Received: 28 October 2018 | Accepted: 28 February 2019

DOI: 10.1111/1365-2745.13164

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Spartina alterniflora invasion drastically increases methane production potential by shifting methanogenesis from hydrogenotrophic to methylotrophic pathway in a coastal marsh

Junji Yuan[1] | **Deyan Liu**[1] | **Yang Ji**[2] | **Jian Xiang**[1] | **Yongxin Lin**[1] | **Ming Wu**[3] | **Weixin Ding**[1]

[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China

[2]College of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing, China

[3]Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang, China

Correspondence
Weixin Ding
Email: wxding@issas.ac.cn

Funding information
Special Fund for Forest Scientific Research in Public Welfare, Grant/Award Number: 201404210; National Key Research and Development Program of China, Grant/Award Number: 2016YFD0200304 and 2016YFD0200309; National Natural Science Foundation of China; Natural Science Foundation of Jiangsu Province, Grant/Award Number: BK20151056

Handling Editor: Gabriela Bielefeld Nardoto

Abstract

1. Plant invasion can strongly influence carbon (C) cycling processes, thus it may affect climate change by altering C sequestration and greenhouse gas emissions in the invaded ecosystem. Since 1979, the exotic *Spartina alterniflora* has rapidly expanded in China's coastal areas, where significant increase in methane (CH_4) emissions has been documented from post-invaded sites. However, a mechanistic understanding of the structural and functional changes of associated methanogens accompanying this invasion remains elusive.
2. Here we conducted integrated biogeochemical investigations on methanogenic substrates, activity, and diversity to identify implications of *S. alterniflora* invasion for methanogenesis in coastal wetlands. To do this, we collected and analysed 0–50 cm soil profiles from an uncolonised tidal flat (TF) and salt marshes that *S. alterniflora* has invaded for 1 year (SA-1) and 12 years (SA-12) in Jiangsu, China. Methanogenic community composition was characterised by massive parallel sequencing. The rates and pathways of methanogenesis were determined by adding trace concentrations of ^{13}C-labelled substrates to anaerobic incubated samples.
3. Our results revealed that 12-year invasion of *S. alterniflora* drastically increased CH_4 production potential by one order of magnitude over that of TF. This substantial increase was primarily attributed to methanogenesis from trimethylamine; its rates increased by two orders of magnitude over TF whereas those from acetate and H_2/CO_2 increased far less. Hydrogenotrophic methanogenesis was the dominant pathway operating in the TF, but methanotrophic pathway contributed most to CH_4 production in the surface layer of SA-1 and uppermost 40-cm layers of SA-12. Consistent with these observations, the dominant methanogens shifted from obligate hydrogenotrophic *Methanococcales* in TF to potential methylamine-utilising *Methanosarcinaceae* in SA-12. Our Mantel analysis indicated that 'non-competitive' trimethylamine, derived from cytoplasmic osmolytes of *S. alterniflora*, was the major driver of this change in methanogenic community composition.

4. *Synthesis*. Our results suggest that invasive *Spartina alterniflora* plants gradually facilitated the local dominance of methylotrophic *Methanosarcinaceae* by changing the key type of methanogenic substrate in coastal marshes. Shifts in methanogen communities and enhanced availability of trimethylamine elevated the rates and importance of methylotrophic methanogenesis, thereby markedly increasing CH_4 production potential and emission rates in this type of ecosystem.

KEYWORDS

^{13}C tracing, methanogen, methanogenesis pathway, methanogenic substrates, plant invasion, salt marsh, *Spartina alterniflora*, trimethylamine

1 | INTRODUCTION

Invasive species pose a grave threat to the biodiversity and stability of native ecosystems worldwide (Lambertini et al., 2011). By altering resource availability, trophic structure, and subterranean microbial communities, invasive plants can profoundly affect ecosystem functioning and processes, with implications for climate change effects (Norton et al., 2008). On the one hand, compared with native plants, invasive plants typically increase net primary productivity (Liao et al., 2008) and accelerate nitrogen (N) fluxes (Castro-Díez, Godoy, Alonso, Gallardo, & Saldaña, 2014), thereby increasing soil carbon (C) and N pools in post-invaded ecosystems. On the other hand, this newly altered resource environment may strongly influence soil microbial communities and their dynamics (Piper, Siciliano, Winsley, & Lamb, 2015; Ramirez, Lauber, Knight, Bradford, & Fierer, 2010), which regulate the stability of soil organic C (SOC) and greenhouse gas (nitrous oxide (N_2O) and CH_4) emissions in the invaded ecosystems (Tamura & Tharayil, 2014; Yuan et al., 2015). Since climate change and exotic plant invasion are predicted to strongly interact with catastrophic outcomes (Dawson, Rohr, van Kleunen, & Fischer, 2012), a mechanistic understanding of how plant invasions influence greenhouse gas production remains an important ecological and environmental issue (Tylianakis, Didham, Bascompte, & Wardle, 2008).

Plant invasion can alter the composition and activity of soil microbial communities by altering the quality and quantity of leaf litter, as well as the composition of root exudates (Eilers, Lauber, Knight, & Fierer, 2010). For instance, grass invasion increased the abundance and changed the composition of ammonia-oxidising bacteria by augmenting the mineralisation rates of exogenous organic materials and the availability of ammonium, thereby increasing nitrification rates (Hawkes, Wrem, Herman, & Firestone, 2005) and N_2O emissions in soil (Norton et al., 2008). In contrast, weak or negligible influences on soil microbial composition and activity under plant invasion have also been reported (e.g., Cruz-Martinez et al., 2009).

Compared with other terrestrial ecosystems, such as shrubland and grassland, natural wetlands are more susceptible to invasive plants and more prone to becoming monocultures after an invasion (McCary, Mores, Farfan, & Wise, 2016). In wetlands, invasive plants have a higher net primary productivity than native plants, and they can have more pronounced effects on trophic structure and subterranean C cycles than in other ecosystems (Angeloni, Jankowski, Tuchman, & Kelly, 2006; McCary et al., 2016). As a major source of atmospheric CH_4, biogenic CH_4 production in wetlands is regulated by methanogens (Conrad, 2007,2009): these microbes not only differ in their CH_4 production pathway and potential but may also respond differently to trophic and environmental changes (Jetten, Stams, & Zehnder, 1992). Therefore, linking methanogenic community composition to its functioning in CH_4 production is essential for understanding the cascade effect of plant invasions on CH_4 emissions in wetland ecosystems.

Spartina alterniflora is a C_4 plant native to the Atlantic and Gulf coasts of North America that was intentionally introduced to China in 1979 for coastal erosion control (Qin & Zhong, 1992). Since then it has spread rapidly in Chinese coastal regions and becomes one of the dominant plants in the land-water ecotone, as it did in Pacific Coast estuaries of the USA and other East Asian countries (Bertness, 1991; Davis, Taylor, Civille, & Strong, 2004; Henmi, Fuchimoto, Kasahara, & Shimanaga, 2017). In these invaded wetlands not only is this plant a threat to birds, fish, and shellfish that depend on open habitats (Grevstad, 2005), but it also has dramatically changed the cycling of C and nutrients (Benner, Fogel, & Sprague, 1991; Liao et al., 2008). *S. alterniflora* has greater net primary productivity than co-occurring native plants, which presumably could lead to enlarged C stocks in plant biomass and soils (Zhang, Ding, Luo, & Donnison, 2010). Yet, although the elevated organic C input has increased CH_4 emissions in the salt marshes (Yuan et al., 2015), this was disproportionate to any increase in SOC. For example, when compared with a nearby bare tidal flat (TF), 12 years of *S. alterniflora* invasion augmented the soil C stock in the top 1m profile by 2.24 times while the corresponding CH_4 emissions have increased by 5.05 times (Yuan et al., 2015). Hence, relative to SOC content, ensuing changes to the methanogenic substrate type and methanogen community composition might be more responsible for the stimulated functioning of CH_4 production typical of invaded marshlands (Buckley, Baumgartner, & Visscher, 2008; Emery & Fulweiler, 2014).

Nevertheless, microbial processes in salt marshes are strictly governed by the ordering of oxygen, nitrate, Mn (IV), Fe (III), and sulphate along with the redox gradient, with sulphate being the

most common acceptor (Wilms, Köpke et al., 2006). Thus salt marshes are typically redox-stratified and feature depth-related chemical gradients (Lambais, Otero, & Cury, 2008; Wilms, Sass et al., 2006). Thermodynamically, methanogens are out-competed by sulphate reducers for H_2 and acetate, and CH_4 production via these 'competitive' substrates is generally inhibited until the available sulphate is depleted (King, 1984). However, methylotrophic methanogenesis that utilises 'non-competitive' substrates – for example, methylated compounds, such as, methylated amines, methanol, and dimethyl sulfide – is known to co-occur in the presence of sulphate (Oremland, Marsh, & Desmarais, 1982), and was found to be of greater importance in sulphate-rich coastal sediments (Parkes et al., 2012; Zhuang et al., 2016). In the Atlantic coasts of North America, *S. alterniflora* was found to release amines, especially trimethylamine, into salt-marsh sediment during decomposition (Wang & Lee, 1994). Moreover, this plant is a unique salt marsh macrophyte in that it absorbs sulphate from tidewater to synthesise dimethylsulfoniopropionate (DMSP) (Husband & Kiene, 2007; Rhodes & Hanson, 1993). Conversely, the decomposition of DMSP can release sulphur, which can be further oxidised by *S. alterniflora* roots, thereby increasing the storage capacity of sulphate in salt marsh soils (Dacey, King, & Wakeham, 1987; Lee, Kraus, & Doeller, 1999; Zhou et al., 2009).

Here, we hypothesised that *S. alterniflora* gradually alters chemical and trophic gradients and the abundance and community composition of methanogens in its invaded sites, thus augmenting the rates and pathways of methanogenesis. This study had two objectives: (a) to evaluate the influence of *S. alterniflora* on methanogenic community composition and the rates and pathways of CH_4 production in profiled soils; and (b) to better understand how the composition and functioning of methanogens is linked to geochemical characteristics of a wetland under *S. alterniflora* invasion.

2 | MATERIALS AND METHODS

2.1 | Site description and soil sampling

The sites located in the Yancheng National Wetland Reserve, in Jiangsu Province, China (33°36'N, 120°36'E) – details of its hydrological, vegetation, and soil characteristics were previously described (Yuan, Ding, Liu, Xiang, & Lin, 2014). Briefly, the coastal salt marsh features typical alluvial mudflats with semidiurnal tidal periodicity, with a tidewater salinity of 30.0‰–32.0‰. The native plant species *Suaeda salsa* dominated this area until 1982 before *S. alterniflora* was intentionally introduced (Chung, Zhuo, & Xu, 2004). Since then, this invasive species has not only gradually replaced the native plants but also rapidly spread over the intertidal mudflats. During 1992–2007, >75% of the new invaded marsh in this region was initially derived from bare flats (Zheng, Zhang, Jiang, & Wang, 2009).

