Seasonal variation in CH_4 emission and its ^{13}C-isotopic signature from *Spartina alterniflora* and *Scirpus mariqueter* soils in an estuarine wetland

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Abstract Although invasions by non-native species represent a major threat to biodiversity and ecosystem functioning, little attention has been paid to the potential impacts of these invasions on methane

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(CH_4) emission and its ^{13}C-CH_4-isotope signature in salt marshes. An invasive perennial C_4 grass *Spartina alterniflora* has spread rapidly along the east coast of China since its introduction from North America in 1979. Since its intentional introduction to the Jiuduansha Island in the Yangtze River estuary in 1997, *S. alterniflora* monocultures have become the dominant component of the Jiuduansha's vegetation, where monocultures of the native plant *Scirpus mariqueter* (a C_3 grass) used to dominate the vegetation for more than 30 years. We investigated seasonal variation in soil CH_4 emission and its ^{13}C-CH_4-isotope signature from *S. alterniflora* and *S. mariqueter* marshes. The results obtained here show that *S. alterniflora* invasion increased soil CH_4 emissions compared to native *S. mariqueter,* possibly resulting from great belowground biomass of *S. alterniflora*, which might have affected soil micro-environments and /or CH_4 production pathways. CH_4 emissions from soils in both marshes followed similar seasonal patterns in CH_4 emissions that increased significantly from April to August and then decreased from August to October. CH_4 emissions were positively correlated with soil temperature, but negatively correlated with soil moisture for both *S. alterniflora* and *S. mariqueter* soils ($p<0.05$). The $\delta^{13}C$ values of CH_4 from *S. alterniflora,* and *S. mariqueter* soils ranged from -39.0‰ to -45.0‰, and -37.3‰ to -45.7‰, respectively, with the lowest $\delta^{13}C$ values occurring in August in both marshes. Although the

leaves, roots and soil organic matter of *S. alterniflora* had significantly higher $\delta^{13}C$ values than those of *S. mariqueter*, *S. alterniflora* invasion did not significantly change the ^{13}C- isotopic signature of soil emitted CH_4 (p>0.05). Generally, the CH_4 emissions from both invasive *S. alterniflora* and native *S. mariqueter* soils in the salt marshes of Jiuduansha Island were very low (0.01–0.26 mg m^{-2} h^{-1}), suggesting that *S. alterniflora* invasion along the east coast of China may not be a significant potential source of atmospheric CH_4.

Introduction

Methane is the second most important greenhouse gas and its global warming potential is 25 times greater than CO_2 on a mass basis (IPCC 2007; Dalal et al. 2008). Natural wetlands play an important role in contributing methane (CH_4) to the atmosphere (Bartlett and Harriss 1993; Edwards et al. 2000). The CH_4 produced from wetlands accounts for approximately 20% of the total atmospheric budget (Rodhe 1990; IPCC 2007). Previous studies have reported that CH_4 emissions from wetlands result from the complex interactions between the processes of CH_4 production, consumption, and transport, which are governed by various interrelated environmental factors, such as temperature, vegetation, soil properties, and microbial activities (e.g., Conrad 1999; Kutzbach et al. 2004; Laine et al. 2007).

Vegetation is one of the important factors that affect CH_4 emissions from wetland ecosystems (e.g., Kutzbach et al. 2004; Minkkinen and Laine 2006). While an increasing number of studies has reported that vegetation affects gas emissions through regulating production and transport in wetland ecosystems worldwide (e.g., Van der Nat and Middelburg 2000; Cheng et al. 2007; Wilson et al. 2009), several studies have indicated that belowground parts of vegetation (decaying plant materials and fresh root exudates) could provide the substrates for methanogenesis (Whiting and Chanton 1993; Joabsson et al. 1999), which promote CH_4 production (Whiting and Chanton 1993; Van der Nat and Middelburg 2000).

Soil temperature can also change decomposition of soil organic matter through regulating soil respiration rates and microbial activities (Rustad et al. 2001; Laine et al. 2007), and can eventually regulate the amount and seasonal pattern of CH_4 emission in wetlands (Flessa et al. 2008). Additionally, soil moisture influences CH_4 fluxes directly by controlling the relative extent of oxic and anoxic environments within soils, and indirectly by affecting soil genesis and vegetation composition, which are important additional factors controlling CH_4 fluxes (Kutzbach et al. 2004). Thus, the changes in the relative importance of these processes, for example, species composition caused by plant invasions induced by human activities, may change soil organic matter input and quality (Ehrenfeld et al. 2001; Ehrenfeld 2003), which can potentially lead to large changes in the magnitude of CH_4 flux from wetland ecosystems.

Furthermore, numerous studies have reported that isotopic fractionation occurs during CH_4 emissions from the submerged anoxic soil into the atmosphere (Chanton et al. 1997; Bilek et al. 1999; Fey et al. 2005; Venkiteswaran and Schiff 2005). Stable carbon isotope ratios ($\delta^{13}C$) of CH_4 have proven to be a useful technique for partitioning individual atmospheric CH_4 sources and sinks (Blair 1998; Edward et al. 2000), which is based on the fact that the CH_4 production is almost exclusively derived from acetate fermentation and CO_2 reduction (H_2/CO_2 pathway) in the anoxic wetland environments (Conrad 2005; Penning et al. 2005). Production of CH_4 from the CO_2 reduction pathway exhibits larger isotope fractionation than that from acetate fermentation because the ^{13}C-CH_4 produced by acetate fermentation is more enriched (-30 ~ -60‰) than that produced by CO_2 reduction (-60 ~ -110‰) (Fey et al. 2005; Conrad 2005). Thus, this difference in carbon isotope fractionation can be used for partitioning C-flux sources in methanogenic environments (Conrad 2005). However, Penning et al. (2005) have demonstrated that the $\delta^{13}C$ values of CH_4 produced from either CO_2 reduction or acetate fermentation have a characteristic signature, and the magnitude of fractionation is usually considerably different among natural ecosystems. Meanwhile, the pathway of CH_4 production from either CO_2 or acetate can vary seasonally under field conditions, but the causes of the variations are not fully understood (Fey et al. 2005).

