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Influence of niche differentiation on the abundance of methanogenic archaea and methane production potential in natural wetland ecosystems across China

D. Liu, W. Ding, Z. Jia, and Z. Cai

State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

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Correspondence to: W. Ding (wxding@mail.issas.ac.cn)

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Abstract

Methane (CH_4) emissions from natural wetland ecosystems exhibit large spatial variability. To understand the underlying factors that induce differences in CH_4 emissions from natural wetlands around China, we measured the CH_4 production potential and the abundance of methanogenic archaea in vertical profile soils sampled from the Poyang wetland in the subtropical zone, the Hongze wetland in the warm temperate zone, the Sanjiang marsh in the cold temperate zone, and the Ruoergai peatland in the Qinghai-Tibetan Plateau. The top soil layer had the highest population of methanogens ($1.07-8.29\times10^9$ cells g^{-1} soil) in all wetlands except the Ruoergai peatland and exhibited the maximum CH_4 production potential measured at the mean in situ summer temperature. There is a significant logarithmic correlation between the abundance of methanogenic archaea and the soil organic carbon ($R^2=0.718$, $P<0.001$, $n=13$) and between the abundance of methanogenic archaea and the total nitrogen concentrations ($R^2=0.758$, $P<0.001$, $n=13$) in wetland soils. This indicates that the amount of soil organic carbon may affect the population of methanogens in wetland ecosystems. While the CH_4 production potential is not significantly related to methanogen population ($R^2=0.011$, $P>0.05$, $n=13$), it is related to the dissolved organic carbon concentration ($R^2=0.305$, $P=0.05$, $n=13$). This suggests that the methanogen population is not an effective index for predicting the CH_4 production in wetland ecosystems. The CH_4 production rate of the top soil layer increases with increasing latitude, from 274 µg CH_4 kg^{-1} soil d^{-1} in the Poyang wetland to 665 µg CH_4 kg^{-1} soil d^{-1} in the *Carex lasiocarpa* marsh of the Sanjiang Plain. The CH_4 production potential in the freshwater wetlands of Eastern China is affected by the supply of methanogenic substrates rather than by temperature, whereas the supply of substrates was mainly affected by the position and stability of the wetland water table. In contrast, low summer temperatures at high elevations in the Ruoergai peatland of the Qinghai-Tibetan Plateau result in the presence of dominant species of methanogens with low CH_4 production potential rather than the reduction of the supply of methanogenic substrates, which in turn suppresses CH_4 production.

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1 Introduction

The methane (CH_4) concentration in the atmosphere has increased by 153% since 1750. Natural wetlands have emitted 100–231 Tg CH_4 year^{-1}, which accounts for 20–39% of the annual global CH_4 emission (IPCC, 2007). The CH_4 emission from wetlands increased by 7% from 2003 to 2007 (Bloom et al., 2010). Up to date, many studies have focused on the CH_4 emission from wetland ecosystems, in an attempt to quantify the CH_4 source strengths from wetlands. However, there is great uncertainty in the magnitude and distribution of methane sources on the regional, national, and continental scales because of the large spatial and temporal variations in emissions across individual wetlands and wetland types (Melling et al., 2005; Wang and Han, 2005; Chen et al., 2008). Several factors that affect CH_4 emissions have been identified, such as temperature (Westermann, 1993), plant type (Bartlett et al., 1992), primary production, (Whiting and Chanton, 1993), and the water table (Moore and Dalva, 1993; Frenzel and Karofeld, 2000; Ding et al., 2010).

Saarnio et al. (1998) concluded that the decomposition of root exudates is suppressed at low temperatures, and thus, CH_4 production and emission are reduced. Similarly, Ding et al. (2004) suggested that the high elevation (height above sea level: >3400 m) of the Ruoergai peatland in the Qinghai-Tibetan Plateau leads to low temperatures in summer, which in turn lowers the supply of methanogenic substrates and CH_4 production. Shannon and White (1996) and Chasar et al. (2000) concluded that the CH_4 production in wetlands is affected by the acetate supply through acetate fermentation and/or the CO_2 reduction potential. On the basis of a study conducted in an ombrotrophic bog in Michigan, Avery et al. (2003) pointed out that the exponential increase in the rate of CH_4 production with temperature is due to an increase in the number of available substrates and is not associated with changes in the composition and populations of methanogens. On the other hand, Duddleston and Kinney (2002) argued that temporal accumulation of acetate in the Turnagain bog of Alaska is attributable to the absence of acetoclastic methanogenesis. Horn et al. (2003) found that

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no active acetoclastic methanogenesis occurred in an acidic peatland in Germany and that the addition of acetates or other volatile fatty acids reduced rather than increased CH_4 production, possibly because of the inhibition of hydrogenotrophic methanogenesis (Brauer et al., 2004). Cadillo-Quiroz et al. (2006) found that in the case of vertical profiles of the McLean and Chicago bogs in central New York State, USA, the difference between the methanogenesis in different soil layers is strongly associated with the population and activity of methanogens using the quantitative polymerase chain reaction (qPCR). To date, little is known about the size of the methanogenic archaea population and the CH_4 production potential in freshwater natural wetlands in China.

We have examined the spatial variation in the CH_4 production potential and that in the methanogenic population in four typical freshwater wetlands across China. The objectives of this study were (1) to understand the relationship between the population of methanogenic archaea and CH_4 production potential, and (2) to evaluate the underlying factors that determine the spatial variation of the CH_4 production potential in wetland ecosystems. For this purpose, a method based on the qPCR of methanogens was used and soil slurries were incubated at mean summer temperatures in order to simulate in situ conditions.