Soil sampling was carried out in June 2011 at three sites. Bare TF and two sites where *S. alterniflora* colonised in 2010 (SA-1) and 1999 (SA-12), respectively, were identified from Thematic Mapper (TM) satellite images (Figure S1). The *S. alterniflora* sites were formerly pristine bare TF, and all three sites were flooded semidiurnally. The pre-invasion soil contained sand 7.8%, silt 82.2%, and clay 10.0%, with a pH of 8.4. Before sampling, three 5m × 5m plots in each site were randomly located with an inter-plot distance of 50m. Soil cores in each plot (depth to 50 cm) were obtained in triplicate using a Wardenaar® peat profile sampler (Eijelkamp, Netherlands) and divided into 10cm-thick sections in the field (with a plastic spatula). Replicate sections of the same layer in each plot were carefully mixed to form a composite plot-level sample. A subsample from each was placed into a sealed plastic bag, transported on ice to the laboratory, and stored at −20°C until the microbial and geochemical analyses. Another subsample was air-dried for the analysis of soil properties. A 50cm × 50cm quadrat in each plot was sampled to estimate the density of *S. alterniflora* and the weight of its litter and above- and below-ground biomass. Plants and litter in each quadrat were clipped at the soil surface for their height and stem density measurements. Subterranean biomass was determined via the core method (Mazzilli, Kemanian, Ernst, Jackson, & Piñeiro, 2015): soil cores (diameter = 5 cm, length = 50 cm) were taken from each quadrat, with the roots and rhizomes then separated from the soil using water and mesh sieves. The litter and above- and below-ground biomass samples were oven-dried for 24 hr at 70°C and weighed.

2.2 | Soil properties

Concentrations of SOC and total N (TN) were determined by using the wet oxidation redox titration method and the micro-Kjeldahl method, respectively. Dissolved organic and inorganic components of the fresh soils were extracted using boiled distilled water (CO_2 removed) in a soil-to-water ratio of 1:2 (w/v) for 30 min under agitation at 200 rpm in a flask, and centrifuged at 2,500 *g* for 25 min at 4°C. Part of the supernatant was then filtered through a 0.45μm pore size membrane filter (Whatman, Clifton) and its bicarbonate concentration determined by potentiometric titration. The remaining supernatant was immediately acidified with 2 ml of 2 M HCl and filtered. The analysis of sulphate was performed on an ICS-1100 ion chromatography system (Dionex, Camberley, UK) fitted with an AS11-HC 2mm column and a conductivity detector. Dissolved organic C (DOC) was measured on a Multi N/C 3100 TOC analyser (Analytik Jena AG, Jena, Germany). Organic acid concentrations were determined using an LC-2010HT high-performance liquid chromatography apparatus (Shimadzu, Kyoto, Japan), fitted with a Shodex RS-Pak KC-811 column (Waters Corporation, Milford), and an ultraviolet-visible spectrum detector (SPD-20A/20AV, Shimadzu, Kyoto, Japan). Acidified extracts were concentrated to approximately 7–8 ml from 100 ml by diffusion at 55°C. An aliquot (6 ml) of each concentrated extract was pipetted into a 10ml vial that contained 2 g NaCl. The vial was sealed with Teflon-faced septa before injecting 0.5 ml of 10 M NaOH. All vials were individually conditioned for 40 min at 65°C and 500 rpm agitation to volatilise the methylated amines. The concentration of amines was determined by injecting a 1.0ml headspace sample into a gas chromatography-mass spectrometry (GC-MS) system, which consisted of a helium CP 3800 GCs (Varian, Darmstadt, Germany) coupled to a Saturn 2200 MS (Varian, Darmstadt, Germany). The

temperature of the injector, detector, and transfer line was set at 120°C, 250°C, and 280°C, respectively. The column temperature was initially held at 35°C for 3 min, then increased at a rate of 35°C/min to 120°C, at which it was maintained for 2 min to remove any volatile or semi-volatile interference before the next injection. The flow rate of the helium carrier gas was set at 1 ml/min, by using the splitless mode. Electron impact mode at 70 eV was used, and the quantification of amines performed under the selected ion-monitoring mode (Yuan et al., 2014). The method detection limit was approximately 0.22 μM for trimethylamine and 0.35 μM for dimethylamine.

2.3 | Methanogenic activity

The CH_4 production rates were measured using $NaH^{13}CO_3$, [U-^{13}C] sodium acetate and trimethylamine (99% enriched, Cambridge Isotope Laboratories, Woburn). From each fresh soil sample, 10 g (dry weight) was placed into a 100ml incubation jar containing anoxic artificial seawater (soil-to-water ratio of 1:2) and thoroughly mixed into the slurry. Artificial seawater was prepared according to Buckley et al. (2008), but dissolved inorganic C was excluded from the mixture. Each jar was sealed with butyl rubber septa, evacuated with a vacuum pump, and flushed with N_2. Before the injection of ^{13}C-labelled substrates, these jar samples were incubated at 25°C in the dark for 3 days until CH_4 production rates had stabilised. Although the sampling, storage, and mixing procedure might have affected microbial activity in soil samples, it should not have biased the effects of different treatments and temporal behaviour. Then, 0.5 ml of a stock solution containing a ^{13}C-labelled substrate treatment – 40 μM acetate, 40 μM trimethylamine, or 400 μM bicarbonate – was injected into the three replicates to yield a final ^{13}C-substrate concentration of 1.0 μM each for acetate and trimethylamine, and 10 μM for bicarbonate. This range of added substrate concentrations was chosen on purpose to maximise the detection of produced $^{13}CH_4$ without stimulating methanogenesis (i.e., ^{13}C being added in trace quantities) (Kelley, Chanton, & Bebout, 2015; Kelley, Poole, Tazaz, Chanton, & Bebout, 2012). All the samples were incubated at 25°C in the dark for 8 hr after injection. Gas samples were taken and analysed for their concentration and δ^{13}C of CH_4 during incubation. The CH_4 production potential of soil was measured using six replicates of control (i.e., no substrate added).

Methane concentration in the headspace was analysed by GC12A gas chromatography (Shimadzu, Kyoto, Japan) equipped with a flame ionisation detector. The δ^{13}C of CH_4 was analysed on a gas chromatography combustion isotope ratio mass spectrometer system (Thermo Finnigan MAT 253, Bremen, Germany). Precision for δ^{13}C of CH_4 was ± 0.19% based on repeated measurements of a working laboratory standard. The δ^{13}C of the samples was expressed as follows:

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

where R_{sa} is the $^{13}C/^{12}C$ ratio of the sample, and R_{st} is the $^{13}C/^{12}C$ ratio of the Pee Dee Belemnite standard.

The rates of CH_4 production from acetate, H_2/CO_2, and trimethylamine were calculated by considering the proportion of $^{13}CH_4$ produced from the corresponding added ^{13}C substrate (acetate, bicarbonate, or trimethylamine), the concentration of substrates in the incubated slurry, and the incubation time, as follows:

$$R_{sub} = a \times \frac{\left(F_t m_t - F_0 m_0\right) - \left(F_* m_{t*} - F_* m_{0*}\right)}{m_s \times t} \times \frac{n_{sub}}{n_{^{13}C-sub}} \quad (2)$$

where F_0 and F_t are fractional abundances (converted by δ^{13}C) of the C isotopes of CH_4, respectively, at the beginning and end of the incubation in ^{13}C-substrate addition jars; F_* is the fractional abundance of C isotopes of CH_4 in control jars; m_0 and m_t are the amounts (μmol) of CH_4 in the headspace of ^{13}C-substrate addition jars at the beginning and end of the incubation, respectively; m_{0*} and m_{t*} are the amounts (μmol) of CH_4 in the headspace of control jars at the beginning and end of incubation, respectively; n_{sub} and $n_{^{13}C-sub}$ are the initial amounts (μmol) of the substrates and added ^{13}C-substrate; m_s is the mass of incubated soils (kg); t is incubation time (hr); and α is the isotopic fractionation factor (presumed to be 1.04 for bicarbonate, 1.02 for acetate, and 1.06 for trimethylamine; Zhuang et al., 2016).

The relative contribution of methanogenesis pathways was calculated as follows:

$$f_{sub} = R_{sub}/MPP \quad (3)$$

where f_{sub} is the fraction of CH_4 produced from acetate (f_{Ac}), H_2/CO_2 (f_{H2}), and trimethylamine (f_{TMA}); MPP is the CH_4 production potential.

Relative turnover times for all ^{13}C-substrates were expressed as the days required to metabolise to CH_4 from the total amount of added ^{13}C-substrate, as follows (Parkes et al., 2012):

$$\text{Turnover time} = n_{^{13}C-sub}/\left(m_{^{13}CH_4}/t\right) \quad (4)$$

where $n_{^{13}C-sub}$ is the amount of added ^{13}C-substrate (μmol) and $m_{^{13}CH_4}$ is the amount of $^{13}CH_4$ produced (μmol) from the added ^{13}C-substrate.

2.4 | Molecular analysis

Microbial DNA was extracted from soils (0.5 g, in triplicate) using the FastDNA SPIN Kit for Soil (Bio 101, Carlsbad). This extracted DNA was used for qPCR and Illumina Miseq sequencing. The numbers of bacterial and archaeal 16S rRNA gene copies and methanogenic *mcrA* gene copies were determined by qPCR, respectively, using the primer combinations 519F/907R for bacteria (Stubner, 2002), Arch344F/Arch915R for archaea (Zheng et al., 2013), and MLf/MLr for *mcrA* gene (Juottonen, Galand, & Yrjälä, 2006). PCR reactions were performed in a CFX96 Optical Real-Time Detection System (Bio-Rad Laboratories Inc., Hercules, USA) in a total volume of 20 μl that contained 1 × SYBR® *Premix Ex Taq*™ (TaKaRa Biotech, Dalian, China), 0.5 μM of each primer, and 1 μl of the DNA template (1–10 ng). Amplifications for the bacterial and archaeal 16S rRNA gene began with denaturing at 95°C for 5 min, followed by 30 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. The amplification procedure for *mcrA* gene consisted of initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturing at 95°C for 10 s, annealing at 55°C for 30 s, extension at

72°C for 30 s, with the plate finally read at 80°C. Real-time PCR was performed for each of the triplicates. Their standard curves were generated using 10-fold serial dilutions of linearised plasmids containing the bacterial (or archaeal or *mcrA*) gene from the environmental samples.

Specific PCR primers – 524F10ext, 5'-TGYCAGCCGCCGCGGTAA-3' and Arch958R, 5'-YCCGGCGTTGAVTCCAATT-3' – targeting the V4–V5 region of the archaeal 16S rRNA gene were used (Pires et al., 2012). The PCR conditions consisted of initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s, with a final extension at 72°C for 10 min. The purified amplicons were first pooled in an equimolar concentration before their sequencing using a HiSeq 2000 system (Illumina Inc., San Diego). All the raw archaeal reads have been deposited in the DNA Data Bank of Japan under the accession number DRA007727.