Spartina alterniflora was transplanted into tidal marshes of the coastal zone in China in 1979 from its native range in the United States because of its great ability to rapidly colonize mudflats (Qin and Zhong 1992; Wang et al. 2006a). While its rapid growth has greatly helped to stabilize the tidal flats, the negative impacts have also been characterized by the displacement of native species (Chen et al. 2004) and the changes of biotic communities and ecosystem processes (Wang et al. 2006a; Liao et al. 2007, 2008; Li et al. 2009). For example, Jiuduansha is an estuarine island growing from constant deposition of sediments carried from the Yangtze River in China. Invasive *S. alterniflora*, a C_4 plant, was introduced to the island in 1997 under the Green Recovery for Birds project in the Yangtze River estuary, where the native *Scirpus mariqueter* (a C_3 plant) had dominated the island's vegetation for over 30 years (Cheng et al. 2006). Previous studies have reported that *S. alterniflora* grows faster than the native species (Wang et al. 2006b) and its invasion has significantly changed soil organic matter (SOM) (Cheng et al. 2006), resulting in greater residue inputs to coastal marshes. Cheng et al. (2007) have compared trace gas emissions from *S. alterniflora* with those from a native *Phragmites australis* by establishing brackish marsh mesocosms to experimentally assess the effects of plant species, flooding status, and clipping on trace gas emissions. It is well known that the CH_4 emissions via plant pathway contribute significantly to the total CH_4 emission from wetland ecosystems (e.g., Schimel 1995; Van der Nat and Middelburg 2000; Chanton et al. 2002). Little effort, however, has been made to assess the impact of invasive plants on CH_4 emissions and its ^{13}C-isotopic signature from the soil associated with soil substrates in natural marsh ecosystems. We hypothesized that C_4 *S. alterniflora* invasion in the Jiudansha estuarine wetland significantly alters CH_4 emissions and the ^{13}C-isotopic signature because *S. alterniflora* differs from the native C_3 plant in productivity, tissue chemistry, and may affect soil properties such as soil temperature and moisture differently. The aims of this study were to: (1) reveal the seasonal variations of CH_4 emissions and the $\delta^{13}C$-CH_4 signatures from the *S. alterniflora*, and *S. mariqueter* soils; (2) assess the effects of plant traits and environmental variables (soil temperature, soil moisture, and soil substrate) on CH_4 emissions and its ^{13}C-CH_4-isotope signatures.

Materials and methods

Study area

This study was conducted in Shanghai Jiuduansha Wetland Nature Reserve in the Yangtze River estuary (31°03'–31°17'N, and 121°46'–122°15'E). Jiuduansha Island is an alluvial island that was formed from the sediments from the Yangtze River. The Island has an area of 425 km^2 in 2003 and still continues to grow at about 70 m in radius per year (Chen 2003). Its climate is characterized by annual precipitation of 1,145 mm and annual mean temperature of 15.7°C, with monthly means of 27.3°C for July and 4.2°C for January (Chen 2003). Jiuduansha has developed as a stable island over a half century. The vegetation on the island consists of very few species, and the structure of the plant communities is relatively simple. The native species, *S. mariqueter,* dominated the salt marshes on this island until the invasive *S. alterniflora* was introduced in 1997 (Chen 2003).

Field sample collection

In April 2004, we selected two transects, 3 km long, along a transitional zone from *S. alterniflora* to *S. mariqueter* in the wetland (details given in Liao et al. 2007). Because *S. alterniflora* and *S. mariqueter* do not co-exist, both of the two species form their respective monocultures. In most of Jiuduansha wetland, *S. alterniflora* had replaced *S. mariqueter* over about 8 years since its intentional introduction in 1997, while *S. mariqueter* had dominated the salt marshes for over 30 years (Chen 2003, Cheng et al. 2006). We randomly selected six sampling sites on each transect, each of which measured 50 m wide and 200 m long. At each site, we collected several samples (up to 6) of litter, roots, and soil. One replicate (litter, roots, and soil) was taken from several randomly-selected separate samples at each site (Cheng et al. 2008). Soil samples were separated into two parts; one part was sealed in a soil tin and analyzed for gravimetric water content (percentage water, measured as g water/g dry soil×100), the other was placed in a glass vial for isotopic analysis of soil organic carbon. All measurements were made four times from April to October (i.e. bimonthly) in 2004. We collected gas samples at the same time of day between 8:00 AM and 10:00 AM on each sampling