2 Materials and methods

2.1 Site description and soil sampling

Four typical natural wetlands in China were selected for this study (Fig. 1). The eutrophic freshwater marsh is located at the Chinese Academy of Sciences Sanjiang Mire Wetland Experimental Station in Tongjiang City of the Heilongjiang province (Table 1). The elevation is 56 m and the mean annual precipitation is approximately 600 mm. The mean annual temperature is 1.9 °C, ranging from −18.8 °C in January to 20.8 °C in July. Marsh initiation began during the late-Pleistocene epoch due to convergence of the Heilongjiang River, Songhuajiang River and Wusulijiang River, and blockage of

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water seepage by clayey soil. Wetland vegetation varies from *Calamagrostis angustifolia* to *Carex lasiocarpa* as standing water depth increases (Ding et al., 2002). In the *C. lasiocarpa* marsh, vegetation is 90% covered by *C. lasiocarpa* and 10% covered by *Glyceria spiculosa*, with the profile composed of standing water layer, root layer, peat layer, and grey soil layer. Vegetation in the *C. angustifolia* marsh is purely *C. angustifolia*, but its profiles are absent from the peat layer. The Ruoergai peatland is situated in Ruoergai county, Sichuan province, a typical part of the Qinghai-Tibetan Plateau, and is the largest peatland in China. The dominant vegetation is *Carex muliensis* and *Carex meyeriana*. Similar to the Sanjiang plain, the mean annual temperature is 1 °C and the mean annual precipitation is 650 mm. The elevation in the Ruoergai highlight is, however, up to 3400 m, resulting in the little annual variation of temperature. The lowest and highest average monthly temperatures were −10.5 °C in January and 10.7 °C in July. Low temperatures in summer greatly reduce the decomposition rate of plant litters and accelerate the deposit of peat, thus the depth of peat at the sampling site is more than 1 m. Hongze Lake, with an area of 2069 km^2 and an elevation of 12 m, is located in the northern part of the Jiangsu province and is the fourth largest freshwater lake in China. The mean annual precipitation is approximately 926 mm and the mean low and high monthly temperatures are 0 °C and 27.5 °C, respectively. This lake was formed on an alluvial plain in the middle reaches of the Huaihe River due to river course blockage during the Tang dynasty (Zhu, 1991). At present, the average water depth is approximately 4 m. There is a variety of wetland plants in the land-water ecotone, such as *Phragmites australis*, *Zizania caduciflora*, *Nelumbo nucifera*, *Euvyale ferox*, *Trapa matans*, *Potamogeton malaianus*, and *Myriophyllum spicatum*. Poyang Lake is the largest lake in China, covering 3283 km^2, and lies in the northern part of the Jiangxi Province at the southern bank of the middle reaches of Yangtze River. The average annual temperature and precipitation is 17 °C and 1636 mm, respectively. The mean water depth of the lake is 8.4 m, with a maximum depth of 25.1 m.

Profile soil samples were collected from 25 September to 10 October 2009. Three 5 m × 5 m sampling plots were randomly established at each site. Five soil samples

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were taken for each layer at different positions in each plot using a 2.5 cm diameter stainless steel soil sampler. All samples of the same layer from each plot were then carefully mixed to form a composite. All samples were immediately stored in sterile bags, kept on ice in coolers, and directly transported to the laboratory. One subsample was stored at –20 °C for DNA extraction, another subsample was used for the measurement of CH_4 production potential, and the other was air-dried for the measurement of soil properties.

2.2 Methane production potential measurement

Methane production potential of wetland soils was determined using a slightly modified method from Galand et al. (2003). Ten grams of fresh soil sample (on an oven dried basis) were weighed and placed in a 100 ml glass jar. The ratio of soil to water was adjusted to be 1:5 with distilled water. The contents of the jar were mixed thoroughly using a glass rod. The jars were vacuumed and then injected with pure nitrogen gas (N_2) using an atmospheric pressure balance. The above procedure was replicated three times to obtain completely anoxic conditions. The jars were incubated in the dark at different temperatures. Incubation temperatures were chosen based on mean summer temperatures of wetlands sampled and was 10 °C for Ruoergai, 20 °C for Sanjiang, 28 °C for Hongze, and 30 °C for Poyang. We also measured the CH_4 production potential of the Ruoergai peatland at temperature of 20 °C to evaluate the effect of temperature increases on CH_4 production. All treatments were performed with three replicates. During the incubation, the CH_4 concentration in the jar headspace was measured daily by sampling 1.0 ml headspace gas with a precision sampling syringe (Valco Instruments, Baton Rouge, LA, USA) and applied to a Shimadzu GC12A with FID and a 2 m Porapak Q (80/100 mesh) column. The oven, injector, and detector temperatures were 80 °C, 200 °C, and 200 °C, respectively. The carrier gas (N_2) flow rate was 30 ml min^{-1} and flame gases (H_2 and O_2) were set at 20 and 30 ml min^{-1}, respectively. The standard gas was cross-checked by the National Institute for Agro-environmental Sciences, Japan. The rate of CH_4 production was calculated from the slope of the

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linear regression given by the graph of CH_4 concentration increase over time. The amount of CH_4 produced in the jars was sum of CH_4 in the jar headspace and CH_4 dissolved in water; the latter was calculated based on Bunsen solubility coefficients of CH_4 (Ding et al., 2010).

2.3 Soil analysis

Soil pH was determined using a glass electrode and a soil to water ratio of 1:5. Soil organic carbon (SOC) was measured by wet oxidation using dichromate in acid medium followed by the $FeSO_4$ titration method, and total nitrogen (TN) was determined by the Kjeldahl method (Lu, 2000). For dissolved organic carbon (DOC), 10 g fresh soil (on an oven dried basis) was incubated with 50 ml distilled water for 30 min on an end-over-end shaker at 25 °C, then centrifuged for 20 min at 8000 rpm. The extracted solutions were passed through a 0.45-µm filter paper and analyzed on a Shimadzu C analyzer (TOC Vcph, Shimadzu, Kyoto, Japan).

