Soil methane production, anaerobic and aerobic oxidation in porewater of wetland soils of the Minjiang River estuarine, China

W. Wang1,2*, C. Zeng1,2, J. Sardans3,4, C. Wang1, C. Tong1,2, J. Peñuelas3,4

1Institute of Geography, Fujian Normal University, Fuzhou 350007, China
2Key Laboratory of Humid Subtropical Eco-geographical Process, Ministry of Education, Fujian Normal University, Fuzhou 350007, China
3CSIC, Global Ecology Unit CREA-CSIC-UAB. 08913 Bellaterra, Catalonia. Spain
4CREAF. 08913 Cerdanyola del Vallès. Catalonia. Spain

*Author to whom correspondence should be addressed; E-Mail: wangweiqi15@163.com
Tel.: +86-591-83465214; Fax: +86-591-83465397.

Abstract

Wetlands are important sources of methane emission. Methane anaerobic oxidation, aerobic oxidation and production, and dissolved methane are important process of methane metabolism. We studied methane metabolism and the soil influencing factors. Potential soil methane production, anaerobic oxidation and aerobic oxidation rates, and dissolved methane in soil porewater changed seasonally and the annual average was 21.1±5.1 μg g⁻¹d⁻¹, 11.0±3.9 μg g⁻¹d⁻¹, 20.9±5.8 μg g⁻¹d⁻¹, and 62.9±20.6 μmol l⁻¹, respectively. Potential soil methane production and anaerobic and aerobic oxidation were positively correlated among them and with soil pH and negatively correlated with soil redox potential (Eh). Potential soil methane production and aerobic and anaerobic oxidation rates were negatively related to pore soil methane concentration. Thus, the more water saturated the soil (the lower Eh), the higher its capacity to methane production was, but even higher was soil potential capacity to methane oxidation both in the same anaerobic circumstances and when the soil was suddenly submitted to aerobic conditions. All these results suggested a buffer effect in the methane balance in wetland areas, the environmental circumstances favoring methane production are also favorable to methane anaerobic oxidation.

Keywords Methane production · methane anaerobic oxidation · methane aerobic oxidation · dissolved methane · Minjiang River estuarine wetland
Introduction

Although, the total area occupied by wetlands currently accounts for only 4.6% of the total land area (Costanza et al. 1997), their ecosystem services value accounts for 32% of the total value of the world, especially coastal wetlands whose ecosystem service value accounts for 17% of the global total value (Costanza et al. 1997). Wetland is sensitive to the external stress and becomes the ideal area for global change research (Simas et al. 2001). Methane is one of the important greenhouse gases affecting global climate change. Although, wetlands only represent a small fraction of the Earth's land surface, they are the main sources of methane to the atmosphere, representing between 20%-39% of global methane emissions (Laanbroek 2010). The relative increase of methane at the scale of 100-year is about 25 times than that of carbon dioxide (IPCC, 2007). Thus, improving the knowledge of methane metabolism in wetland soils warrants intense research.

Methane metabolism is in several phases: production, oxidation, dissolved methane, transport and emission (Buckley et al. 2008). Methane production, oxidation and dissolved methane in water have great impacts on the ultimate reduction of methane emissions in wetlands (Singh 2011). In the 21st century, methane anaerobic oxidation has become the core and hot issues (Raghoebarsing et al. 2006; Kniemeyer et al. 2007). However, the reports about methane anaerobic oxidation in coastal estuarine wetlands are few. Furthermore, the relationships between potential soil methane anaerobic oxidation capacity with potential soil methane production and with potential soil methane aerobic oxidation capacity are uncertain in wetland areas.
Whether relationships exist between the soil capacity to produce methane and the soil capacity to oxidize methane when the soil is in anaerobic conditions and whether between production and oxidation in wet conditions in dry-aerobic periods are two important questions that warrant research.