occasion to minimize any effects of diurnal variation in emissions. We used the static closed chamber technique to measure CH_4 emissions (Cheng et al. 2007). Briefly, we first inserted stainless-steel collars into the soil to a depth of 50 mm one day prior to each gas sampling. Every care was taken to minimize disturbance to the soil, particularly inside the chamber, during insertion. The small transparent acrylic resin chambers covering an area of 10×10 cm with a height of 10 cm were placed on the notched collars, and an airtight closure was ensured by water sealing during the measurements. Air inside the chambers was circulated with battery-driven fans during the measurement in order to ensure gas samples were well-mixed. The gas fluxes of CH_4 in all plots were measured simultaneously. Generally, five gas samples of chamber air were taken using 25 ml polypropylene syringes at 0 min, 10 min, 20 min, 30 min and 40 min after enclosure. In order to equilibrate air pressure inside chamber, the same amount of air (25 ml) was pulled into the chamber through polypropylene syringes immediately after taking sample at 0 min, 10 min, 20 min and 30 min. Samples were injected into 10 ml pre-evacuated vials for laboratory analysis. The air temperature inside the chamber and soil temperature in the top soil layer (0–20 cm depth) was taken during each gas sample.

Laboratory analysis

The CH_4 concentrations in the gas samples were analyzed by using gas chromatography (GC/FID Shimadzu 14 B), with a unibead-C column. Column, injection, and FID temperature were set at 100°C, 120°C, and 300°C, respectively, with a carrier gas flow rate of 65 ml min^{-1}, and an injection volume of 0.5 ml (Towprayoon et al. 2005; Cheng et al. 2007). The CH_4 fluxes were calculated by linear regression analysis of the change of gas concentration in the mixed chamber with time over a 40-minute period (Rolston 1986; Cheng et al. 2007).

Samples of roots, litter, and soil were dried at 50°C to constant weight and ground to pass through 20-mesh (0.85m m) sieves (Lin et al. 1999; Cheng et al. 2006). Subsamples of roots, litter, and soil were measured on an isotope ratio mass spectrometer (Thermo Finnigen, Delta-Plus, Flash, EA, 1112 Series, USA) for carbon isotopes. Urea and glycine were analyzed as a check on the accuracy and precision of isotopic ratios by the elemental composition analyzer. Precision for $\delta^{13}C$ was±0.15 ‰.

Stable carbon isotope ratio ($\delta^{13}C$) in gas samples collected at initial (0), and final (40) minutes after enclosure was measured using a gas chromatograph combustion isotope ratio mass spectrometer (GCC-IRMS) system (Finnigan MAT model delta plus, Thermoquest, Bremen, Germany). The method requires only very small samples, namely several picolitres of the vapor (for principal operations see Brand (1996)). Briefly, the CH_4 in the gas samples (10–400 μl) were first separated in a Hewlett Packard 6,890 gas chromatograph operating with a Pora Plot Q column (27.5 m length; 0.32 mm i.d.; 10 μm film thinkness; Chrompack, Frankfurt, Germany) at 25°C with He (99.996% purity; 2.6 ml min-1) as carrier gas. After conversion of CH_4 to CO_2 in the Finnigan Standard GC Combustion Interface III, the gases were transferred into the IRMS (Fey et al. 2005; Conrad and Claus 2005; Penning et al. 2005). The working standards were CO_2 (99.998% purity; Messer-Griessheim, Düsseldorf, Germany) and methylstearate (Merck). The latter was intercalibrated at the Max-Planck-Institut für Biogeochemie, Jena, Germany (courtesy of Dr. W. Brand) against NBS22 (National Bureau of Standards 22) and expressed in the delta notation versus PDB (Pee Dee Belemnite) carbonate:

$$\delta^{13}C = 10^3 (R_{sa}/R_{st} - 1)$$

where $R = {}^{13}C/{}^{12}C$ of sample (sa) and standard (st), respectively. The precision of repeated analysis was± 0.5‰ when 1.3 nmol CH_4 was injected. The $\delta^{13}C$ values from emitted CH_4 were calculated by a mass balance model using both initial and final concentration and isotopic data (Chanton and Liptay 2000; Powelson et al. 2007).

Statistical analyses

The data analyses were performed using Stat Soft's Statistica software for Windows (Version 6.0, Stat-Soft, Inc. 2001) and Microsoft Excel software for analysis of variance and t-test with a significance level of $P < 0.05$.

The $\delta^{13}C$ values of plant matter and CH_4, CH_4 flux rates, and soil temperature and moisture reported here are the means of six measurements. Two-way ANOVA was performed to examine the differences

in soil temperature, soil moisture, CH_4 flux rates, the $\delta^{13}C$ values of CH_4 between the two marshes in relation to time. One-way ANOVA was further employed to examine the variation in soil temperature, soil moisture, CH_4 flux rates, the $\delta^{13}C$ values of CH_4 with time within each marsh. The t-test was used to examine the differences in $\delta^{13}C$ values of plant materials and soil organic matter between the two marshes.