2.4 DNA extraction and real-time PCR

Total DNA from three replicates of soil samples was extracted using the FastDNA SPIN Kit for soils (BIO 101, Qbiogene, Carlsbad, CA, USA) according to the manufacturer's instructions (Cahyani et al., 2008). Real-time PCR was carried out to quantify the methanogenic archaea 16S rRNA genes in soil samples using LightCycler ST300, LightCycler Software Version 3.5 (Roche Diagnostics, Germany) and SYBR Premix Ex Taq (TaKaRa, Japan). The primer pair 1106F (forward) and 1378R (reverse) was used for PCR amplification targeting the 16S rRNA gene of methanogenic archaea (Watanabe et al., 2006). Each reaction mixture (25 µl) consisted of 12.5 µl 1 × SYBR Premix Ex Taq, 0.25 µl of each primer, 1 µl of DNA template diluted 20 times, and sterilize distilled water. The real-time PCR program was initiated by a denaturation step at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 10 s, annealing at 57 °C for 10 s, and extension at 72 °C for 6 s. A standard curve based on known

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methanogenic archaea copy numbers (1.97 to 19.7×10^8 copies μl^{-1}) was generated using purified PCR product (Jia and Conrad, 2009).

2.5 Statistical analyses

All data were expressed on the basis of oven-dried soil. The means and standard errors were calculated with three to six replicates. All statistical analyses were performed with SPSS 11.0 software. Statistically significant differences of means in all soils were judged by one-way analysis of variance (ANOVA) and least significant difference (LSD) calculations at a 5% significance level. The P-values for the effects between different wetlands and between different depths of the same wetland were adjusted using a Bonferroni correction. Regression analyses were used to test relationships between population of methanogenic archaea and SOC, TN, and the average CH_4 production potential.

3 Results

3.1 Biogeochemical characteristics of soils

Soil properties of different natural wetlands are shown in Table 1. Soil pH in the top layer of the Sanjiang marsh and Poyang wetland is significantly lower than the top-layer pH of the Hongze wetland and the Ruoergai peatland. SOC in the top layer of the Ruoergai peatland is 134.83 g C kg^{-1} soil, significantly higher than that of other wetlands, and the lowest SOC is measured in the Poyang wetland in the subtropical zone. SOC concentration in the top layer of the Sanjiang marsh in the cold temperate zone varies greatly with plant types and is 38.73 g C kg^{-1} soil in the *C. angustifolia* marsh, which is only one third of the value in the *C. lasiocarpa* marsh, and also dramatically lower than in the Hongze wetland in the warm temperate zone. SOC concentration in the vertical profile sharply increases with depth in the Ruoergai peatland, but to some

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extent decreases in other wetlands. Soil TN shows a similar pattern to SOC (Table 1). The concentrations of DOC increase with soil depth in all wetlands except the Sanjiang plain *C. lasiocarpa* marsh where most roots are distributed in the top layer. The highest top layer soil DOC concentration appears in the *C. lasiocarpa* marsh, whereas the lowest is in the Poyang wetland. The sulfate (SO_4^{2-}) concentration ranges from 0.04 to 1.37 g kg^{-1} soil, and is much higher in the Hongze wetland than in other wetlands, which were below 0.12 g kg^{-1}.

3.2 Methane production potential

In the vertical profile, the highest CH_4 production potential occurs in the top soil layer, and is significantly higher than the values in the lower soil layers in all wetlands except the Ruoergai peatland (Fig. 2). The CH_4 production potential of the 0–20 cm soil in the *C. angustifolia* marsh amounts to 191 µg kg^{-1} soil d^{-1}, and is 8.86 and 7.23 times greater than those of the 20–40 and 40–60 cm soils, respectively. Methane production potential of 0–30 cm soil in the *C. lasiocarpa* marsh is 184-fold higher than that of the lower layer soils, and is 3.48 times as much as the value in the corresponding layer of soil in the *C. angustifolia* marsh. In the top soil layer of the Hongze wetland, CH_4 production potential is 6.40 times that of the lower soil layer, and 5.12–15.76 times greater than that of the Poyang wetland. In the Ruoergai peatland, CH_4 production potential decreases as depth increases at both 10 °C and 20 °C, however difference between soil layers is not significant at the 10 °C incubation temperature, but is significant at 20 °C. When comparing the same soil layer at different temperatures, no significant difference is observed between 10 °C and 20 °C (Fig. 2).

The highest CH_4 production potential in the top soil layer is observed in the Sanjiang Plain *C. lasiocarpa* marsh, and is observed to be 665 µg CH_4 kg^{-1} soil d^{-1}, whereas the lowest values occur in the Ruoergai peatland. The CH_4 production potential in the top soil layer does not increase with the increase of wetland in situ temperature in China (Fig. 3).

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3.3 Methanogen population

The population of methanogenic archaea in the top soil layer of the Hongze wetland is highest relative to other wetlands at 8.29×10^9 cell g^{-1} soil, and the lowest is in the Poyang wetland (1.07×10^9 cell g^{-1} soil) (Fig. 4). In the Sanjiang Plain, the population of methanogens in the top soil layer of the *C. lasiocarpa* marsh is statistically significantly lower than that in the corresponding soil layer of *C. angustifolia* marsh. Both, however, are dramatically lower than those in the Ruoergai peatland. In the vertical profile, the population of methanogens in the top soil layer is significantly higher than that in the Sanjiang marsh lower soil layer, as well as of the Hongze and Poyang wetlands. In contrast, the diversity of the methanogenic community increases with depth in the Ruoergai peatland.