The obtained sequence data were processed by QIIME v1.8.0 and low quality archaeal sequences deleted (Bates et al., 2011), with any chimeric archaeal sequences screened by the UCHIME algorithm (Edgar, Haas, Clemente, Quince, & Knight, 2011). The remaining sequences were de-noised and clustered into operational taxonomic units (OTUs), with a 97% similarity cut-off, by using UPARSE v7.0.1001 (Edgar, 2013). A representative sequence within each OTU was classified in the mothur software platform (Schloss et al., 2009) and aligned against the SILVA archaeal 16S rRNA gene database. Only those OTUs belonging to methanogens and anaerobic methane-oxidising archaea (ANME) were retained for further analyses.

2.5 | Statistical analyses

All the statistical analyses were performed using R v3.2.2 (R Core Team, 2015), with all the data expressed in terms of the oven-dried soil. One-way and two-way analysis of variance (ANOVA), followed by post-hoc Tukey's HSD tests, were used to compare plant characteristics, soil properties, gene abundances, relative abundances of methanogenic archaeal taxa, and rates and fractions of CH_4 produced from substrates among the coastal sites (TF, SA-1, SA-12) and their soil depth layers (five 10cm intervals in the 0–50 cm profile) within the same site. Before this analysis, the response variables above were evaluated for normality and homogeneity and ln-transformed when necessary to satisfy ANOVA assumptions.

For beta-diversity, nonmetric multidimensional scaling (NMDS) was carried out using the 'metaMDS' function. Differences in methanogenic community composition among sites and soil layers were assessed in a permutational multivariate analysis of variance (PERMANOVA), which relied on Bray–Curtis dissimilarity, by using the 'adonis' function. Using these same dissimilarity matrices, we evaluated relationships between soil variables and methanogenic community composition with a Mantel test, with 999 permutations, by using the 'mantel' function. A heatmap was constructed, using the 'ggplot2' package, for the OTUs that explained most of the differences found between the site and soil layer samples. Pearson correlations were used to explore the associations between soil properties and microorganism abundances. Regression models (linear and polynomial) were used to quantify how the f_{TMA}, rates of methanogenesis, and turnover time of trimethylamine depended on the relative abundance of *Methanosarcinaceae*. Differences between group means and fitted regressions were deemed significant at $p < 0.05$. One caveat to note is that we applied statistical tests to data from same-site samples used as replicates for testing the main effect of the site; since the three types of sites could not be truly replicated for logistical reasons, this may limit the inferences we can appropriately draw.

3 | RESULTS

3.1 | Plant and soil characteristics

The above ground plant biomass of the invasive *S. alterniflora* in SA-1 was 75.3% greater than that in SA-12 (Table 1). By contrast, its below ground biomass was significantly higher in SA-12 than in SA-1; in the latter, there was barely any litter (near zero), unlike for SA-12 (>1 kg/m^2).

The average concentrations of SOC, TN, and DOC increased with the invasion duration of *S. alterniflora*, and decreased with soil depth layers at all three sites (Figure 1). They were significantly higher in SA-12 for all layers analysed, but were only significantly higher in the 0–20 cm layer of SA-1 than in the corresponding layer of TF ($p < 0.05$). Sulphate concentration in soils decreased markedly with depth in SA-12, from 32.4 to 9.87 mmol/kg, yet it was notably higher than in TF and SA-1 wherein its profile was less changed. Similarly, the mean concentrations of acetate and trimethylamine increased with invasion duration. Acetate concentration was significantly ($p < 0.05$) higher in SA-12 than in TF and SA-1 in all layers analysed, and it was

TABLE 1 Biological characteristics of the *Spartina alterniflora* community in the Yancheng coastal salt marsh, Jiangsu Province, China

Site	Invasion duration (years)	Aboveground biomass (kg/m^2)	Belowground biomass (kg/m^2)	A/B ratio*	Litter (kg/m^2)
SA-1	1	2.68 ± 0.39a	2.85 ± 0.20b	0.94 ± 0.08a	0.02 ± 0.00b
SA-12	12	1.53 ± 0.29b	3.37 ± 0.23a	0.45 ± 0.05b	1.53 ± 0.29a
F-value	—	17.15	8.74	80.40	100.18
p-value	—	0.014	0.042	0.001	0.001

Note. Values are means ± SE ($n = 3$). Different letters (a or b) within the same column indicate significant differences at $p < 0.05$.
*Ratio of aboveground to belowground biomass.

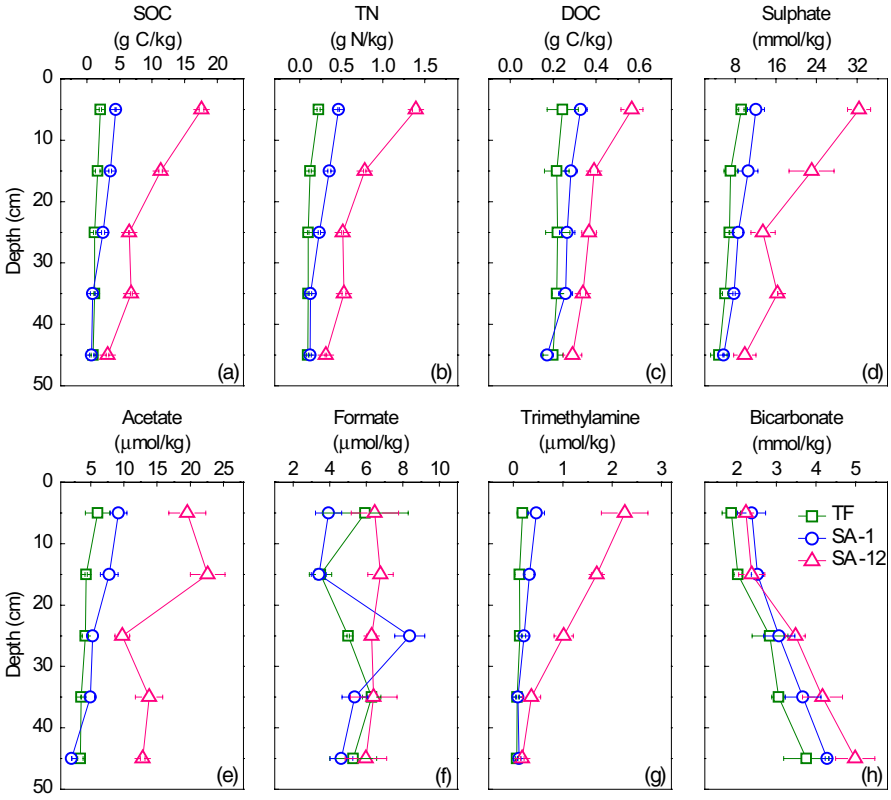

FIGURE 1 Geochemical vertical profiles of a bare tidal flat (TF), and two coastal marshes invaded by *Spartina alterniflora* in 2010 (SA-1) and 1999 (SA-12), respectively. (a) soil organic carbon (SOC), (b) total nitrogen (TN), (c) dissolved organic carbon (DOC), (d) sulphate, (e) acetate, (f) formate, (g) trimethylamine, and (h) bicarbonate. Horizontal bars denote standard errors of means (n = 3) [Colour figure can be viewed at wileyonlinelibrary.com]

generally higher in the upper layers of SA-12, peaking in the 10–20 cm layer (Figure 1e); it was also significantly higher in the 0–20 cm layer of SA-1 than that of TF ($p < 0.05$). Compared with TF, the mean concentration of trimethylamine in SA-1 and SA-12 increased by 208% and 935%, respectively, with both maxima occurring in the surface layer. A significant ($p < 0.05$) difference in trimethylamine was found in the 0–40 cm layers between SA-12 and SA-1 or TF, but only so in the 0–20 cm layers between SA-1 and TF (Figure 1g). The highest formate concentration was measured in the 20–30 cm layer of SA-1, yet its concentrations were similar among the three sites.

3.2 | Abundance of microorganisms

Two-factor ANOVA revealed significant effects of site, layer and their interaction on the abundance of soil microorganisms (Table S1). *S. alterniflora* invasion significantly increased bacterial abundance by 145%–782% and 79.0%–290% in SA-12 and SA-1, respectively, compared with TF (Figure 2a). However, these numbers dropped drastically with soil depth, especially in SA-12. The archaea numbers were generally lower than those of bacteria by one order of magnitude, but their soil depth profile patterns were similar (Figure 2b). The *S. alterniflora* invasion had a stronger positive effect on archaea, as showed by their 261%–1,830% increase in SA-12 over TF. Similarly, when compared with TF, 12-year invasion by *S. alterniflora* increased the abundance of *mcrA* genes by 614%–3,380% whereas the 1-year invasion only increased them by 4%–122% (Figure 2c). Abundances of bacteria, archaea, and *mcrA* genes were significantly correlated with SOC, TN, DOC, trimethylamine, acetate, and sulphate ($p < 0.05$), but not with formate or bicarbonate (Table S2).

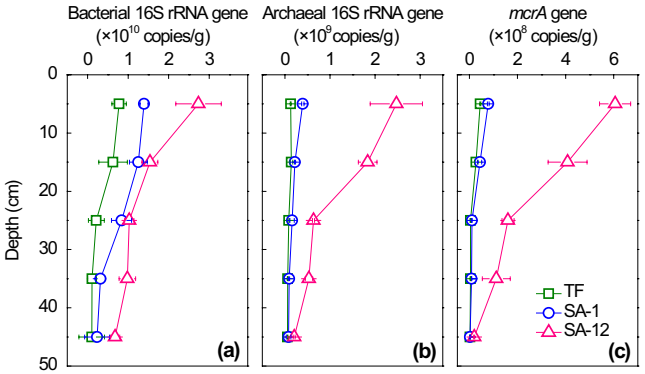

FIGURE 2 Vertical profiles for the abundance of (a) bacterial 16S rRNA gene copies, (b) archaeal 16S rRNA gene copies, and (c) *mcrA* gene copies determined using 'real-time' PCR. Horizontal bars denote standard errors of means (n = 3). Tidal flat (TF) is a bare tidal flat; SA-1 and SA-12 are coastal marshes invaded for 1 and 12 years by *Spartina alterniflora*, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Methanogenic community composition and diversity

High-throughput sequencing of the archaeal 16S rRNA gene was used to determine the major methanogen taxa in marsh soils. Total OTUs consisted of six different orders of methanogens in the

phyla Euryarchaeota, including putative hydrogenotrophic (all except *Methanosaetaceae* and *Methanomassiliicoccales*), acetoclastic (*Methanosaetaceae* and *Methanosarcinaceae*), and methylotrophic (*Methanosarcinaceae* and *Methanomassiliicoccales*) organisms; yet methanogens in both Bathyarchaeota and Verstraetearchaeota phyla were not detected.