Results
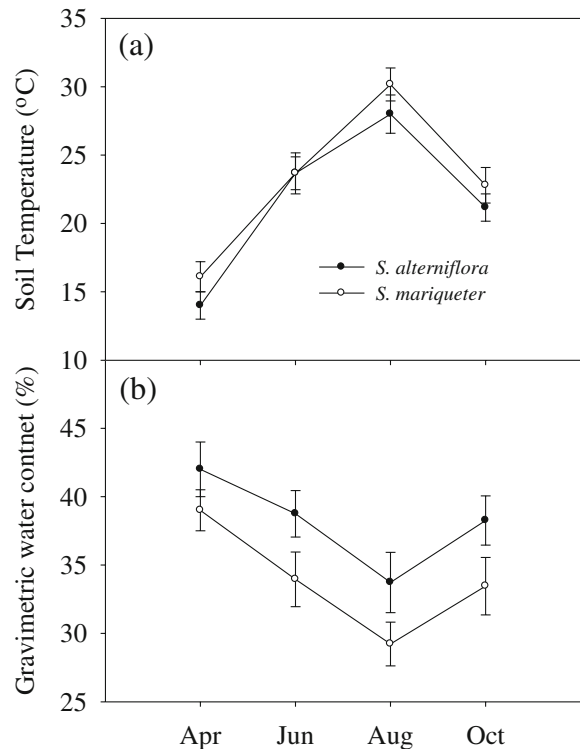
Organic matter, soil temperature and soil moisture

The plant matter of S. *alterniflora*, and S. *mariqueter* had the mean $\delta^{13}C$ values of -13.0±0.4 ‰, and - 27.4±0.7 ‰, respectively (Table 1). Based on the $\delta^{13}C$ values, vegetation in the S. *alterniflora* marsh consisted exclusively of C_4 plant species while vegetation in the S. *mariqueter* marsh consisted exclusively of C_3 plant species. The SOM in S. *alterniflora,* and S. *mariqueter* marshes had the mean $\delta^{13}C$ values of - 23.5±0.3 ‰, and - 24.3±0.2 ‰, respectively (Table 1). Basically, the $\delta^{13}C$ values of plant matter and SOM did not change significantly from April to October within each marsh (Table 1, p>0.05).

Significant seasonal changes in soil temperature and moisture were recorded from April to October (Fig. 1), but soil temperature was not different

Fig. 1 Seasonal variation of soil temperature and moisture in S. *alterniflora* and S. *mariqueter* marshes in Jiuduansha wetland in Yangtze River estuary, China. Error bars represent SE (standard error) of the means (n=6)

between the two marshes during the same time period (Fig. 1a; Table 2; p>0.05). In contrast, soil moisture in both marshes decreased from April to August and increased from August to October with greater soil moisture in S. *alterniflora* marsh compared to S. *mariqueter* marsh (Fig. 1b; Table 2; p<0.05).

CH_4 flux rates and the $^{13}C - CH_4 -$ isotopic signature

The CH_4 flux rates from the two marshes followed similar seasonal patterns, and varied significantly from April to October, with the highest CH_4 flux rate being observed in August and the lowest CH_4 flux rate in October (Fig. 2a). The flux rates of CH_4 from the S. *alterniflora* and S. *mariqueter* marshes ranged from 0.02 to 0.26 mg m^{-2} h^{-1}, and from 0.01 to 0.23 mg m^{-2} h^{-1}, respectively (Fig. 2a). The flux rate of CH_4 emission from the S. *alterniflora* marsh was generally higher than that from the S. *mariqueter* marsh (Fig. 2a; Table 2; p<0.05). Overall, CH_4 emissions from S. *alterniflora* and S. *mariqueter* soils

Table 1 The values of $\delta^{13}C$ (‰) of SOM, litter and roots in S. *alterniflora* and S. *mariqueter* marshes in Jiuduansha wetland in Yangtze River estuary, China

Marsh type	Month	SOM	Litter	Root
S. *alterniflora*	Apr	-23.4±0.2[a]	-12.7±0.3[a]	-13.2±0.2[a]
	Jun	-23.7±0.2[a]	-13.5±0.3[a]	-12.5±0.3[a]
	Aug	-23.8±0.1[a]	-13.3±0.2[a]	-12.2±0.3[a]
	Oct	-23.1±0.2[a]	-13.4±0.4[a]	-12.8±0.3[a]
S. *mariqueter*	Apr	-24.1±0.1[b]	-28.7±0.3[b]	-26.6±0.2[b]
	Jun	-24.3±0.2[b]	-27.5±0.4[b]	-27.3±0.3[b]
	Aug	-24.6±0.2[b]	-27.3±0.3[b]	-26.8±0.2[b]
	Oct	-24.0±0.1[b]	-27.9±0.3[b]	-26.7±0.2[b]

Values are means (n=6) with standard errors. Different suffixes indicate significant differences between two marshes (*t*-test, p< 0.05)

Table 2 The p-values of two-way ANOVA for testing the effects of marsh type and sampling time on CH_4 flux rates, $\delta^{13}C$ of CH_4, soil temperature, and soil moisture between *S. alterniflora* and *S. mariqueter* marshes in Jiuduansha wetland in Yangtze River estuary, China

Source of variation	CH_4 Flux	^{13}C-CH_4	Soil T	Soil M
Plant community (PC)	0.037	0.761	0.49	0.043
Sampling month (SM)	<0.0005	<0.005	<0.005	<0.0005
PC × SM	0.049	0.813	0.45	0.091

*Soil T*: soil temperature; *Soil M*: soil moisture

were positively correlated with soil temperature, but negatively correlated with soil moisture (p<0.05) (Table 3).

The $\delta^{13}C$ values of CH_4 from *S. alterniflora,* and *S. mariqueter* soils ranged from -39.0‰ to -45.0‰, and from -37.3‰ to -45.7‰, respectively, with the lowest $\delta^{13}C$ values occurring in August for both marshes (Fig. 2b). Although the $\delta^{13}C$ values of CH_4 from *S. alterniflora* soil were higher in April and lower in October than those from *S. mariqueter* soil

(Fig. 2b), two-way ANOVA showed no significant seasonal difference in the $\delta^{13}C$ value of CH_4 between the two marshes (Fig. 2b; p>0.05).