Regression analysis shows that DOC significantly increases exponentially with SOC concentration in the soils of all wetlands ($R^2 = 0.320$, $P = 0.044$, $n = 13$), while CH_4 production potential is significantly correlated with DOC ($R^2 = 0.305$, $P = 0.05$, $n = 13$), rather than SOC ($R^2 = 0.001$, $P = 0.92$, $n = 13$). There is a significant logarithmic correlation between the population of methanogens and the SOC and TN concentrations (Fig. 5), however the population of methanogens is not significantly associated with CH_4 production potential (Fig. 6). The specific CH_4 production potential in the top soil layer of the Sanjiang Plain *C. lasiocarpa* marsh and the Poyang wetland is significantly higher than those in corresponding soil layers of other wetlands (Fig. 7). It is considerably reduced in the lower soil layer relative to the top soil layer in all wetlands sampled.

4 Discussion

Methane emissions from natural wetland ecosystems show large spatial variations (Yavitt et al., 1988; Crill et al., 1992; Saarnio et al., 1998). Ding et al. (2004) found that CH_4 emissions from the Ruoergai peatland vegetated with *Carex muliensis* and *Carex meyeriana* in the Qinghai-Tibetan Plateau average 2.87 mg CH_4 m^{-2} h^{-1} over

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the growth season, which correlates to only one sixth of the values measured at the *Carex* freshwater marsh in China's Sanjiang Plain. This study concluded that the highest CH_4 production potential occurred in the *C. lasiocarpa* marsh of the Sanjiang Plain and the lowest occurred in the Ruoergai peatland (Fig. 2). This is completely consistent with results of previous field measurements (Ding et al., 2004). However, CH_4 production potential measured at the mean in situ summer temperature roughly decreased as the air temperature increased from the Sanjiang Plain freshwater marsh in the cold temperate zone, to the Hongze wetland in the warm temperate zone, and then to the Poyang wetland in the north subtropical zone (Fig. 3). This indicates that CH_4 production potentials of wetlands may increase as the latitude increases in the eastern part of China. Freitag and Prosser (2009) observed that the CH_4 production rate was significantly correlated with the mcrA transcript:gene ratio in an ombrotrophic peatland in the North Wales, UK. In the present study, a simultaneous decrease in the CH_4 production rate and methanogen population with decreasing soil depth in all wetlands except the Ruoergai peatland suggests that CH_4 production potential is likely determined by the abundance of methanogenic archaea (Figs. 2 and 4). On the national scale, however, no significant relationship is apparent between CH_4 production rate and the population of methanogens in wetlands (Fig. 6). This finding is in agreement with the results of Galand et al. (2003) and Cadillo-Quiroz et al. (2006), who reported that the variation in community and population of methanogens did not change potential CH_4 production. Colwell et al. (2008) also failed to accurately estimate the emission rate using a newly-launched model based on the relationship between in situ CH_4 production rates and the abundance of mcrA genes in marine sediments. Our finding, together with previous results (Galand et al., 2003; Freitag and Prosser, 2009), suggest that the discrepancies in CH_4 production potential among wetlands around China do not completely result from the differences in the abundance of methanogenic archaea.

Bergman et al. (1998, 2000) found that the absence of labile organic carbon in peatland heavily suppressed CH_4 production at low temperature. In a Michigan ombrotrophic bog, Avery et al. (1999, 2003) observed that acetate accumulation stimu-

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lated CH_4 production, which contributed > 80% of total CH_4 production. In the present study, CH_4 production rate was found to be significantly correlated with the presence of DOC ($R^2 = 0.30$, $P = 0.05$, $n = 13$). This indicates that the supply of methanogenic substrates may be responsible for the level of CH_4 production potential in wetlands. We found that both CH_4 production rate and the population of methanogenic archaea in the top soil layer of the Poyang wetland is significantly lower than in the Sanjiang Plain *C. lasiocarpa* marsh and the Hongze wetland (Figs. 2 and 4), while the abundance of methanogens is significantly correlated with SOC and total nitrogen concentrations in soils (Fig. 5). Apparently, low SOC in the Poyang wetland not only suppressed the growth of methanogens but also reduced the supply of substrates for methanogens, resulting in a low CH_4 production rate. The water table in the Poyang wetland fluctuates dramatically with increases or decreases in the Yangze River water level and is generally near or below the soil surface in winter, resulting in strong decomposition of SOC in the subtropical zone. Thus, we argue that intermittently inundated wetlands such as the Poyang wetland may not be a hot source of atmospheric CH_4 compared to permanently inundated Sanjiang Plain marsh in China (Ding et al., 2004, 2010).

In this study, the population of methanogens in the top soil layer of wetlands was measured to be between $1.07 - 8.29 \times 10^9$ cells g^{-1} soil (Fig. 4), which is higher than the $\sim 1 \times 10^8$ cells g^{-1} soil in an acidic bog and a calcareous fen in the UK (Kim et al., 2008) and the $0.5 - 0.9 \times 10^7$ cells g^{-1} fresh peat in an acidic peat bog in West Siberia, Russia (Kotsyurbenko et al., 2004), but similar to the value ($\sim 1.3 \times 10^9$ cells g^{-1} soil) recorded in Japanese paddy field soil (Watanabe et al., 2007). The highest population of methanogen archaea was measured in the Hongze wetland (Fig. 4), indicating that this wetland is particularly favorable to the growth of methanogens. However, the CH_4 production rate and specific CH_4 production potential of per methanogen cell in the top soil layer of the Hongze wetland was significantly lower than in the Sanjiang Plain *C. lasiocarpa* marsh (Figs. 2 and 7). This implies that the methanogenic community in wetlands located in different latitudes shows a distinct capacity for CH_4 production. However, a relatively high SO_4^{2-} concentration was measured in the Hongze wetland but not