The *S. alterniflora* invasion had gradually changed the community composition of archaea potentially involved in CH_4 cycling (i.e., methanogen and ANME; Figure 3). In TF, the obligate hydrogenotrophic methanogens (i.e., *Methanococcales*, *Methanobacteriales*, *Methanomicrobiales*, *Methanocellales*) clearly dominated the community: their relative abundance increased with depth, going from 59.2% (0–10 cm layer) to 81.9% (40–50 cm layer), while that of *Methanococcales* correspondingly went from 27.8% to 56.6%. By contrast, the relative abundance of versatile *Methanosarcinaceae* and obligate acetoclastic *Methanosaetaceae* decreased with depth, from 23.6% (0–10 cm) to 5.82% (40–50 cm) and from 9.37–11.4% (0–20 cm) to 1.69% (40–50 cm), respectively. The H_2-dependant methylotrophic *Methanomassiliicoccales* accounted for 7.09%–23.5% of total methanogens. In SA-1, members of *Methanosarcinaceae* were dominant in the 0–10 cm layer (44.4%) but their relative abundance decreased to 7.00% in the 40–50 cm layer, while hydrogenotrophic methanogens represented 59.3%–75.5% of all methanogens in the 10–50 cm layer. Members of *Methanosarcinaceae* dominated the 0–40 cm layer in SA-12 and their relative abundance was significantly higher than either that of TF or SA-1 ($p < 0.05$). Similarly, the relative abundance of *Methanosaetaceae* in each layer was also higher (7.21%–23.2%) in SA-12 than in TF (1.69%–11.4%) or SA-1 (2.46–11.1%). In contrast, relative abundances of hydrogenotrophic methanogens and *Methanomassiliicoccales* were both lower in SA-12 than in TF or SA-1. However, at all sites the ANME were only barely detected.

For methylotrophic methanogens, OTU1017 (*Methanosarcina*) was dominant in all the samples (Figure S2), and it had a relatively higher abundance in the soil depth profile of SA-12 than that of TF and SA-1, yet at all three sites it showed a similar decreasing trend with depth. The relative abundances of OTUs belonging to the order *Methanomassiliicoccales* (OTU7162, OTU16009, OTU13209) were generally lower than that of OTU1017, and they showed a more equal distribution between sites and between soil depth layers.

Shifts in methanogenic community composition in response to *S. alterniflora* invasion were discerned by the NMDS analysis (Figure 4). Samples were clearly separated by layers across axis 1 and by sites across axis 2; this finding was confirmed by the PERMANOVA analysis (Table 2). Mean distances and the variability in methanogenic community composition were greatest in SA-1, followed by SA-12 and TF. Additionally, the compositions of the methanogenic community in the 40–50 cm layer at each site clustered more closely than those in the upper soil layers in the NMDS plot. The heatmap of the top 32 OTUs suggested the samples were separated into two major clusters: the distribution of OTUs in the 0–40 cm layer of SA-12 and the 0–10 cm layer of SA-1 were distinct from those in the 10–50 cm layers of SA-1 and TF (Figure S3). In the former cluster, *Methanosarcinaceae* OTU1017 was most abundant while in the latter was *Methanococcales* (OTU17825 and OTU6819). Mantel testing revealed the methanogenic

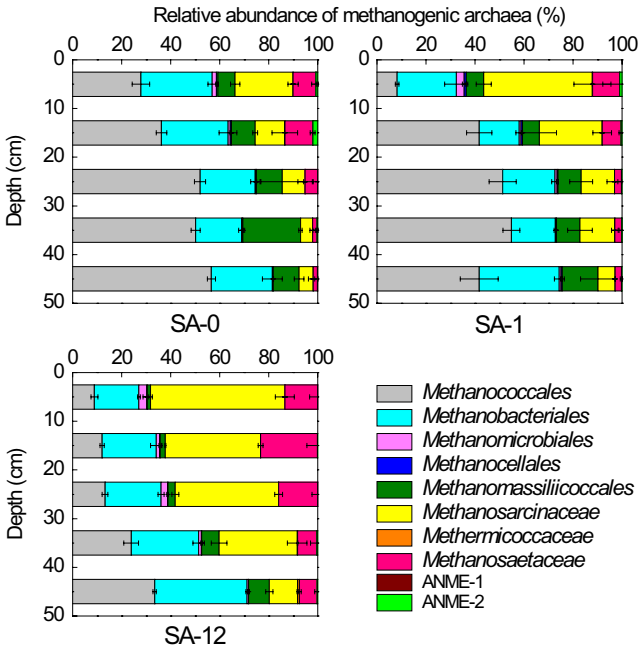

FIGURE 3 Relative abundance of taxonomic groups among total sequences that are potentially involved in CH_4 cycling (methanogen and anaerobic methane-oxidising archaea, ANME), as determined by high-throughput sequencing. Error bars are standard errors of means ($n = 3$). Tidal flat (TF) is a bare tidal flat; SA-1 and SA-12 are coastal marshes invaded for 1 and 12 years by *Spartina alterniflora*, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

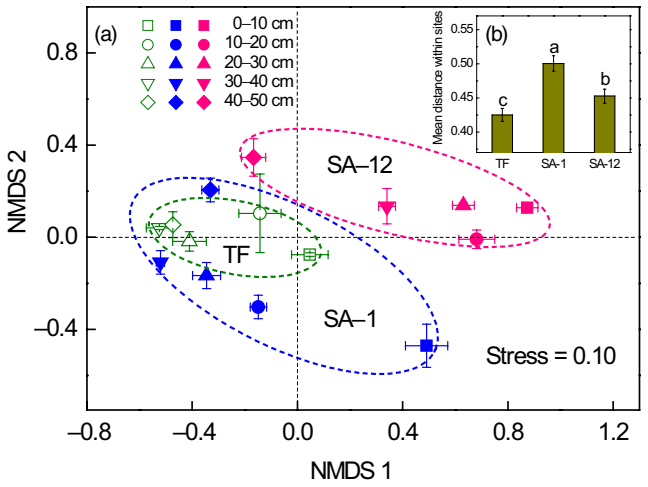

FIGURE 4 (a) Nonmetric multidimensional scaling (NMDS) plot of methanogen community composition, calculated from a distance matrix of the relative abundances of methanogenic archaeal 16S rRNA gene OTUs (with at least 97% sequence similarity). Symbols are the mean values of the replicated individual horizons of each site. Error bars are standard errors of means ($n = 3$). The insert in the upper right corner (b) shows the corresponding mean (±*SE*) distances within each site. The open olive, solid blue, and solid pink symbols respectively represent TF (bare tidal flat), SA-1 (1-year *Spartina alterniflora* invasion), and SA-12 (12-year *S. alterniflora* invasion) [Colour figure can be viewed at wileyonlinelibrary.com]

community composition as being significantly correlated with concentrations of SOC, TN, DOC, acetate, sulphate, and especially trimethylamine, but not with formate or bicarbonate (Table 3).

3.4 | Methanogenesis

The rate of methanogenesis measured in soils without ^{13}C-substrate addition (i.e., CH_4 production potential) was highest in the upper layer, decreasing with depth at all sites (Figure 5a and Figure S4). In the SA-12 layers the potential rates ranged from 0.89 to 6.42 μmol CH_4 kg^{-1} d^{-1}, or about 6.56–14.9 times higher than those of TF, while they only increased by 166%–315% in the 0–30 cm layer of the site invaded for 1 year by *S. alterniflora*. The $\delta^{13}C$ values of CH_4 produced during the incubation were similar in all layers of each site, but they generally increased with soil depth in unamended controls (Figure 5b and Figure S5). By contrast, CH_4 became more ^{13}C-depleted under a longer duration of invasion (Figure 5b).

The highest rates of methanogenesis from each substrate (as measured by trace ^{13}C-substrate additions) were always detected in the 0–10 cm layer of the three sites (Figure 6). In TF, rates for trimethylamine were significantly lower than those for acetate and H_2/CO_2 in each layer; at this site, H_2/CO_2 was the main substrate used, contributing 34.2% (0–10 cm) to 53.3% (40–50 cm) of the total CH_4 produced (f_{H2}). However, the contribution of acetate (f_{Ac}) and trimethylamine (f_{TMA}) showed a decreasing pattern with depth. Compared with TF, the methanogenesis rate for trimethylamine in the 0–30 cm layers of SA-1 was one order of magnitude higher; however, the corresponding rates of acetoclastic and hydrogenotrophic methanogenesis only increased by 104%–126% and 82.8%–129%, respectively.

In SA-12, the methanogenesis rate for trimethylamine in the 0–40 cm layer was 83.5%–890% and 3.02%–841% higher than that for acetate and H_2/CO_2, respectively, with a f_{TMA} of

TABLE 2 PERMANOVA tests of methanogen community composition dissimilarity based on a Bray–Curtis distance matrix

Source of variation	*df*	*F*-value	R^2	*p*-value
Site	2	28.70	0.337	0.001
Depth	4	9.85	0.232	0.001
Site × depth	8	5.42	0.255	0.001
Residuals	30	—	0.176	—

TABLE 3 Mantel correlations between eight soil properties and methanogen community composition

Soil properties	R_{Mantel}	*p*-value
SOC	−0.372	0.002
TN	−0.307	0.008
DOC	−0.253	0.036
Sulphate	−0.356	0.005
Acetate	−0.334	0.005
Formate	−0.096	0.353
Trimethylamine	−0.418	<0.001
Bicarbonate	0.098	0.817

Note. Values were ln-transformed prior to the statistical analyses to meet ANOVA assumptions.
SOC, soil organic carbon; TN, total nitrogen; DOC, dissolved organic carbon.

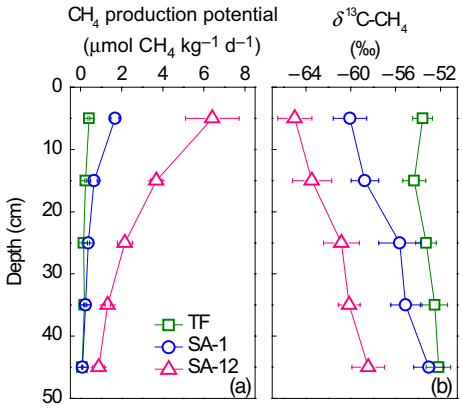

FIGURE 5 Vertical profiles of (a) CH_4 production potential and (b) ^{13}C-isotope signatures of CH_4 produced in anoxic coastal marsh soil slurries. Horizontal bars denote standard errors of means (n = 6). Tidal flat (TF) is a bare tidal flat; SA-1 and SA-12 are coastal marshes invaded for 1 and 12 years by *Spartina alterniflora*, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

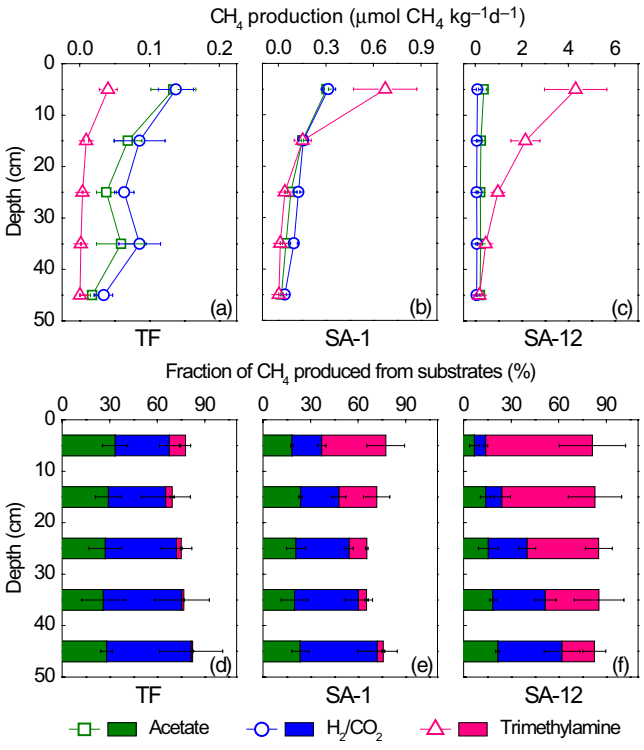

FIGURE 6 Vertical profiles of rates of CH_4 production and fraction of CH_4 produced from (a, d) acetate, (b, e) H_2/CO_2, and (c, f) trimethylamine in a bare tidal flat (TF) and *Spartina alterniflora*-invaded marsh soils (SA-1, 1-year invasion; SA-12, 12-year invasion) [Colour figure can be viewed at wileyonlinelibrary.com]

33.9%–67.3% (Figure 6c,e). However, f_{TMA} decreased with depth, to 20.3% in the 40–50 cm layer; by contrast, f_{H2} increased with depth, reaching its maximum in this deep layer. Overall, 12-year invasion by *S. alterniflora* significantly stimulated the methanogenesis rates from trimethylamine, the rates of which were 5.41–56.5 and 104–270 times higher than those in SA-1 and TF, respectively. Rates of acetoclastic and hydrogenotrophic methanogenesis in SA-12 were also significantly higher than those in SA-1 and TF, especially in the deep soil layers. In SA-12, trimethylamine was the key substrate in the upper 0–40 cm layers, while H_2/CO_2 became more important for methanogenesis below 40 cm (Figure 6f).