Discussion

This study compared the effects of an invasive species (*S. alterniflora*) and a native species (*S. mariqueter*) on CH_4 emissions and its ^{13}C-CH_4-isotopic signature in marshlands in the Yangtze River estuary, China. Our results obtained here showed that CH_4 emission was greater from the *S. alterniflora* soil than that from the *S. mariqueter* soil (Fig. 2a). Numerous studies have reported that vegetation can foster CH_4 production in anoxic wetland environments by contributing soil organic matter and/or acting as the pathway to allow CH_4 to escape to the atmosphere without passing through the aerobic layer of sediments (e.g., Fey et al. 2005; Kutzbach et al. 2004). Our previous study has demonstrated that the invasive species *S. alterniflora* has greatly increased root biomass in the Jiuduansha estuarine wetland (Liao et al. 2007) and hence increased labile soil organic matter (Cheng et al. 2008) and the pathway to emit CH_4 from soil (Cheng et al. 2007), which may result in a higher CH_4 emission production from the *S. alterniflora* soil. Moreover, being consistent with the previous observations that the CH_4 emission from salt marshes (e.g. Lovley and Phillips 1987; Roden and Wetzwel 1996; Liikanen et al. 2002) is relatively low compared with that from other wetlands like freshwater wetlands, and rice fields (e.g. Ding et al. 2003; Towprayoon et al. 2005), this study shows that the flux rates of CH_4 in salt marsh ecosystems were quite low, ranging from 0.01 to 0.26 mg m^{-2} h^{-1} (Fig. 2a). There are two possible explanations for the very low CH_4 emission rates from salt marsh soils. One explanation is that the
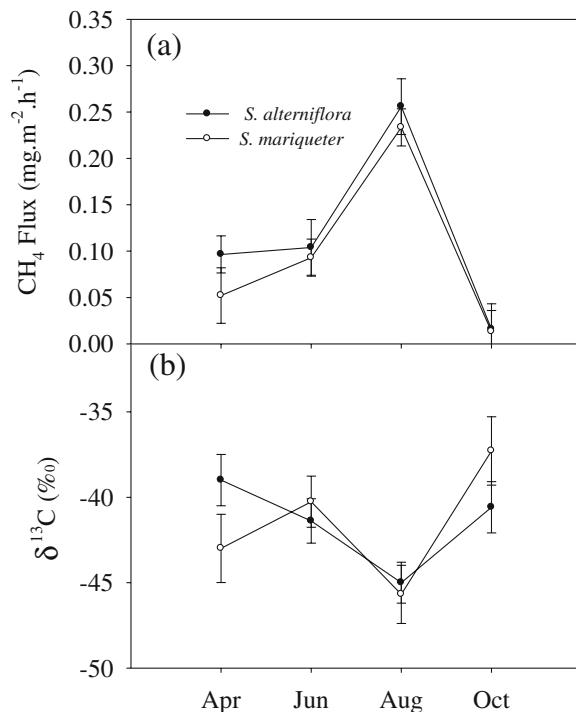
Fig. 2 Seasonal variation of CH_4 emission rates and its ^{13}C-isotopic signatures of *S. alterniflora* and *S. mariqueter* marshes in Jiuduansha wetland in Yangtze River estuary, China. Error bars represent SE (standard error) of the means (n=6)

Table 3 Summary of regression analyses between CH_4 emissions and soil properties of *S. alterniflora* and *S. mariqueter* marshes in Jiuduansha wetland in Yangtze River estuary, China

CH_4 Emission	Species	Independent Variable	Regression Equation	R^2 value	p value
	S. alterniflora	Soil T	Y=0.0088x - 0.08	0.33	0.049
		Soil M	Y=-0.0142x + 0.29	0.48	0.013
	S. mariqueter	Soil T	Y=0.029x - 0.66	0.80	0.0001
		Soil M	Y=-0.013x + 0.56	0.42	0.022

Soil T: soil temperature; *Soil M*: soil moisture

low emission rates from salt marsh soils in this study could be partly due to omission of CH_4 produced through the plant aerenchyma tissues (e.g., Ding et al. 2003; Cheng et al. 2007). Another explanation is that the low CH_4 emissions in the salt marshes might reflect the fact that the greater amounts of HS^- (or S^{2-}) and Fe^{3-} in the salt marsh soil limit CH_4 production (e.g., Kostka et al. 2002; Neubauer et al. 2005) compared to other freshwater wetlands. These HS^- (or S^{2-}) and Fe^{3-} reducers can outcompete methanogens for common substrates, thus, CH_4 emissions in coastal wetlands are generally lower than those in freshwater ones (e.g., Kostka et al. 2002; Neubauer et al. 2005).