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in other wetlands (Table 1). Sulfate-reducing bacteria could outcompete methanogens for substrates such as acetate and H_2 (Chin and Conrad, 1995), thereby lowering CH_4 production or possibly masking potential CH_4 production of individual metahnogen cells in the Hongze wetland. Alternatively, Thauer (1998) and Cadillo-Quiroz et al. (2006) observed a higher proportion of dead or inactive methanogens in wetland soils with low levels of functional mcrA mRNA (methyl coenzyme M reductase). Thus, it is possible that a difference exists between the composition and the physiology of dominant methanogens in different wetlands (Galand et al., 2003). Further experiments are necessary to evaluate the methanogen community in Chinese wetlands.

Although there are more methanogenic archaea in the *C. angustifolia* marsh than in the *C. lasiocarpa* marsh of the Sanjiang Plain (Fig. 4), a significantly lower amount of DOC in the top soil layer results in a low CH_4 production rate (Fig. 2). Previous studies have shown that *Eriophorum* plants provide more root exudates for methanogens than *Carex* and *Juncus* (Ström et al., 2005; Koelbener et al., 2010). Ding et al. (2005) verified that *C. angustifolia* may make a larger contribution to CH_4 production than to CH_4 oxidation, and in contrast, while *C. lasiocarpa* has the opposite effect. This indicates that higher CH_4 production in the *C. lasiocarpa* marsh is not attributable to organic materials such as root exudates released by living *C. lasiocarpa*. Ding et al. (2002) suggested that the deep standing water in the *C. lasiocarpa* marsh inundates more plant litter, which in turn provides more substrates for methanogens. Therefore, we propose that SOC in Eastern China wetlands affects the population of methanogens and the supply of DOC, rather than temperature controlled CH_4 production potentials, while SOC in wetland soils is mainly affected by the position and stability of the water table through varying vegetation cover and SOC decomposition rate (Galand et al., 2003).

On the contrary, DOC concentration in the Ruoergai peatland is much higher than that in the Hongze and Poyang wetlands (Table 1), indicating that low CH_4 production potential in the Ruoergai peatland is not be attributable to the absence of substrates for methanogenesis. Saarnio et al. (1998) and Frenzel and Karofeld (2000)

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recognized that methanogens are quite sensitive to soil temperature, especially temperatures lower than 20 °C. Chin et al. (1999) observed a significant reduction in CH_4 production and a subsequent increase in the accumulation of acetates as incubation temperature was lowered from 30 °C to 15 °C in an Italian paddy soil. Further, they found that the reduction in CH_4 production potential resulted from changes in dominant species of methanogens. Zhang et al. (2008) identified a dominant uncultured methanogen in the Ruoergai peatland and named it a Zoige cluster I (ZC-I). They found that this type of methanogen is suitable only for living at low temperature. It follows that low CH_4 production potential in the Ruoergai peatland is mainly due to a low cell-specific rate of CH_4 production by distinct methanogens (Fig. 7). Results of the present study indicate that CH_4 production potential in the Ruoergai peatland did not change significantly as the incubation temperature increased from 10 °C to 20 °C (Fig. 2). This is in agreement with the result of Zhang et al. (2008), who found that there is no significant difference in the CH_4 production rate and the increased rate of population of ZC-I between incubation temperatures of 15 °C and 30 °C. These results suggest that the predicted future increase (0.6–4.0 °C) of global temperatures (IPCC, 2007) may not strongly stimulate CH_4 emissions from peatlands in the Qinghai-Tibetan Plateau as in natural wetlands with low elevation (Kettunen et al., 1996) unless the shift in dominant species of methanogens occurred in a peatland. A detailed study is required to evaluate the influence of rising temperatures on CH_4 emission from peatlands in the Qinghai-Tibetan Plateau.

5 Conclusions

The top soil layer has the maximum population of methanogenic archaea in all wetlands except the Ruoergai peatland and exhibits the highest CH_4 production potential, as measured at the mean in situ summer temperature for all wetlands in China. There is a significant relationship between the population of methanogens and SOC or TN concentrations in wetland soils. This indicates that soil organic carbon and/or nitrogen

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may control the abundance of methanogens in wetland ecosystems. However, the CH_4 production potential is not significantly related to the methanogen population; it is related to the DOC concentration, and this indicates that the abundance of methanogens is not an effective index for predicting the CH_4 production potential in wetlands. In China, the CH_4 production potential in the top soil layer of wetlands increases with increasing latitude. This suggests that the CH_4 production potential in the wetlands of Eastern China is not affected by temperature; rather, it is affected by the supply of substrates for methanogens, which may depend on the wetland niche such as the position and stability of the water table. In contrast, low temperatures at high elevations may result in methanogens with low CH_4 production potential to become the dominant species, which in turn results in the suppression of the CH_4 production in the Ruoergai peatland, rather than the deficiency of substrates for methanogensis.

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Table 1. Sampling site characteristics and soil properties.