Methane metabolism in wetlands is strongly influenced by environmental factors that vary both spatially and temporally (Datta et al. 2013). The availability of electron acceptors and donors in soils plays a key role in regulating CH$_4$ production and consumption (Moran et al. 2008; Ettwig et al. 2010; Ro et al. 2011) and thereby controlling dissolved methane and the emission. Electron acceptors (e.g. Fe$^{3+}$, NO$_3^-$, and sulfate) are reduced during wet periods, but regenerated (oxidized) during dry periods (Neubauer et al. 2007). Soils can also provide carbon substrates to microbes for mediating CH$_4$ production and enhancing plant growth that in turn governs more than 90% of CH$_4$ transport (Le Mer and Roger 2001). Other environmental variables, include soil temperature, pH, redox potential (Eh) and salinity also influences CH$_4$ metabolism (Song et al. 2008; Wang et al. 2017). Better knowledge and characterization of CH$_4$ metabolism and transport of CH$_4$, are essential for better understanding and characterizing of GHG emissions from wetlands advancing in the knowledge of soil and soil pore water circumstances can favor the production and oxidation processes in soil media and thus, the final methane contents in soil-atmosphere interface and emission can give clues to choose between soil and plant community management strategies to diminish as much as possible the methane emissions. This information can also provide clues to improve the models and
projections of methane production and emissions on regional and global scale.

China has a coastline of 18,000 km in length, with numerous estuaries and bays and a diversity of coastal wetlands which are important component of China's wetlands, as well as the world's wetlands. Minjiang River estuarine wetland is the main natural wetland of southeast coast of China. The wetland of Minjiang River estuary is rich in biological species and abundant in waterbird resources (Liu et al., 2006). Moreover, the Minjiang River estuary is an important tidal wetland ecosystem due to its unique location at the transition between central and southern subtropical climatic zones (Zheng et al. 2006). The tidal wetlands are rich in animal and plant biodiversity (Zhou et al. 2006) being an excellent site representing the wide coastal wetland areas of this part of China coast-

We studied the: (1) the seasonal variation potential of soil methane production, soil methane anaerobic oxidation, soil methane aerobic oxidation, dissolved methane, emission and the relationships among these variables and (2) the soil variables that have significant relationships with methane-related variables in Minjiang River estuarine wetland along the year. The results obtained in this study were also aimed to provide a scientific basis for a suitable management of wetland avoiding as much as possible CH₄ emissions.

Materials and Methods

Study area

This study was conducted in the Shanyutan wetland (26°01′46″N; 119°37′31″E,
Fig. 1), the largest tidal estuarine wetland (approximately 3120 ha) in the estuary of the Minjiang River.

The climate in this region is relatively warm and wet with a mean annual temperature of 19.6 °C and a mean annual precipitation of 1346 mm (Zheng et al. 2006). The soil surface is submerged across the study site beneath 10-120 cm of water for 3-3.5 h during each tidal inundation. Soil surfaces of the entire wetland are exposed at low tide during 24 h and the weight percentage of water in the soil and soil redox potential are 116.39% and 12.57 mV respectively and soil remains flooded at some depths. The average salinity of the tidal water between May and December 2007 was 4.2 ± 2.5‰.

*C. malaccensis* is one of the two dominant species of plants in this estuarine wetland. *C. malaccensis* is a native plant typically found in the upper (mid to high) portions of mudflats that grow between April and October, the highest population height is about 1.5 m and the density is about 1000 m². Below-ground rhizomes are creeping growth in the topsoil layers.

**Experimental design**

Seasonal variation samples were collected from April in 2012 to March in 2013 from Shanyutan wetland in Minjiang River estuary. We established a plot of 900 m² in Shanyutan wetland and then collected the *C. malaccensis* wetland soil randomly after selecting three quadrats (100 m²) within the big plot. Soil samples of 0-20 cm were collected with a small core sampler (length and diameter of 0.3 and 0.1 m). The sampling was conducted every month during one-year. Thus, a total of 36 soil
samples (one wetland type × one soil layer × twelve months’ × three replicate plots) were thus collected.