Twelve-year invasion by *S. alterniflora* enhanced the turnover of all three substrates to CH_4, expect for acetate in the 0–10 cm layer (Figure S6); however, these turnover times tended to increase with depth. Trimethylamine's turnover time in SA-12 was only 0.25–0.50 days (about 1–3 orders of magnitude faster than other substrates) followed by acetate, with bicarbonate having the longest turnover time. Nevertheless, the turnover time of trimethylamine was comparable to that of acetate in the deep soil layers of the TF and SA-1 sites.

The rates of methanogenesis and f_{TMA} were positively ($p < 0.01$) correlated with the concentrations of SOC, DOC, acetate, trimethylamine, and sulphate, as well as the abundance of archaeal 16S rRNA genes and *mcrA* genes, whereas these variables were negatively correlated with f_{H2} and f_{Ac} (Table S3). The f_{TMA}, rate of methanogenesis and turnover time for trimethylamine strongly ($p < 0.01$) depended on the relative abundance of *Methanosarcinaceae* in marsh soils (Figure S7).

4 | DISCUSSION

4.1 | Importance of methylotrophic methanogenesis

Twelve-year invasion of a coastal marsh by *S. alterniflora* plants increased its CH_4 production potential by 6.56–14.9 times compared with the non-invaded control site (TF). This result is consistent with previously measured field CH_4 emissions that increased by 57.4%–505% under *S. alterniflora* invasion when compared with bare TF and native plant marshes (Tong, Morris, Huang, Xu, & Wan, 2018; Yuan et al., 2015). Here, we derived the contribution of different substrates to total CH_4 production, and we found that trimethylamine was the key substrate for CH_4 production at SA-12, despite the observed much lower concentrations of trimethylamine than acetate at this site. Generally, sulphate reducers outcompete methanogens for H_2 and acetate in sulphate-rich coastal marshes (Froelich et al., 1979). The higher CH_4 production rate of trimethylamine than H_2/CO_2 and acetate in SA-12 suggested methylated compounds were not being competitively utilised by other microorganisms (King, 1984). Compared with TF, the 1-year and 12-year invasion by *S. alterniflora* increased the trimethylamine content by 1.45- and 10.9-fold in the 0–10 cm soil layer of SA-1 and SA-12, respectively. Early studies reported that *S. alterniflora* could release cytoplasmic osmolytes – for example, glycine betaine, choline, creatine, and proline – during senescence, which can be subsequently decomposed into methylamines, especially trimethylamine, in salt marsh sediments (Wang & Lee, 1994,1995). Notably, up to 27%–30% of the N assimilated by *S. alterniflora* is used to synthesise osmolytes, with glycine betaine and choline being two of the most important ones in *S. alterniflora* plants (Cavalieri & Huang, 1981). Thus, we consider that the rapid increase in CH_4 production rate at SA-12 was primarily due to the increased trimethylamine production from *S. alterniflora*.

In SA-12, the increase in methanogenesis rates appeared to coincide with the remarkable increase in *mcrA* genes in the soil. However, the mean specific rates for methanogens at TF, SA-1, and SA-12 were similar at about 0.37–1.73, 0.89–1.54, and 0.38–1.86 amol CH_4 h^{-1} $copy^{-1}$ of genes, respectively. These values are only one thousandth of the maintenance coefficients required for methanogens (Scholten & Conrad, 2000). Presumably, substantial *mcrA* gene copies detected in this study could be derived from dormant (inactive) methanogens (Conrad et al., 2014). The abundance of methanogenic genes in soil is relatively resistant to environmental changes, but their functional gene transcription is sensitive and responsive to it (Reim et al., 2017). It follows that an altered abundance of *mcrA* transcripts most likely reflects the response of active methanogens to *S. alterniflora* invasion, so we argue such changes should be included in future studies (Yuan, Conrad, & Lu, 2011).

We also found that the potentially methylamine-utilising methanogens *Methanosarcinaceae* dominated the 0–40 layer of SA-12, unlike in TF where the hydrogenotrophic *Methanococcales* were dominant in all layers analysed (Figure 3). *Methanosarcinaceae* are common methylotrophic methanogens that grow and subsist on methylated compounds and *Methanomassiliicoccales* are the newly discovered H_2-dependent methylotrophic methanogens (requiring both H_2 and methylated compounds). However, *Methanosarcinaceae* are identified as a unique group, one able to survive by carrying on H_2-independent methylotrophic methanogenesis (Lang et al., 2015). Our results also showed the SA-12 soil had faster responses to, and greater CH_4 production rates of, amended trimethylamine than either acetate or H_2/CO_2, consistent with our previous study (Yuan et al., 2016). Recently, Xiao, Beulig, Røy, Jørgensen, and Risgaard-Petersen (2018) reported that only a supplement of trimethylamine rather than H_2 and acetate quickly accelerated CH_4 production in a marine sediment, regardless of whether sulphate-reducing bacteria had been inhibited or not. Zhuang et al. (2016) found that the energy yields of hydrogenotrophic and acetoclastic methanogenesis were just barely able or insufficient to meet the presumed rate needed for sustaining life (–15 kJ/mol) in a hypersaline sediment, whereas methanogenesis from methylated compounds was more energetically favourable ($\Delta G < -180$ kJ/mol), even at extremely low porewater concentrations (<1 μm). Hence, we speculate that methylotrophic methanogens are capable of maintaining greater CH_4 production capacity than either hydrogenotrophic or acetoclastic methanogens in saline environments.

Further evidence for a shift in the predominant methanogenic pathway to methylotrophic methanogenesis at SA-12 came from the more ^{13}C-depleted CH_4 produced in this site. The $\delta^{13}C$ of *Spartina* leaves (–14.5%) that we found exceeded that of *Phragmites* leaves (–26.1%) (Kim et al., 2018), which likely generated heavier C input to soils than from native plants (Zhang et al., 2010). Although the $\delta^{13}C$ of methanogenic substrates was not measured here, we posit that differences in the isotopes of substrates may not be responsible for the isotopically lighter CH_4 produced in SA-12. Rather, the more ^{13}C-depleted CH_4 in SA-12 could be explained by stronger C isotope fractionation during methylotrophic methanogenesis (ε = –94% to –56%) than that occurring in acetoclastic (ε = –35% to –5%) and hydrogenotrophic methanogenesis (ε = –79% to –28%) (Conrad & Claus, 2005; Penger, Conrad, & Blaser, 2012). We should note that the contribution of anaerobic oxidation of CH_4 (AOM) to more ^{13}C-enriched CH_4 at both TF and SA-1 is a process that cannot be ruled out, even though ANME occurred in a very small proportion (Figure 3). Similar barely detectable or absent ANMEs were also found in other coastal wetland sediments (e.g., salt marsh, mangrove, mudflat, or estuarine sediments) (Devereux et al., 2015; Lazar et al., 2015). Nonetheless, Lloyd, Alperin, and Teske (2011) suggested that ANME cells constituted a numerically small yet active part of the archaeal population in coastal wetlands. Thus, the observed isotopic composition of CH_4 could be a collective result of methanogenesis from different pathways supplemented by the AOM process.

We also found that, in SA-12, the turnover time of trimethylamine to CH_4 was shorter than that for CO_2 and acetate. This result implied that although the trimethylamine concentration was relatively low compared with bicarbonate and acetate concentrations there, the fast turnover of trimethylamine by *Methanosarcinaceae* maintained methylotrophic methanogenesis at high levels in *S. alterniflora*-invaded sites, especially in the upper soil layers (Zhuang et al., 2018).

Despite methanogen abundance differing among the three sites, they had a similar profile pattern for the variation in structure and function of methanogens: decreasing relative abundance of *Methanosarcinaceae* and f_{TMA} and increasing relative abundance of hydrogenotrophic methanogens and f_{H2} with depth. Parkes et al. (2012) also found that relative abundance of *Methanosarcinales* decreased with depth in a salt marsh of England; they suggested the dominance of methanogenesis from competitive substrates in deep layer sediments arose from sulphate depletion. However, sulphate concentrations in the 0–50 cm layer of our three sites were >20 times greater than the 1mM threshold for methanogens to utilise acetate or H_2/CO_2 (King, 1984). Hence, sulphate depletion was not applicable in our case. Yet we did find that trimethylamine was only enriched in the upper soil layers, while H_2 could be produced from more recalcitrant organic compounds in deep-layer sediments (Chan et al., 2005). We postulate that the supply of trimethylamine became comparatively lower as depth increased, leaving the abundance of *Methanosarcinaceae* not efficiently stimulated in the deep layers of the *S. alterniflora*-invaded sites but allowing for the populations of hydrogenotrophic methanogens to remain relatively constant and much like that of the non-invaded site. In this way, trimethylamine, not sulphate, could primarily drive the vertical changes in community composition and associated functioning of methanogens in our studied marsh ecosystem.

4.2 | Contribution of competitive substrates

The 12-year invasion by *S. alterniflora* also significantly increased rates of acetoclastic and hydrogenotrophic methanogenesis, along with the abundance of acetoclastic and hydrogenotrophic methanogens, thus suggesting that both forms of methanogenesis were not completely inhibited by sulphate reducers with an increasing sulphate concentration. This result and interpretation are consistent with those of sulphate-rich mangroves (Arai et al., 2016), estuarine and marine sediments (Sela-Adler et al., 2017; Treude et al., 2014), and oilfield fluids (Lv et al., 2015). It has been suggested that the co-existence of methanogenesis and sulphate reduction is possible on competitive substrates and is primarily controlled via organic C input levels (Egger et al., 2016). When the concentration of acetate or H_2 in sulphate-rich wetlands exceeds the competition level of sulphate reduction and/or was used non-competitively, such co-existence should occur (Ozuolmez et al., 2015). For example, Sela-Adler et al. (2017) found that acetate at 40 mM, or lactate at 10 mM, simultaneously drove sulphate reduction and methanogenesis in sulphate-rich (10 mM) estuarine sediments. Thus, we argue that an enhanced labile organic C supply under the *S. alterniflora* invasion was responsible for the increased methanogenesis from competitive substrates, especially acetate.