Location	Vegetation	Water depth (cm)	Site	Soil depth (cm)	pH	SOC ($g\,C\,kg^{-1}$)	TN ($g\,N\,kg^{-1}$)	DOC ($g\,C\,kg^{-1}$)	SO_4^{2-} ($g\,kg^{-1}$)
Sanjiang, Heilongjiang (47°34′N, 133°30′E)	*Calamagrostis angustifolia*	5	SJA1	0–20	5.07 ± 0.05a	38.73 ± 0.15d	2.63 ± 0.11e	0.43 ± 0.01ab	0.04 ± 0.00a
			SJA2	20–40	5.49 ± 0.03d	29.00 ± 0.29c	2.24 ± 0.00d	0.64 ± 0.16abc	0.08 ± 0.01c
			SJA3	40–60	5.86 ± 0.02f	35.05 ± 0.37cd	2.46 ± 0.04e	0.77 ± 0.15abc	0.12 ± 0.02c
Sanjiang, Heilongjiang (47°34′N, 133°29′E)	*Carex lasiocarpa*	25	SJL1	0–30	5.51 ± 0.03d	128.01 ± 10.48g	6.92 ± 0.02h	2.74 ± 0.56d	0.06 ± 0.00b
			SJL2	30–60	6.36 ± 0.03g	7.69 ± 0.05a	0.74 ± 0.04a	0.96 ± 0.06bc	0.04 ± 0.01a
Hongze, Jiangsu (33°13′N, 118°19′E)	*Potamogeton malaianus*	120	HZ1	0–20	7.84 ± 0.05h	78.86 ± 0.53f	6.54 ± 0.07g	0.47 ± 0.03ab	1.37 ± 0.01e
			HZ2	20–40	8.02 ± 0.03i	60.29 ± 0.74e	4.81 ± 0.02f	0.64 ± 0.12abc	0.67 ± 0.00d
Poyang, Jiangxi (29°26′N, 116°01′E)	*Cyperus glomeratus* L.	2	PY1	0–10	5.33 ± 0.02c	19.69 ± 0.17b	1.62 ± 0.01c	0.15 ± 0.01a	0.05 ± 0.00ab
			PY2	10–20	5.16 ± 0.00b	13.83 ± 0.14ab	1.21 ± 0.01b	0.19 ± 0.02a	0.06 ± 0.00b
			PY3	20–40	5.61±0.01e	9.04±0.16a	0.83 ± 0.02a	0.24 ± 0.02a	0.04 ± 0.00ab
Ruoergai, Sichuan (33°54′N, 102°49′E)	*Carex muliensis* *Eleochalis valleculosa*	5	REG1	0–10	8.21 ± 0.01j	134.83 ± 3.13g	8.55 ± 0.04i	0.69 ± 0.09abc	0.09 ± 0.01c
			REG2	10–20	8.21 ± 0.03j	187.53 ± 6.88h	10.25 ± 0.12j	0.75 ± 0.15abc	0.10 ± 0.00c
			REG3	20–40	8.15 ± 0.00j	256.55 ± 4.67i	12.28 ± 0.06k	1.20 ± 0.07c	0.11 ± 0.00c
F values	–	–	–	–	2160	672	4156	11.1	3029
P	–	–	–	–	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Values are means ($n = 3$) with standard error. Different letters within the same column indicate significant differences at $P < 0.05$.

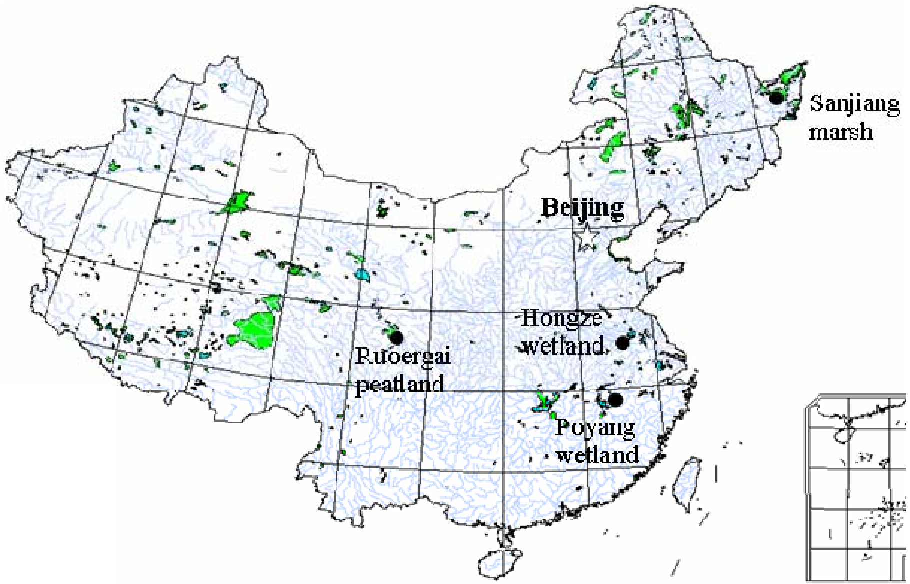

Fig. 1. Location of studied natural wetlands in China.

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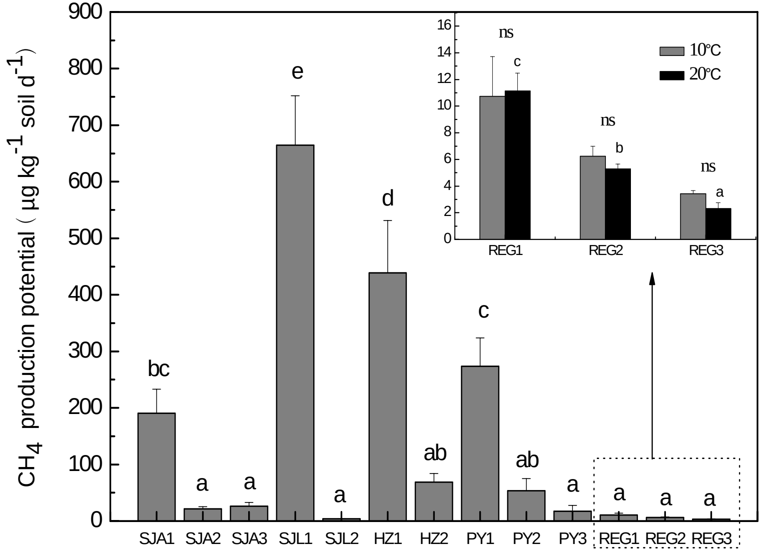

