \text{h}^{-1}) + 24.546$  ( $R^2 = 0.4211$ ,  $p < 0.001$ ).

[22] N<sub>2</sub>O production in freshwater marshes was clearly limited by nitrogen deficiency [Zhang *et al.*, 2007]. Under field conditions, concentrations of inorganic nitrogen compounds are usually low in the root zone in the growing season due to plant uptake of nitrogen [Bodelier *et al.*, 1996; Van der Nat *et al.*, 1997]. The growth of *S. mariqueter* might suppress N<sub>2</sub>O emission by taking up nitrogen in sediment, which could directly inhibit the N<sub>2</sub>O production by diminishing the substrate for nitrification and denitrification. When denitrifiers and nitrifiers in the sediment were suffering the NO<sub>3</sub><sup>-</sup> limitation, they would consume the N<sub>2</sub>O diffusing from the atmosphere [Frasier *et al.*, 2010]. Although

this process is poorly understood, some researches found that N<sub>2</sub>O was consumed by the soil [Chapuis-Lardy *et al.*, 2007; Peichle *et al.*, 2010; Ullah and Moore, 2011]. On the other hand, Zhang *et al.* [2007] assumed that plant uptake can reduce some N<sub>2</sub>O, most likely by taking up available nitrogen. Furthermore, our explanation is also in agreement observations on *Spartina alterniflora* and *Phragmites australis* reported by Cheng *et al.* [2007] in experimental mesocosms using plants and soils from the Jiuduansha salt marsh in the Yangtze River estuary.

[23] Beside the competition between *S. mariqueter* and microbes for nitrogen, O<sub>2</sub> is transported and diffused into *S. mariqueter* rhizosphere during photosynthesis. It effectively increases the aerobic-anaerobic surface area and influences anaerobic metabolism in the wetland soil [Magonigal *et al.*, 2004]. In another research report [Wang *et al.*, 2009], CH<sub>4</sub> flux in light chamber was higher than in dark chamber from July to September, but there were no significant difference in July and August, and in October, CH<sub>4</sub> flux in light chamber was slightly lower than in dark chamber. Photosynthesis of the plant or the O<sub>2</sub> transported into sediment did not inhibit methanogenesis process greatly because more organic materials would also be transported into root and exuded [Zhang *et al.*, 2007]. Denitrification is often tightly coupled to nitrification in the high redox area [Kettunen *et al.*, 2005] that predominates in the rhizosphere, while methanogenesis dominates at lower reducer environment only when other electron acceptors are almost exhausted. Basing on this spatial distribution, it is reasonable to assume that the denitrification is more sensitive to the rhizospheric O<sub>2</sub> delivery.

[24] In sediment, because of nitrate limitation coupled nitrification-denitrification was the main process of N<sub>2</sub>O production [Moseman-Valtierra *et al.*, 2011]. LaMontagne *et al.* [2003] found that there was a higher N<sub>2</sub>O uptake rates in the opaque chamber deployed on macroalgae covered sediments, and benthic N<sub>2</sub>O sink can be explained by a close coupling of nitrification and denitrification [LaMontagne *et al.*, 2003]. When coupled nitrification provides the nitrate for denitrifier, N<sub>2</sub>O uptake can occur [van Raaphorst *et al.*, 1992], denitrifiers would consume N<sub>2</sub>O during hypoxic conditions [Usui *et al.*, 2001]. When *S. mariqueter* photosynthesized under light conditions, more O<sub>2</sub> was

released from the root to sediment meeting the demand of nitrification, stimulated denitrifier consumed the N<sub>2</sub>O inducing to it diffusing from atmosphere into sediment. While in the dark chambers, although denitrification process would be slowed down because of the nitrate limitation, the consumption was decreased quickly and there was a net N<sub>2</sub>O production from denitrification. On the other hand, the stimulation of CH<sub>4</sub> production by root exudates of *S. mariqueter* [Wang et al., 2009] indicates that the root exudates should also fuel the denitrifiers resulting in greater denitrification rate [Zhang et al., 2007] and more N<sub>2</sub>O consumption.

[25] From July to October, under light conditions, besides the diffusing into marsh sediment directly, N<sub>2</sub>O could diffuse with O<sub>2</sub> from the stomata to the rhizosphere when *S. mariqueter* photosynthesized and the stomata were open, where it was consumed by coupled nitrify denitrification [Vieten et al., 2008; Moseman-Valtierra et al., 2011]. Under dark conditions, molecular diffusion was not the main transporting mechanism of N<sub>2</sub>O emission. Clipping the aboveground portion of *S. mariqueter* had no obviously effect N<sub>2</sub>O flux, facilitating N<sub>2</sub>O diffusing from rhizosphere to atmosphere. In July, the flux in clipped light and dark chambers was significantly lower than in unclipped dark chambers ( $p < 0.001$ , Figure 3), indicating that the positive effect of respiration of *S. mariqueter* on N<sub>2</sub>O emission exceeding the negative effect by the resistance of leaf and stem on N<sub>2</sub>O diffusion. But there was no significant difference between flux in unclipped dark chambers and clipped light and dark chambers from August to October (Figure 3). N<sub>2</sub>O diffusing out or into by molecular diffusion method through intercellular gas space was depended on its gradient between rhizosphere and atmosphere. The transport of N<sub>2</sub>O by *S. mariqueter* is not only simple molecular diffusion but the result associated with the complex effects of nitrogen cycling in rhizosphere. More detailed studies on N<sub>2</sub>O transport and its production and consumption in the rhizosphere via nitrification and denitrification process are needed in order to fully understand such effects.

## 5. Conclusion

[26] Wetland plant has not only the physical effect on N<sub>2</sub>O transporting and diffusing by facilitating it emission from sediment to atmosphere or providing a pass way which N<sub>2</sub>O diffuse into rhizosphere with O<sub>2</sub> when there is a concentration gradient because of N<sub>2</sub>O consumption in sediment, it also control N<sub>2</sub>O production and consumption by influencing the biogeochemical processes in sediment. Under light condition, competition with the microbe for nitrogen and directly using of N<sub>2</sub>O, and providing O<sub>2</sub> and decomposable organic carbon for nitrifiers and denitrifier are the two main mechanisms inducing the N<sub>2</sub>O absorption. While in the dark chamber, the higher N<sub>2</sub>O emission flux indicated that Yangtze estuarine wetland is a net source of atmospheric N<sub>2</sub>O; it is must to carefully consider the temporal and spatial change of N<sub>2</sub>O flux in calculating N<sub>2</sub>O budget in an area.

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