Although the $\delta^{13}C$ value of *S. alterniflora* plant materials was significantly different from that of *S. mariqueter* (Table 1), the $\delta^{13}C$-CH_4 signature did not differ significantly between *S. alterniflora* and *S. mariqueter* soils (Fig. 2b). Since short-term *S. alterniflora* invasion only contributed less than 10% to the soil labile organic C in the top 100 cm soil layer and caused slightly higher $\delta^{13}C$ value of SOM compared with *S. mariqueter* (Cheng et al. 2008), we tentatively concluded that most of the emitted CH_4 might have been produced from soil organic carbon rather than from root exudates and residues. Meanwhile, in salt marsh sediments containing rich labile organic carbon (Cheng et al. 2008), CH_4 production occurs predominantly by the acetate fermentation pathway (Blair 1998; Edward et al. 2000), which is also the case in this study, reflected by $\delta^{13}C$-CH_4 values. Generally, soils containing an abundance of labile organic carbon follow the acetate fermentation pathway while soils with more recalcitrant organic matter follow the CO_2 reduction pathway (Edward et al. 2000). We found that the values of $\delta^{13}C$-CH_4 were lower in August compared to those in other months within each marsh (Fig. 2b). The lower value of $\delta^{13}C$-CH_4 is probably ascribed to reduced labile substrate for methanogenic

bacteria (e.g., Kutzbach et al. 2004; Conrad and Claus 2005). Additionally, the $\delta^{13}C$ values of surface emitted CH_4 is regulated by CH_4 oxidation, which tends to enrich ^{13}C in residual CH_4 (Krüger et al. 2002). The lowest $\delta^{13}C$ values corresponded to the highest CH_4 flux in August (Fig.2a vs. b), indicating that CH_4 production was greater than CH_4 oxidation.

Previous studies have reported that CH_4 emissions from wetlands are affected greatly by both soil temperature and moisture (e.g., Christensen 1993; Moosavi and Crill 1998; Kutzbach et al. 2004; Laine et al. 2007). For example, several studies have indicated that CH_4 production has a strong temperature response with reported Q_{10} values of 2.7–20.5 (e.g. Dunfield et al. 1993; Moosavi and Crill 1998). Our results showed that CH_4 emissions increased from April to August and decreased from August to October (Fig.2a), and were positively correlated with temperature (Table 3; P<0.05). This result is consistent with those of other studies in which CH_4 emissions are correlated with soil temperature in wetland ecosystems (e.g., Christensen 1993; Nykǎnen et al. 1998). Kutzbach et al. (2004) have reported that the ratios between CH_4 production and CH_4 oxidation are controlled directly by soil moisture which regulates the relative extent of oxic and anoxic environments within soils. Our results showed that the CH_4 emissions were negatively correlated with soil moisture in both *S. alterniflora* and *S. mariqueter* soils (Table 3; P<0.05). Christensen et al. (2001) have indicated that the impact of soil water on CH_4 emissions from wetlands depends on the water table status which can be envisaged as an on–off switch. When the water table falls below the soil surface, low soil moisture can drastically increase microbial CH_4 oxidation, which reduces CH_4 emissions (Kutzbach et al. 2004).

In summary, we found that *S. alterniflora* invasion on Jiuduansha Island increased CH_4 emissions com-

pared to the native *S. mariqueter* marsh. We assume that an increase in belowground biomass of *S. alterniflora* marsh changes soil microenvironments and/or the CH_4 production pathway. However, *S. alterniflora* invasion did not significantly change ^{13}C-CH_4-isotopic signature. CH_4 emissions from soils in both of two marshes followed similar seasonal patterns, and increased significantly from April to August and decreased from August to October. CH_4 emissions from *S. alterniflora* and *S. mariqueter* soils were positively correlated with soil temperature ($p<0.05$), but negatively correlated with soil moisture ($P<0.05$). Overall, the CH_4 emission from both *S. alterniflora* and *S. mariqueter* soils are relatively low compared to other types of wetlands, which is consistent with our previous results from the mesocosm study with *S. alterniflora* (Cheng et al. 2007). Although *S. alterniflora* invasion altered C and N dynamics in the salt marsh (Cheng et al. 2006, 2008; Liao et al. 2007), the results of this study, together with those of our early study (Cheng et al. 2007), tentatively suggest *S. alterniflora* invasion along the east coast of China may not be a significant potential source of atmospheric CH_4. Nevertheless, we should acknowledge a limited number of samples in this study that may not sufficiently address the possible *S. alterniflora* invasion effects on CH_4 emissions. More field studies and related modeling work are still needed to quantify the effects of *S. alterniflora* invasion on potential CH_4 emissions and its feedbacks to the future climate change on larger scales.

References

Bartlett KB, Harriss RC (1993) Review and assessment of methane emissions from wetlands. Chemosphere 26:261–320. doi:10.1016/0045-6535(93)90427-7

Blair N (1998) The δ^{13}C of biogenic methane in marine sediments: the influence of C_{org} deposition rate. Chem Geol 152:139–150. doi:10.1016/S0009-2541(98)00102-8

Bilek RS, Tyler SC, Sass RL et al (1999) Difference in CH_4 oxidation and pathways of production between rice cultivars deduced from measurements of CH_4 flux and δ^{13}C of CH_4

and CO_2. Global Biogeochem Cycles 13:1029–1044. doi:10.1029/1999GB900040

Brand WA (1996) High precision isotope ratio monitoring techniques in mass spectrometry. J Mass Spectrom 31:225–235. doi:10.1002/(SICI)1096-9888(199603)31:3<225::AID-JMS319>3.0.CO;2-L

Chanton JP, Whiting GJ, Blair NE et al (1997) Methane emission from rice: Stable isotopes, diurnal variations, and CO_2 exchange. Global Biogeochem Cycles 11:15–27. doi:10.1029/96GB03761