Fig. 2. CH_4 production potential of vertical profile soil slurries in the Sanjiang Plain *C. angustifolia* (SJA1 = 0–20 cm, SJA2 = 20–40 cm, SJA3 = 40–60 cm) and *C. lasiocarpa* (SJL1 = 0–30 cm, SJL2 = 30–60 cm) marshes, Hongze wetland (HZ1 = 0–20 cm, HZ2 = 20–40 cm), Poyang wetland (PY1 = 0–10 cm, PY2 = 10–20 cm, PY3 = 20–40 cm) and Ruoergai peatland (REG1 = 0–10 cm, REG2 = 10–20 cm, REG3 = 20–40 cm). Vertical bars denote standard errors of means ($n = 3$). Different letters indicate significant differences at $P < 0.05$. The incubation temperature was 20 °C for the Sanjiang marsh, 28 °C for the Hongze wetland, 30 °C for the Poyang wetland, and 10 °C or 20 °C for the Ruoergai peatland. The ns indicates no significant difference between incubation temperature of 10 °C and 20 °C for the same soil layer at $P < 0.05$.

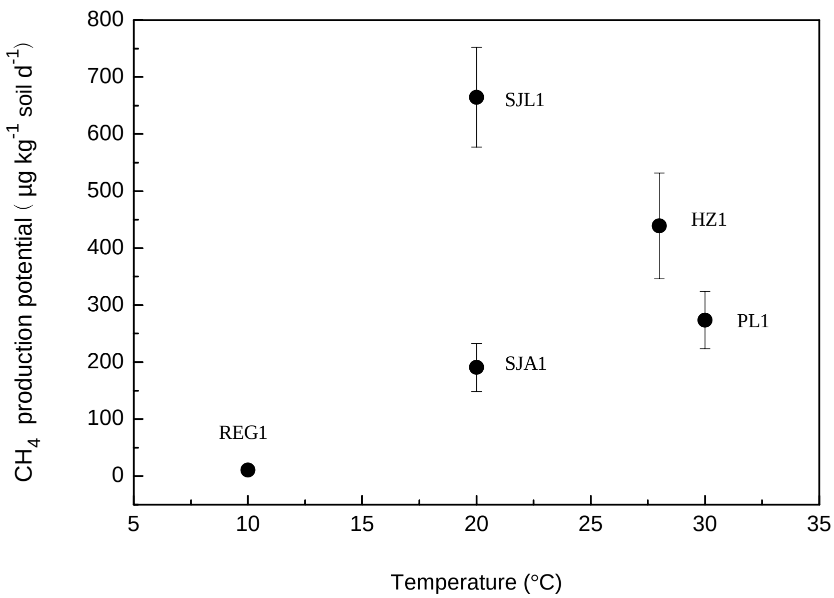

Fig. 3. Relationship between CH_4 production potential of the top soil layer in different wetlands across China and incubation temperature. Vertical bars denote standard errors of means (n = 3). SJA1 = 0–20 cm soil in the Sanjiang Plain *C. angustifolia* marsh, SJL1 = 0–30 cm soil in the Sanjiang Plain *C. lasiocarpa* marsh, HZ1 = 0–20 cm soil in the Hongze wetland, PY1 = 0–10 cm soil in the Poyang wetland, and REG1 = 0–10 cm soil in the Ruoergai peatland.

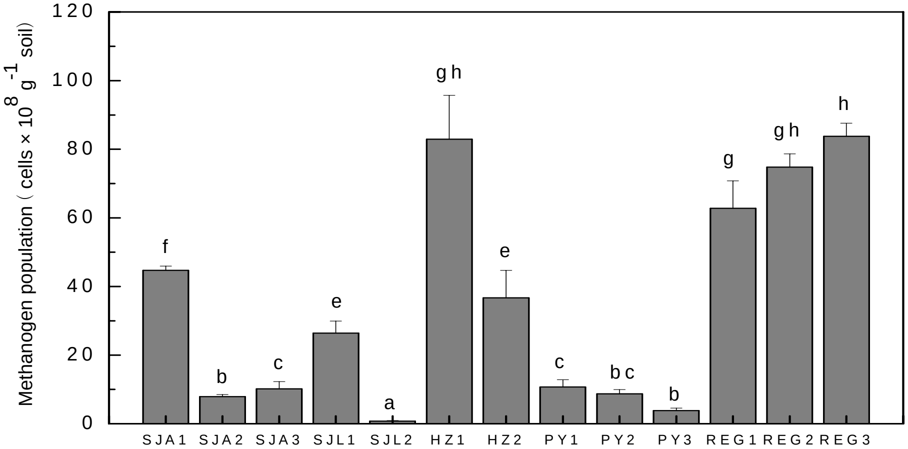

Fig. 4. Population of methanogenic archea in vertical profile soils of the Sanjiang Plain *C. angustifolia* (SJA1 = 0–20 cm, SJA2 = 20–40 cm, SJA3 = 40–60 cm) and *C. lasiocarpa* (SJL1 = 0–30 cm, SJL2 = 30–60 cm) marshes, Hongze wetland (HZ1 = 0–20 cm, HZ2 = 20–40 cm), Poyang wetland (PY1 = 0–10 cm, PY2 = 10–20 cm, PY3 = 20–40 cm) and Ruoergai peatland (REG1 = 0–10 cm, REG2 = 10–20 cm, REG3 = 20–40 cm). Vertical bars denote standard errors of means ($n = 3$). Different letters indicate significant differences at $P < 0.05$.