Although the overall in situ concentration of competitive substrates might not reach the level for co-existence of methanogenesis and sulphate reduction, non-equilibrium dynamics of acetate and H_2 can sometimes generate abundant niches, thereby permitting the co-existence of methanogenesis and sulphate reduction. For example, Hoehler, Bebout, and Des Marais (2001) and Buckley et al. (2008) have showed that H_2 at the surface of microbial mats varied in concentration by as much as four orders of magnitude over a diurnal cycle, reaching as high as 10% (pH_2 = 10 kPa). Another possible explanation is that sulphate-depleted microenvironments could form in sulphate-rich layers around living or dead plant rhizome and roots (King & Wiebe, 1980), where sulphate levels are not equilibrated with pore-water sulphate or any sulphate there is quickly consumed (Bojanowski & Clarkson, 2012). In this way, acetoclastic and hydrogenotrophic methanogens could survive and maintain their biochemical activity in such microenvironments of salt marshes (Yuan et al., 2016). A plausible third mechanism to explain this co-occurrence of methanogenesis and sulphate reduction is the syntrophic cooperation between soil-dwelling microbes. Hydrogenotrophic methanogens were found to be fueled by H_2 leakage from co-cultured methylotrophic methanogens and acetoclastic sulphate reducers (Finke, Hoehler, & Jorgensen, 2007; Ozuolmez et al., 2015). Nonetheless, the intensity of

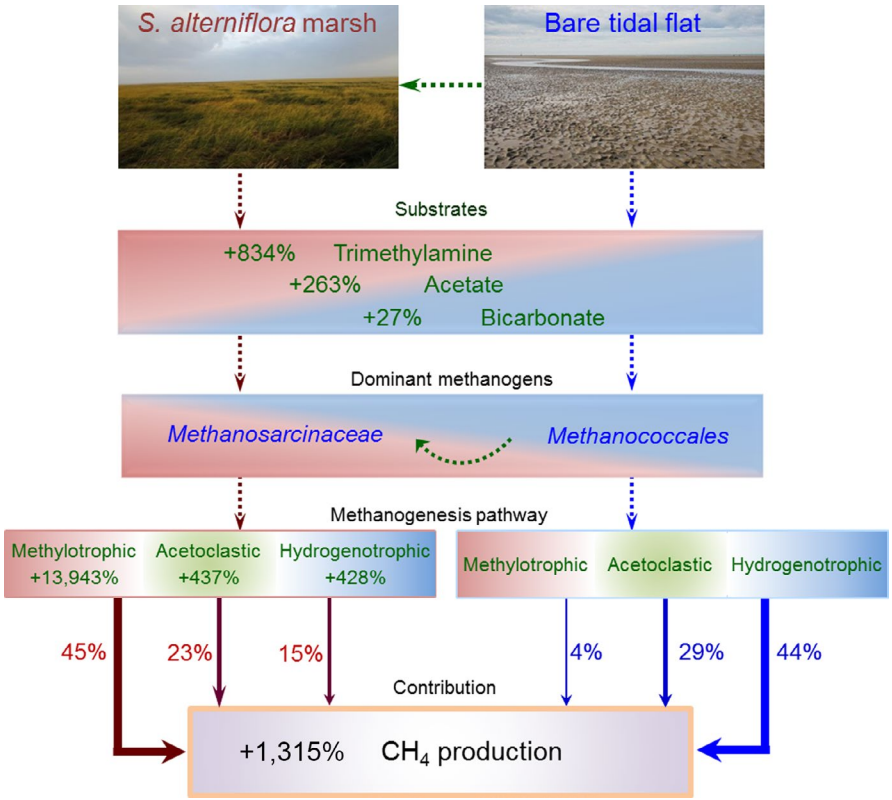

FIGURE 7 Schematic diagram showing the *Spartina alterniflora* invasion-induced shifts of mathanogenesis processes in the coastal wetlands. The two green-dashed arrows represent the changes of landscape and dominant methanogens between bare tidal flats and coastal marshes at 12 years post-invasion by *S. alterniflora*. The weight of solid arrows indicates the relative contribution of different methanogenesis pathways to total CH_4 production. The increases shown represent changes of concentrations of methanogenic substrates and rates of methanogenesis between SA-12 and TF. Data are soil profile averages (n = 3) [Colour figure can be viewed at wileyonlinelibrary.com]

methanogenesis is substantially constrained by competition from sulphate reducers for common substrates, leaving rates of methanogenesis steadily lower than those of sulphate reduction by 1–2 orders in sulphate-rich environments in spite of a sustained labile organic matter supply under plant invasion (Maltby, Sommer, Dale, & Treude, 2016; O'Sullivan et al., 2013).

In this study, however, we only provide indirect evidence that methanogenesis in the tested wetlands is closely related to the physiology of invasive *S. alterniflora* (producing precursors of 'non-competitive' substrates). These precursors are primarily produced for cytoplasmic osmoregulation (as discussed above). By using the ^{13}C-methyl labelled glycine betaine or choline as substrates, the contribution of *S. alterniflora*-derived methylated amines to CH_4 production can be reliably quantified. Moreover, the active methanogens carrying out methylotrophic methanogenesis can be identified with the DNA stable isotope probing (DNA-SIP) techniques (Eyice et al., 2015). Hence, a direct linkage between the structure and function of methanogens and plant physiology could well be established in future studies and therefore help to accurately and mechanistically understand the implications of *S. alterniflora* invasion upon CH_4 emissions in coastal wetlands.

5 | CONCLUSIONS

In summary, a 12-year invasion by exotic *S. alterniflora* plants remarkably altered the key type of methanogenic substrate, the abundance and community composition of methanogens, and the dominant methanogenesis pathway in the 0–40 cm layer of a coastal marsh in China (Figure 7). The dominant methanogens shifted to methylamine-utilising *Methanosarcinaceae* in the 0–10 cm layer of SA-1 and 0–40 cm layers of SA-12 from hydrogenotrophic ones in the TF (and deep layers of *S. alterniflora* sites). Accordingly, the dominant methanogenic pathway shifted from hydrogenotrophic at TF to methylotrophic in 0–40 cm layers of SA-12. Our complementary findings suggest the quantity of 'non-competitive' trimethylamine under *S. alterniflora* invasion was crucial for driving this shift in methanogenic community composition, which enhanced the capacity of methanogenesis by increasing the relative importance of methylotrophic methanogenesis in this coastal marsh ecosystem.

ACKNOWLEDGEMENTS

We are grateful to H. Chen for support with the methylated amines analysis and to Y. Cao for the CH_4 stable carbon isotopic measurements. We also thank SE David Gibson, AE Gabriela Nardoto, and two anonymous reviewers for their detailed and constructive comments on our manuscript. This work was supported by the Special Fund for Forest Scientific Research in Public Welfare (grant number 201404210), the National Key Research and Development Program of China (grant numbers 2016YFD0200304, 2016YFD0200309), the Natural Science Foundation of China (grant numbers 41501274, 41471077), and Jiangsu Province (grant number BK20151056).The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

W.D., J.Y., and M.W. designed the study; J.Y., D.L., J.X., and Y.L. collected field samples and data, and performed the research; J.Y. and W.D. wrote the first version of the manuscript, and Y.J. contributed to the final writing and presentation of the data; all authors contributed to revisions.

DATA AVAILABILITY STATEMENT

Data used in this study are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.6f60v3q (Yuan et al., 2019).

ORCID

Junji Yuan https://orcid.org/0000-0002-8339-699X

REFERENCES

Angeloni, N. L., Jankowski, K. J., Tuchman, N. C., & Kelly, J. J. (2006). Effects of an invasive cattail species (*Typha* × *glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland. *FEMS Microbiology Letters*, *263*, 86–92. https://doi.org/10.1111/j.1574-6968.2006.00409.x

Arai, H., Yoshioka, R., Hanazawa, S., Minh, V. Q., Tuan, V. Q., Tinh, T. K., … Inubushi, K. (2016). Function of the methanogenic community in mangrove soils as influenced by the chemical properties of the hydrosphere. *Soil Science and Plant Nutrition*, *62*, 150–163. https://doi.org/10.1080/00380768.2016.1165598

Bates, S. T., Berg-Lyons, D., Caporaso, J. G., Walters, W. A., Knight, R., & Fierer, N. (2011). Examining the global distribution of dominant archaeal populations in soil. *The ISME Journal*, *5*, 908–917. https://doi.org/10.1038/ismej.2010.171

Benner, R. B., Fogel, M. L., & Sprague, E. K. (1991). Diagenesis of belowground biomass of *Spartina alterniflora* in salt-marsh sediments. *Limnology and Oceanography*, *36*, 1358–1374. https://doi.org/10.4319/lo.1991.36.7.1358

Bertness, M. D. (1991). Zonation of *Spartina patens* and *Spartina alterniflora* in New England salt marsh. *Ecology*, *72*, 138–148. https://doi.org/10.2307/1938909

Bojanowski, M. J., & Clarkson, E. N. K. (2012). Origin of siderite concretions in microenvironments of methanogenesis developed in a sulfate reduction zone: An exception or a rule? *Journal of Sedimentary Research*, *82*, 585–598. https://doi.org/10.2110/jsr.2012.50

Buckley, D. H., Baumgartner, L. K., & Visscher, P. T. (2008). Vertical distribution of methane metabolism in microbial mats of the Great Sippewissett Salt Marsh. *Environmental Microbiology*, *10*, 967–977. https://doi.org/10.1111/j.1462-2920.2007.01517.x

Castro-Díez, P., Godoy, O., Alonso, A., Gallardo, A., & Saldaña, A. (2014). What explains variation in the impacts of exotic plant invasions on the nitrogen cycle? A meta-analysis. *Ecology Letters*, *17*, 1–12. https://doi.org/10.1111/ele.12197

Cavalieri, A. J., & Huang, A. H. (1981). Accumulation of proline and glycinebetaine in *Spartina alterniflora* Loisel. in response to NaCl and nitrogen in the marsh. *Oecologia*, *49*, 224–228. https://doi.org/10.1007/BF00349192

Chan, O. C., Claus, P., Casper, P., Ulrich, A., Lueders, T., & Conrad, R. (2005). Vertical distribution of structure and function of the methanogenic archaeal community in Lake Dagow sediment. *Environmental Microbiology*, *7*, 1139–1149. https://doi.org/10.1111/j.1462-2920.2005.00790.x

Chung, C. H., Zhuo, R. Z., & Xu, G. W. (2004). Creation of *Spartina* plantations for reclaiming Dongtai, China, tidal flats and offshore sands. *Ecological Engineering*, *23*, 135–150. https://doi.org/10.1016/j.ecoleng.2004.07.004