Chanton J, Liptay K (2000) Seasonal variation in methane oxidation in a landfill cover soil as determined by an in situ stable isotope technique. Global Biogeochem Cycles 14:51–60. doi:10.1029/1999GB900087

Chanton JP, Arkebauer TJ, Harden HS et al (2002) Diel variation in lacunal CH_4 and CO_2 concentration and d ^{13}C in *Phragmites australis*. Biogeochemistry 59:287–301. doi:10.1023/A:1016067610783

Chen JK (2003) A comprehensive Survey on Jiuduansha Island. Science Press, Beijing

Chen ZY, Li B, Zhong Y et al (2004) Local competitive effects of *Spartina alterniflora* invasions on *Scirpus mariqueter* in the Yangtze River estuary, China. Hydrobiologia 528:99–106. doi:10.1007/s10750-004-1888-9

Cheng X, Luo Y, Chen J et al (2006) Short-term C_4 plant *Spartina alterniflora* invasions change the soil carbon in C_3 plant-dominated tidal wetlands on a growing estuarine Island. Soil Biol Biochem 38:3380–3386. doi:10.1016/j.soilbio.2006.05.016

Cheng X, Peng R, Chen J et al (2007) CH_4 and N_2O emissions from *Spartina alterniflora* and *Phragmites australis* in experimental mesocosms. Chemosphere 68:420–427. doi:10.1016/j.chemosphere.2007.01.004

Cheng X, Chen J, Luo Y et al (2008) Assessing the effects of short-term *Spartina alterniflora* invasion on labile and recalcitrant C and N pools by means of soil fractionation and stable C and N isotopes. Geoderma 145:177–184. doi:10.1016/j.geoderma.2008.02.013

Christensen TR (1993) Methane emission from arctic tundra. Biogeochemistry 21:117–139. doi:10.1007/BF00000874

Christensen TR, Lloyd D, Svensson B et al (2001) Biogenic controls on trace gas fluxes in northern wetlands. IGBP-Global Change News Lett 51:9–15

Conrad R (1999) Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. FEMS Microbiol Ecol 28:193–202. doi:10.1111/j.1574-6941.1999.tb00575.x

Conrad R (2005) Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. Chem Geol 36:739–752

Conrad R, Claus P (2005) Contribution of methanol to the production of methane and its ^{13}C-isotopic signature in anoxic rice field soil. Biogeochemistry 73:381–393. doi:10.1007/s10533-004-0366-9

Dalal RC, Allen DE, Livesley SJ et al (2008) Magnitude and biophysical regulators of methane emission and consumption in the Australian agricultural, forest, and submerged landscapes: a review. Plant Soil 309:43–76. doi:10.1007/s11104-007-9446-7

Ding WX, Cai ZC, Tsurata H et al (2003) Key factors affecting spatial variation of methane emissions from freshwater

marshes. Chemosphere 51:167–173. doi:10.1016/S0045-6535(02)00804-4

Dunfield P, Knowles R, Dumont R et al (1993) Methane production and consumption in temperature and subarctic peat soils: response to temperature and pH. Soil boil Biochem 25:321–326

Edward R, Hornibrook C, Jongstaffe FJ et al (2000) Evolution of stable carbon isotope composition for methane and carbon dioxide in freshwater wetlands and other anaerobic environments. Geochim Cosmochim Acta 64:1013–1027. doi:10.1016/S0016-7037(99)00321-X

Ehrenfeld JG, Kourtev P, Huang W (2001) Changes in soil functions following invasions of exotic understory plants in deciduous forests. Ecol Appl 11:1287–1300. doi:10.1890/1051-0761(2001)011[1287:CISFFI]2.0.CO;2

Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems (N Y, Print) 6:503–523. doi:10.1007/s10021-002-0151-3

Fey A, Claus P, Conrad R (2005) Temporal change of ^{13}C-isoyope signatures and methanogenic pathway in rice field soil incubated anoxically at different temperatures. Geochim Cosmochim Acta 68:293–306. doi:10.1016/S0016-7037(03)00426-5

Flessa H, Rodionov A, Guggenberger G et al (2008) Landscape controls of CH_4 fluxes in a catchment of the forest tundra ecotone in northern Siberia. Glob Change Biol 14:2040–2056. doi:10.1111/j.1365-2486.2008.01633.x

IPCC (2007) Climate Change 2007: The Scientific Basis. Cambridge University Press, New York, USA

Joabsson A, Christensen TR, Wallén B (1999) Vascular plant controls on methane emissions from northern peatforming wetlands. Trends Ecol Evol 14:385–388. doi:10.1016/S0169-5347(99)01649-3

Kostka JE, Roychoudhury A, Van Cappellen P (2002) Rates and controls of anaerobic microbial respiration across spatial and temporal gradient in salt marsh sediments. Biogeochemistry 60:49–76. doi:10.1023/A:1016525216426

Krüger M, Eller G, Conrad R et al (2002) Seasonal variation in pathways of CH_4 production and in CH_4 oxidation in rice fields determined by stable carbon isotopes and specific inhibitors. Glob Change Biol 8:265–280. doi:10.1046/j.1365-2486.2002.00476.x

Kutzbach L, Wagner D, Pfeifer EM (2004) Effect of microrelief and vegetation on methane emission from wet polygonal tundra, Lena Delta, Northern Siberia. Biogeochemistry 69:341–362. doi:10.1023/B:BIOG.0000031053.81520.db