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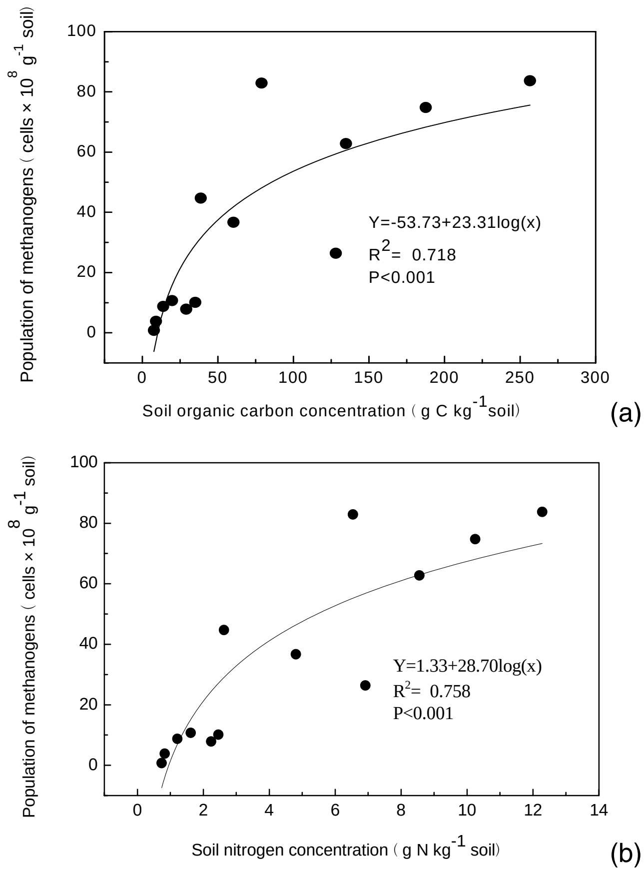

Fig. 5. Relationships between population of methanogenic archaea and SOC **(a)** or total nitrogen **(b)** concentrations in different soil layers of four wetlands across China.

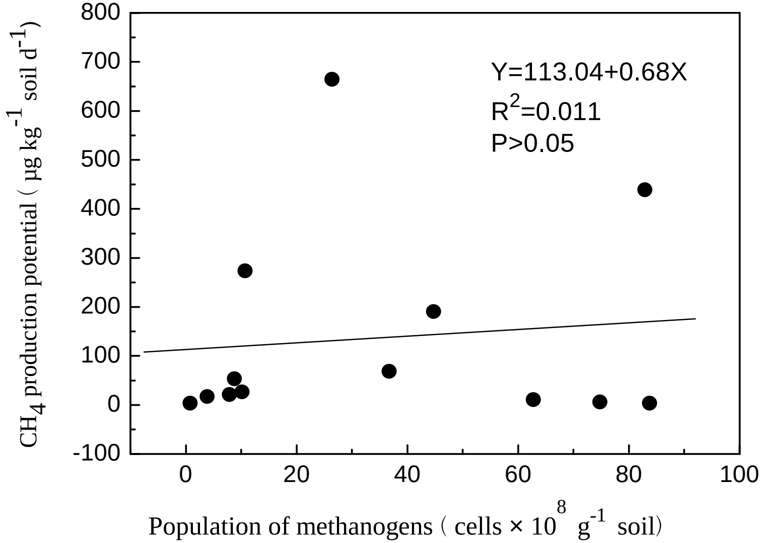

Fig. 6. Relationship between population of methanogenic archaea and mean CH_4 production potential in different soil layers of four wetlands across China.

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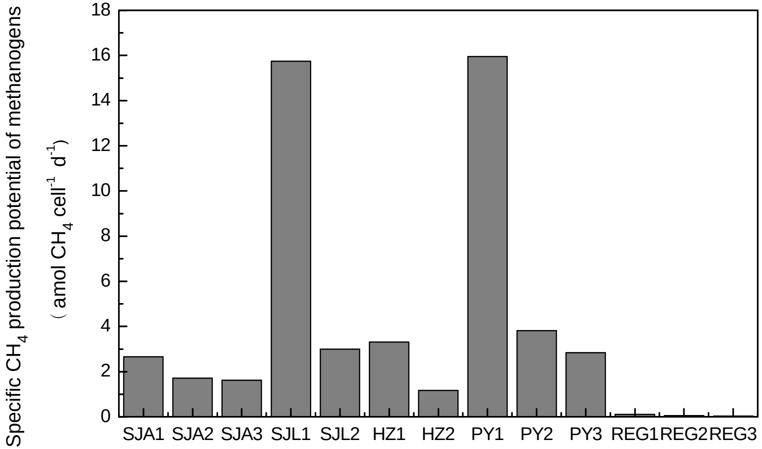

Fig. 7. Specific CH_4 production potential of methanogens (CH_4 production potential per cell) in different soil layers of the Sanjiang Plain *C. angustifolia* (SJA1 = 0–20 cm, SJA2 = 20–40 cm, SJA3 = 40–60 cm) and *C. lasiocarpa* (SJL1 = 0–30 cm, SJL2 = 30–60 cm) marshes, Hongze wetland (HZ1 = 0–20 cm, HZ2 = 20–40 cm), Poyang wetland (PY1 = 0–10 cm, PY2 = 10–20 cm, PY3 = 20–40 cm) and Ruoergai peatland (REG1 = 0–10 cm, REG2 = 10–20 cm, REG3 = 20–40 cm).

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