Conrad, R. (2007). Microbial ecology of methanogens and methanotrophs. *Advances in Agronomy*, *96*, 1–63. https://doi.org/10.1016/S0065-2113(07)96005-8

Conrad, R. (2009). The global methane cycle: Recent advances in understanding the microbial processes involved. *Environmental Microbiology Reports*, *1*, 285–292. https://doi.org/10.1111/j.1758-2229.2009.00038.x

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. https://doi.org/10.1007/s10533-004-0366-9

Conrad, R., Ji, Y., Noll, M., Klose, M., Claus, P., & Enrich-Prast, A. (2014). Response of the methanogenic microbial communities in Amazonian oxbow lake sediments to desiccation stress. *Environmental Microbiology*, *16*, 1682–1694. https://doi.org/10.1111/1462-2920.12267

Core Team, R. (2015). *R: A Language and Environment for Statistical Computing*. Vienna, Austria. http://www.R-project.org/

Cruz-Martinez, K., Suttle, K. B., Brodie, E. L., Power, M. E., Andersen, G. L., & Banfield, J. F. (2009). Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. *The ISME Journal*, *3*, 738–744. https://doi.org/10.1038/ismej.2009.16

Dacey, J. W., King, G. M., & Wakeham, S. G. (1987). Factors controlling emission of dimethylsulphide from salt marshes. *Nature*, *330*, 643–645. https://doi.org/10.1038/330643a0

Davis, H. G., Taylor, G. M., Civille, J. C., & Strong, D. R. (2004). An Allee effect at the front of a plant invasion: *Spartina* in a Pacific estuary. *Journal of Ecology*, *92*, 321–327. https://doi.org/10.1111/j.0022-0477.2004.00873.x

Dawson, W., Rohr, R. P., van Kleunen, M., & Fischer, M. (2012). Alien plant species with a wider global distribution are better able to capitalize on increased resource availability. *New Phytologist*, *194*, 859–867. https://doi.org/10.1111/j.1469-8137.2012.04104.x

Devereux, R., Mosher, J. J., Vishnivetskaya, T. A., Brown, S. D., Beddick, D. L. Jr, Yates, D. F., & Palumbo, A. V. (2015). Changes in northern Gulf of Mexico sediment bacterial and archaeal communities exposed to hypoxia. *Geobiology*, *13*, 478–493. https://doi.org/10.1111/gbi.12142

Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, *10*, 996–998. https://doi.org/10.1038/nmeth.2604

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, *27*, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Egger, M., Lenstra, W., Jong, D., Meysman, F. J. R., Sapart, C. J., van der Veen, C., … Slomp, C. P. (2016). Rapid sediment accumulation results in high methane effluxes from coastal sediments. *PLoS ONE*, *11*, e0161609. https://doi.org/10.1371/journal.pone.0161609

Eilers, K. G., Lauber, C. L., Knight, R., & Fierer, N. (2010). Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biology and Biochemistry*, *42*, 896–903. https://doi.org/10.1016/j.soilbio.2010.02.003

Emery, H. E., & Fulweiler, R. W. (2014). *Spartina alterniflora* and invasive *Phragmites australis* stands have similar greenhouse gas emissions in a New England marsh. *Aquatic Botany*, *116*, 83–92. https://doi.org/10.1016/j.aquabot.2014.01.010

Eyice, Ö., Namura, M., Chen, Y., Mead, A., Samavedam, S., & Schäfer, H. (2015). SIP metagenomics identifies uncultivated *Methylophilaceae* as dimethylsulphide degrading bacteria in soil

and lake sediment. *The ISME Journal*, *9*, 2336–2348. https://doi.org/10.1038/ismej.2015.37

Finke, N., Hoehler, T. M., & Jorgensen, B. B. (2007). Hydrogen 'leakage' during methanogenesis from methanol and methylamine: Implications for anaerobic carbon degradation pathways in aquatic sediments. *Environmental Microbiology*, *9*, 1060–1071. https://doi.org/10.1111/j.1462-2920.2007.01248.x

Froelich, P. N., Klinkhammer, G. P., Bender, M. l., Luedtke, N. A., Heath, G. R., Cullen, D., … Maynard, V. (1979). Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: Suboxic diagenesis. *Geochimica et Cosmochimica Acta*, *43*, 1075–1090. https://doi.org/10.1016/0016-7037(79)90095-4

Grevstad, F. S. (2005). Simulating control strategies for a spatially structured weed invasion: *Spartina alterniflora* (Loisel) in Pacific Coast estuaries. *Biological Invasions*, *7*, 665–677. https://doi.org/10.1007/s10530-004-5855-1

Hawkes, C. V., Wrem, I. F., Herman, D. J., & Firestone, M. K. (2005). Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecology Letters*, *8*, 976–985. https://doi.org/10.1111/j.1461-0248.2005.00802.x

Henmi, Y., Fuchimoto, D., Kasahara, Y., & Shimanaga, M. (2017). Community structures of halophytic plants, gastropods and brachyurans in salt marshes in Ariake and Yatsushiro seas of Japan. *Plankton and Benthos Research*, *12*, 224–237. https://doi.org/10.3800/pbr.12.224

Hoehler, T. M., Bebout, B. M., & Des Marais, D. J. (2001). The role of microbial mats in the production of reduced gases on the early Earth. *Nature*, *412*, 324–327. https://doi.org/10.1038/35085554

Husband, J. D., & Kiene, R. P. (2007). Occurrence of dimethylsulfoxide in leaves, stems, and roots of *Spartina alterniflora*. *Wetlands*, *27*, 224–229. https://doi.org/10.1672/0277-5212(2007)27[224:OODILS]2.0.CO;2

Jetten, M. S. M., Stams, A. J. M., & Zehnder, A. J. B. (1992). Methanogenesis from acetate: A comparison of the acetate metabolism in *Methanothrix soehngenii* and *Methanosarcina* spp. *FEMS Microbiology Reviews*, *88*, 181–197. https://doi.org/10.1016/0378-1097(92)90802-U

Juottonen, H., Galand, P. E., & Yrjälä, K. (2006). Detection of methanogenic Archaea in peat: Comparison of PCR primers targeting the *mcrA* gene. *Research in Microbiology*, *157*, 914–921. https://doi.org/10.1016/j.resmic.2006.08.006

Kelley, C. A., Chanton, J. P., & Bebout, B. M. (2015). Rates and pathways of methanogenesis in hypersaline environments as determined by ^{13}C-labeling. *Biogeochemistry*, *126*, 329–341. https://doi.org/10.1007/s10533-015-0161-9

Kelley, C. A., Poole, J. A., Tazaz, A. M., Chanton, J. P., & Bebout, B. M. (2012). Substrate limitation for methanogenesis in hypersaline environments. *Astrobiology*, *12*, 89–97. https://doi.org/10.1089/ast.2011.0703

Kim, S., Kang, H., Megonigal, J. P., Kang, H., Seo, J., & Ding, W. X. (2018). Impacts of *Phragmites australis* invasion on soil enzyme activities and microbial abundance of tidal marshes. *Microbial Ecology*, *76*, 782–790. https://doi.org/10.1007/s00248-018-1168-2

King, G. M. (1984). Metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. *Applied and Environmental Microbiology*, *48*, 719–725.

King, G. M., & Wiebe, W. J. (1980). Regulation of sulfate concentrations and methanogenesis in salt marsh soils. *Estuarine and Coastal Marine Science*, *10*, 215–223. https://doi.org/10.1016/S0302-3524(80)80059-4

Lambais, M. R., Otero, X. L., & Cury, J. C. (2008). Bacterial communities and biogeochemical transformations of iron and sulfur in a high saltmarsh soil profile. *Soil Biology and Biochemistry*, *40*, 2854–2864. https://doi.org/10.1016/j.soilbio.2008.08.014

Lambertini, M., Leape, J., Marton-Lefevre, J., Mittermeier, R. A., Rose, M., Robinson, J. G., … Genovesi, P. (2011). Invasives: A major conservation threat. *Science*, *333*, 404–405. https://doi.org/10.1126/science.333.6041.404-b

Lang, K., Schuldes, J., Klingl, A., Poehlein, A., Daniel, R., & Brune, A. (2015). New mode of energy metabolism in the seventh order of methanogens as revealed by comparative genome analysis of "*Candidatus* Methanoplasma termitum". *Applied and Environmental Microbiology*, *81*, 1338–1352. https://doi.org/10.1128/AEM.03389-14

Lazar, C. S., Biddle, J. F., Meador, T. B., Blair, N., Hinrichs, K. U., & Teske, A. P. (2015). Environmental controls on intragroup diversity of the uncultured benthic *archaea* of the miscellaneous Crenarchaeotal group lineage naturally enriched in anoxic sediments of the White Oak River estuary (North Carolina, USA). *Environmental Microbiology*, *17*, 2228–2238. https://doi.org/10.1111/1462-2920.12659

Lee, R. W., Kraus, D. W., & Doeller, J. E. (1999). Oxidation of sulfide by *Spartina alterniflora* roots. *Limnology and Oceanography*, *44*, 1155–1159. https://doi.org/10.4319/lo.1999.44.4.1155

Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., … Li, B. O. (2008). Altered ecosystem carbon and nitrogen cycles by plant invasion: A meta-analysis. *New Phytologist*, *177*, 706–714. https://doi.org/10.1111/j.1469-8137.2007.02290.x

Lloyd, K. G., Alperin, M. J., & Teske, A. (2011). Environmental evidence for net methane production and oxidation in putative ANaerobic MEthanotrophic (ANME) archaea. *Environmental Microbiology*, *13*, 2548–2564. https://doi.org/10.1111/j.1462-2920.2011.02526.x

Lv, L., Mbadinga, S. M., Wang, L. Y., Liu, J. F., Gu, J. D., Mu, B. Z., & Yang, S. Z. (2015). Acetoclastic methanogenesis is likely the dominant biochemical pathway of palmitate degradation in the presence of sulfate. *Applied Microbiology and Biotechnology*, *99*, 7757–7769. https://doi.org/10.1007/s00253-015-6669-z

Maltby, J., Sommer, S., Dale, A. W., & Treude, T. (2016). Microbial methanogenesis in the sulfate-reducing zone of surface sediments traversing the Peruvian margin. *Biogeosciences*, *13*, 283–299. https://doi.org/10.5194/bg-13-283-2016

Mazzilli, S. R., Kemanian, A. R., Ernst, O. R., Jackson, R. B., & Piñeiro, G. (2015). Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops. *Soil Biology and Biochemistry*, *85*, 22–30. https://doi.org/10.1016/j.soilbio.2015.02.014

McCary, M. A., Mores, R., Farfan, M. A., & Wise, D. H. (2016). Invasive plants have different effects on trophic structure of green and brown food webs in terrestrial ecosystems: A meta-analysis. *Ecology Letters*, *19*, 328–335. https://doi.org/10.1111/ele.12562

Norton, U., Mosier, A., Morgan, J., Derner, J., Ingram, L., & Stahl, P. (2008). Moisture pulses, trace gas emissions and soil C and N in cheatgrass and native grass-dominated sagebrush-steppe in Wyoming, USA. *Soil Biology and Biochemistry*, *40*, 1421–1431. https://doi.org/10.1016/j.soilbio.2007.12.021

Oremland, R. S., Marsh, L., & Desmarais, D. J. (1982). Methanogenesis in Big Soda Lake, Nevada: An alkaline, moderately hypersaline desert lake. *Applied and Environmental Microbiology*, *43*, 462–468.