Measurements of potential methane production

In each sampled soil, potential soil methane production rate was determined by placing 30 g of the fresh soil sample in a 120 ml incubation bottle and injecting 30 ml of distilled water (water: soil ratio was thus 1:1) (Wang et al. 2010; Bergman et al. 2000). The incubation bottles were filled with oxygen-free nitrogen through a small hole in the bottle stopper to eliminate the possibility of methane consumption caused by carrying oxygen during the sampling process and slowly equilibrated with the atmospheric pressure for 24 h to consume the residual oxygen in incubation bottles, which ensures the soil sample is in a strictly anaerobic environment (Smemo and Yavitt 2007; Wrede et al. 2012). Three replicates were set and placed in an anaerobic incubator (YQX- II, Shanghai Yuejin Medical Equipment Factory) in the dark place using the average soil temperature in situ. Then the gas samples were taken at 0, 24, 48, 72, and 96 h, and the sample incubation bottles were gently swirled for 1-2 min before gas sampling. Each extraction was 2 ml and supplemented with the corresponding volume of oxygen-free nitrogen. Methane concentration was determined by a GC-2010 gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan). The potential methane production rate was calculated by the methane concentration increment during the incubation time in the incubation bottles.

Measurements of potential methane anaerobic oxidation

In each collected soil sample, potential soil methane anaerobic oxidation rate was
determined by placing 30 g of fresh soil sample in a 120 ml incubation bottles and
injecting 30 ml of 40 mmol l\(^{-1}\) of methane production inhibitor (BES, bromoethanesulfonate) solution (Müller et al. 1993; Hoehler et al. 1994) at a water:
soil ratio of 1:1 (Bergman et al. 2000). The incubation bottles were filled with oxygen-free nitrogen through a small hole in the bottle stopper to eliminate the possibility of methane consumption caused by carrying oxygen during the sampling process and slowly equilibrated with the atmospheric pressure for 24 h to consume the residual oxygen in the incubation bottles, ensuring the soil sample is in a strictly anaerobic environment (Smemo and Yavitt 2007; Wrede et al. 2012). Then, pure Methane standard gas was injected into each incubation bottles so that the concentration of methane in the incubation bottle was 10000 μmol mol\(^{-1}\), and 3 replicates were set and placed in an anaerobic incubator (YQX-Ⅱ, Shanghai Yuejin Medical Equipment Factory) in the dark place using the average soil temperature in situ. Then the gas samples were taken at 0, 24, 48, 72, and 96 h, and the sampled incubation bottles were gently swirled for 1-2 min before gas sampling. Each extraction was 2 ml and supplemented with the corresponding volume of oxygen-free nitrogen. Methane concentration was determined by a GC-2010 gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan). Potential methane anaerobic oxidation rate was calculated by the methane concentration decrement as the incubation time in the incubation bottles.

**Measurements of potential methane aerobic oxidation**

In each soil sampled potential soil methane aerobic oxidation rate was determined by Krüger et al. (2002) and Supparattanapan et al. (2009), by placing 30 g of fresh soil sample in a 120 ml incubation bottle and injecting 30 ml of distilled water into it, the water: soil ratio was 1:1 (Wang et al. 2010; Bergman et al. 2000). Then, pure methane
standard gas was injected into each incubation bottles so that the concentration of methane in the incubation bottles was 10000 μmol mol\(^{-1}\), and three replicates were set and incubation in the dark place using the average soil temperature in situ. Then the gas samples were taken at 0, 24, 48, 72, and 96 h, and the sampled incubation bottles were gently swirled for 1-2 min before gas sampling. Each extraction was 2 ml and supplemented with the corresponding volume of oxygen-nitrogen. Methane concentration was determined by a GC-2010 gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan). Potential soil methane anaerobic oxidation rate was calculated by the methane concentration decrement as the incubation time in the incubation bottles.