Laine A, Wilson D, Kiely G et al (2007) Methane flux dynamics in an Irish lowland blanket bog. Plant Soil 299:181–193. doi:10.1007/s11104-007-9374-6

Li B, Liao C, Zhao XD et al (2009) Spartina alterniflora invasions in the Yangtze River estuary, China: an overview of current status and ecosystem effects. Ecol Eng 35:511–520. doi:10.1016/j.ecoleng.2008.05.013

Liao CZ, Luo YQ, Fang CM et al (2008) Litter pool sizes, decomposition, and nitrogen dynamics in Spartina alterniflora-invaded and native coastal marshlands of the Yangtze Estuary. Oecologia 156:589–600. doi:10.1007/s00442-008-1007-0

Liao C, Luo Y, Jiang L et al (2007) Invasion of Spartina alteriflora enhanced ecosystem carbon and nitrogen stocks in the Yangtze Estuary, China. Ecosystems (N Y, Print) 10:1351–1361. doi:10.1007/s10021-007-9103-2

Lin GH, Ehleringer JR, Rygiewicz PT et al (1999) Elevated CO_2 and temperature impacts on different components of soil CO_2 efflux in Douglas-fir terracosms. Glob Change Biol 5:157–168. doi:10.1046/j.1365-2486.1999.00211.x

Liikanen A, Flŏjt L, Martikainen PJ (2002) Gas dynamics in eutropic lake sediments affected by oxygen, nitrate, and sulfate. J Environ Qual 31:338–349

Lovley DR, Phillips EJP (1987) Competitive mechanism for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Appl Environ Microbiol 53:2636–2641

Minkkinen K, Laine J (2006) Vegetation heterogeneity and ditches create spatial variability in methane fluxes from peatlands drained for forestry. Plant Soil 285:289–304. doi:10.1007/s11104-006-9016-4

Moosavi SC, Crill PM (1998) CH_4 oxidation by tundra wetlands as measured by a selective inhibitor technique. J Geophys Res 103(D22):29093–29106. doi:10.1029/97JD03519

Neubauer S, Givler K, Valentine S et al (2005) Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. Ecology 86:3334–3344. doi:10.1890/04-1951

Nykänen H, Alm J, Silvola J et al (1998) Methane fluxes on boreal peatlands of different fertility and the effect of long-term experimental lowering of the water table on flux rates. Global Biogeochem Cycles 12:53–69. doi:10.1029/97GB02732

Penning H, Plugge C, Galand P et al (2005) Variation of carbon isotope fractionation in hydrogenotrphic metahanogenic microbial cultures and environmental samples at different energy status. Glob Change Biol 11:2103–2113. doi:10.1111/j.1365-2486.2005.01076.x

Powelson DK, Charlton JP, Abichou T (2007) Methane oxidation in biofilters measured by mass-balance and stable isotope methods. Environ Sci Technol 41:620–625. doi:10.1021/es061656g

Qin P, Zhong CX (1992) Applied studies on spartina. Ocean Press, China

Rodhe H (1990) A comparison of the contribution of various gases to the greenhouse effect. Science 248:1217–1219. doi:10.1126/science.248.4960.1217

Rolston DE 1986. Gas flux. In Klute A (eds) Methods of Soil Analysis. The 2nd Edition, Monograph No. 9. American Society of Agronomy and Soil Science Society of America, Wisconsin, pp1103-1119.

Roden EE, Wetzwel RG (1996) Organic carbon oxidation and suppression of methane production by microbial Fe (III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnol Oceanogr 41:1733–1748

Rustad JC, Skinner JH, Marion GM et al (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and above-ground plant growth to experimental ecosystem warming. Oecologia 126:543–562. doi:10.1007/s004420000544

Schimel JP (1995) Plant transport and methane production as controls on methane flux from arctic wet meadow tundra. Biogeochemistry 28:183–200. doi:10.1007/BF02186458

Towprayoon S, Smakgahn K, Poonkaew S (2005) Mitigation of methane and nitrous oxide emissions from drained

irrigated rice fields. Chemosphere 59:1547–1556. doi:10.1016/j.chemosphere.2005.02.009

Van der Nat FJWA, Middelburg JJ (2000) Methane emission from tidal freshwater marshes. Biogeochemistry 49:103–121. doi:10.1023/A:1006333225100

Venkiteswaran JJ, Schiff S (2005) Methane oxidation: isotopic enrichment factors in freshwater boreal reservoirs. Appl Geochem 20:683–690. doi:10.1016/j.apgeochem.2004.11.007

Wang Q, An S, Ma Z et al (2006a) Invasive *Spartina alterniflora*: biology, ecology and management. Acta Phytotaxon. Sin. 44:559–588. doi:10.1360/aps06044

Wang Q, Wang CH, Zhao B et al (2006b) Effects of growing conditions on the growth of and interactions between salt marsh plants: implications for invasibility of habitats. Biol Invasions 8:1547–1560. doi:10.1007/s10530-005-5846-x

Whiting GJ, Chanton JP (1993) Primary production control of methane emission from wetlands. Nature 364:794–795. doi:10.1038/364794a0

Wilson D, Alm J, Laine J et al (2009) Rewetting of cutaway peatlands: Are we re-creating hot spots of methane Emissions? Restor Ecol. . doi:10.1111/j.1526-100X.2008.00416.x