O'Sullivan, L. A., Sass, A. M., Webster, G., Fry, J. C., Parkes, R. J., & Weightman, A. J. (2013). Contrasting relationships between biogeochemistry and prokaryotic diversity depth profiles along an estuarine sediment gradient. *FEMS Microbiology Ecology*, *85*, 143–157. https://doi.org/10.1111/1574-6941.12106

Ozuolmez, D., Na, H., Lever, M. A., Kjeldsen, K. U., Jorgensen, B. B., & Plugge, C. M. (2015). Methanogenic archaea and sulfate reducing bacteria co-cultured on acetate: Teamwork or coexistence? *Frontiers in Microbiology*, *6*, 492. https://doi.org/10.3389/fmicb.2015.00492

Parkes, R. J., Brock, F., Banning, N., Hornibrook, E. R. C., Roussel, E. G., Weightman, A. J., & Fry, J. C. (2012). Changes in methanogenic substrate utilization and communities with depth in a salt-marsh, creek sediment in southern England. *Estuarine, Coastal and Shelf Science*, *96*, 170–178. https://doi.org/10.1016/j.ecss.2011.10.025

Penger, J., Conrad, R., & Blaser, M. (2012). Stable carbon isotope fractionation by methylotrophic methanogenic archaea. *Applied and Environmental Microbiology*, *78*, 7596–7602. https://doi.org/10.1128/AEM.01773-12

Piper, C. L., Siciliano, S. D., Winsley, T., & Lamb, E. G. (2015). Smooth brome invasion increases rare soil bacterial species prevalence, bacterial species richness and evenness. *Journal of Ecology*, *103*, 386–396. https://doi.org/10.1111/1365-2745.12356

Pires, A. C. C., Cleary, D. F. R., Almeida, A., Cunha, Â., Dealtry, S., Mendonça-Hagler, L. C. S., … Gomes, N. C. M. (2012). Denaturing gradient gel electrophoresis and barcoded pyrosequencing reveal unprecedented archaeal diversity in mangrove sediment and rhizosphere samples. *Applied and Environmental Microbiology*, *78*, 5520–5528. https://doi.org/10.1128/AEM.00386-12

Qin, P., & Zhong, C. (1992). *Applied studies on* Spartina. China, Beijing: Ocean Press.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., & Fierer, N. (2010). Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology*, *91*, 3463–3470. https://doi.org/10.1890/10-0426.1

Reim, A., Hernández, M., Klose, M., Chidthaisong, A., Yuttitham, M., & Conrad, R. (2017). Response of methanogenic microbial communities to desiccation stress in flooded and rain-fed paddy soil from Thailand. *Frontiers in Microbiology*, *8*, 785. https://doi.org/10.3389/fmicb.2017.00785

Rhodes, D., & Hanson, A. (1993). Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual Review of Plant Biology*, *44*, 357–384. https://doi.org/10.1146/annurev. pp. 44.060193.002041

Schloss, P. D., Westcott, S. l., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: Opensource, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*, 7537–7541. https://doi.org/10.1128/AEM.01541-09

Scholten, J. C. M., & Conrad, R. (2000). Energetics of syntrophic propionate oxidation in defined batch and chemostat cocultures. *Applied and Environmental Microbiology*, *66*, 2934–2942. https://doi.org/10.1128/AEM.66.7.2934-2942.2000

Sela-Adler, M., Ronen, Z., Herut, B., Antler, G., Vigderovich, H., Eckert, W., & Sivan, O. (2017). Co-existence of methanogenesis and sulfate reduction with common substrates in sulfate-rich estuarine sediments. *Frontiers in Microbiology*, *8*, 766. https://doi.org/10.3389/fmicb.2017.00766

Stubner, S. (2002). Enumeration of 16S rDNA of *Desulfotomaculum* lineage 1 in rice field soil by real-time PCR with SybrGreen (TM) detection. *Journal of Microbiological Methods*, *50*, 155–164. https://doi.org/10.1016/S0167-7012(02)00024-6

Tamura, M., & Tharayil, N. (2014). Plant litter chemistry and microbial priming regulate the accrual, composition and stability of soil carbon in invaded ecosystems. *New Phytologist*, *203*, 110–124. https://doi.org/10.1111/nph.12795

Tong, C., Morris, J. T., Huang, J. F., Xu, H., & Wan, S. (2018). Changes in pore-water chemistry and methane emission following the invasion of *Spartina alterniflora* into an oligohaline marsh. *Limnology and Oceanography*, *63*, 384–396. https://doi.org/10.1002/lno.10637

Treude, T., Krause, S., Maltby, J., Dale, A. W., Coffin, R., & Hamdan, L. J. (2014). Sulfate reduction and methane oxidation activity below the sulfate-methane transition zone in Alaskan Beaufort Sea continental margin sediments: Implications for deep sulfur cycling. *Geochimica et Cosmochimica Acta*, *144*, 217–237. https://doi.org/10.1016/j.gca.2014.08.018

Tylianakis, J. M., Didham, R. K., Bascompte, J., & Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, *11*, 1351–1363. https://doi.org/10.1111/j.1461-0248.2008.01250.x

Wang, X. C., & Lee, C. (1994). Sources and distribution of aliphatic-amines in salt-marsh sediment. *Organic Geochemistry*, *22*, 1005–1021. https://doi.org/10.1016/0146-6380(94)90034-5

Wang, X. C., & Lee, C. (1995). Decomposition of aliphatic-amines and amino-acids in anoxic salt-marsh sediment. *Geochimica et Cosmochimica Acta*, *59*, 1787–1797. https://doi.org/10.1016/0016-7037(95)00082-B

Wilms, R., Köpke, B., Sass, H., Chang, T. S., Cypionka, H., & Engelen, B. (2006). Deep biosphere-related bacteria within the subsurface of tidal flat sediments. *Environmental Microbiology*, *8*, 709–719. https://doi.org/10.1111/j.1462-2920.2005.00949.x

Wilms, R., Sass, H., Köpke, B., Köster, J., Cypionka, H., & Engelen, B. (2006). Specific bacterial, archaeal, and eukaryotic communities in tidal-flat sediments along a vertical profile of several meters. *Applied and Environmental Microbiology*, *72*, 2756–2764. https://doi.org/10.1128/AEM.72.4.2756-2764.2006

Xiao, K. Q., Beulig, F., Røy, H., Jørgensen, B. B., & Risgaard-Petersen, N. (2018). Methylotrophic methanogenesis fuels cryptic methane cycling in marine surface sediment. *Limnology and Oceanography*, *63*, 1519–1527. https://doi.org/10.1002/lno.10788

Yuan, J. J., Ding, W. X., Liu, D. Y., Kang, H. J., Freeman, C., Xiang, J., & Lin, Y. X. (2015). Exotic *Spartina alterniflora* invasion alters ecosystem-atmosphere exchange of CH_4 and N_2O and carbon sequestration in a coastal salt marsh in China. *Global Change Biology*, *21*, 1567–1580. https://doi.org/10.1111/gcb.12797

Yuan, J. J., Ding, W. X., Liu, D. Y., Kang, H. J., Xiang, J., & Lin, Y. X. (2016). Shifts in methanogen community structure and function across a coastal marsh transect: Effects of exotic *Spartina alterniflora* invasion. *Scientific Reports*, *6*, 18777. https://doi.org/10.1038/srep18777

Yuan, J. J., Ding, W. X., Liu, D. Y., Xiang, J., & Lin, Y. Y. (2014). Methane production potential and methanogenic archaea community dynamics along the *Spartina alterniflora* invasion chronosequence in a coastal salt marsh. *Applied Microbiology and Biotechnology*, *98*, 1817–1829. https://doi.org/10.1007/s00253-013-5104-6

Yuan, J. J., Liu, D. Y., Ji, Y., Xiang, J., Lin, Y. X., Wu, M., & Ding, W. X. (2019). Data from: *Spartina alterniflora* invasion drastically increases methane production potential by shifting methanogenesis from hydrogenotrophic to methylotrophic pathway in a coastal marsh. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.6f60v3q

Yuan, Y. L., Conrad, R., & Lu, Y. H. (2011). Transcriptional response of methanogen *mcrA* genes to oxygen exposure of rice field soil. *Environmental Microbiology Reports*, *3*, 320–328. https://doi.org/10.1111/j.1758-2229.2010.00228.x

Zhang, Y. H., Ding, W. X., Luo, J. F., & Donnison, A. (2010). Changes in soil organic carbon dynamics in an Eastern Chinese coastal wetland following invasion by a C_4 plant *Spartina alterniflora*. *Soil Biology and Biochemistry*, *42*, 1712–1720. https://doi.org/10.1016/j.soilbio.2010.06.006

Zheng, C. Y., Zhang, S. Q., Jiang, H. X., & Wang, H. (2009). Spatiotemporal dynamics and landscape pattern of alien species *Spartina alterniflora* in Yancheng coastal wetlands of Jiangsu Province, China. *Chinese Journal of Applied Ecology*, *20*, 901–908 (in Chinese with an English abstract). https://doi.org/10.13287/j.1001-9332.2009.0116

Zheng, X., Su, Y. L., Li, X., Xiao, N. D., Wang, D. B., & Chen, Y. G. (2013). Pyrosequencing reveals the key microorganisms involved in sludge alkaline fermentation for efficient short-chain fatty acids production. *Environmental Science & Technology*, *47*, 4262–4268. https://doi.org/10.1021/es400210v

Zhou, C., An, S., Deng, Z., Yin, D., Zhi, Y., Sun, Z., … Qian, C. (2009). Sulfur storage changed by exotic *Spartina alterniflora* in coastal saltmarshes of China. *Ecological Engineering*, *35*, 536–543. https://doi.org/10.1016/j.ecoleng.2008.01.004

Zhuang, G. C., Elling, F. J., Nigr, L. M., Samarkin, V., Joye, S. B., Teske, A., & Hinrichs, K. U. (2016). Multiple evidence for methylotrophic methanogenesis as the dominant methanogenic pathway in hypersaline sediments from the Orca Basin, Gulf of Mexico. *Geochimica*

et Cosmochimica Acta, *187*, 1–20. https://doi.org/10.1016/j.gca.2016.05.005

Zhuang, G.-C., Heuer, V. B., Lazar, C. S., Goldhammer, T., Wendt, J., Samarkin, V. A., … Hinrichs, K.-U. (2018). Relative importance of methylotrophic methanogenesis in sediments of the Western Mediterranean Sea. *Geochimica et Cosmochimica Acta*, *224*, 171–186. https://doi.org/10.1016/j.gca.2017.12.024

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Yuan J, Liu D, Ji Y, et al. *Spartina alterniflora* invasion drastically increases methane production potential by shifting methanogenesis from hydrogenotrophic to methylotrophic pathway in a coastal marsh. *J Ecol*. 2019;107:2436–2450. https://doi.org/10.1111/1365-2745.13164