**Measurement (in situ) of porewater dissolved CH\(_4\) concentration**

Porewater was sampled *in situ* once each month. Three specially designed stainless steel tubes (2.0 cm inner diameter) were installed to a depth of 30 cm in each plot. Porewater samples were collected immediately after the measurements of CH\(_4\) emission using 50-ml syringes to inject it into pre-evacuated vials (20 ml) and stored in a cooling box in the field. After transporting to the laboratory, the samples in the vials were stored at -20 °C until the analysis of CH\(_4\) concentration. Before analysis, the vials were first thawed at room temperature and were then vigorously shaken for 5 min to equilibrate the CH\(_4\) concentrations between the porewater and the headspace. The gas samples were taken from the headspace of the vials and analyzed for CH\(_4\) concentration with the above gas chromatograph (Ding et al. 2003).
Determination of methane concentrations

Methane concentrations in the headspace air samples were determined by gas chromatography (Shimadzu GC-2010, Kyoto, Japan) using a stainless steel Porapak Q column (2 m long, 4 mm outer diameter, 80/100 mesh). A flame ionization detector (FID) was used for the determination of the methane concentrations. The operating temperatures of the column, injector and detector for the determination of methane concentrations were adjusted to 70, 200 and 200 °C. The gas chromatograph was calibrated before and after each set of measurements using 1.01, 7.99 and 50.5 μL methane L⁻¹ in He (CRM/RM Information Center of China) as primary standards.

Calculation of potential methane production, anaerobic oxidation, aerobic oxidation, and porewater dissolved CH₄ concentration

Potential methane production, anaerobic oxidation, and aerobic oxidation rates were estimated by (Wassmann et al., 1998):

\[ P = \frac{dc}{dt} \cdot \frac{V_H}{W_s} \cdot \frac{MW}{MV} \cdot \frac{T_H}{T_{st} + T} \]

where \( P \) is the potential rate of methane anaerobic oxidation, aerobic oxidation and production (μg⁻¹ g⁻¹ d⁻¹), \( dc/dt \) is the recorded change in the mixing ratio of C (methane) in the headspace over time (mmol mol⁻¹ d⁻¹), \( V_H \) is the volume of the headspace (L), \( W_s \) is the dry weight of the soil (g), \( MW \) is the molecular weight of methane (g), \( MV \) is the molecular volume (L), \( T \) is the temperature (K) and \( T_{st} \) is the standard temperature (ºK).
The concentration of CH$_4$ dissolved in the porewater was calculated following (Ding et al. 2003):

$$ C = \frac{Ch \cdot Vh}{22.4 \cdot Vp} $$

where $Ch$ is the CH$_4$ concentration ($\mu$l l$^{-1}$) in the air sample from the vials, $Vh$ is the volume of air in the bottle (ml), and $Vp$ is the volume of the porewater in the bottle (ml).

**Measurements of soil properties**

Total soil porewater (collected by centrifugation at 5000 r min$^{-1}$) dissolved organic-C (DOC) concentration was measured using a TOC-V CPH total carbon analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Porewater (collected by centrifugation at 5000 r min$^{-1}$) NO$_3^-$, SO$_4^{2-}$ and Cl$^-$ concentrations were determined by ICS2100 ion chromatography (American Dionex Production, Sunnyvale, USA). Soil temprature, Eh and pH were measured with an Eh/pH/temprature meter (IQ Scientific Instruments, Carlsbad, USA) and salinity was measured using a 2265FS EC Meter (Spectrum Technologies Inc., Paxinos, USA). Total Fe content was determined by digesting fresh soil samples with 1 mol HCl L$^{-1}$. Ferrous ions were extracted using 1,10-phenanthroline and measured spectrometrically (Wang et al. 2012). Ferric concentration was calculated by subtracting the ferrous concentration from the total Fe concentration.

**Statistical analyses**
The significance of the differences in potential methane production, anaerobic oxidation, aerobic oxidation and dissolved methane, soil variables and other properties among the seasonal variation were assessed by One-Way ANOVA. We analyzed the relationships of the potentials of soil methane production, soil methane anaerobic oxidation, soil methane aerobic oxidation and dissolved methane among them and with soil DOC, soil temperature, Eh, pH, salinity, soil NO$_3^-$, SO$_4^{2-}$ and Cl$^-$ concentrations and plant biomass. Plot and time of sampling (month) were introduced into the models as random factors. If a variable was non-normally distributed we transform it to normalize its distribution. In concrete soil nitrate and soil ferric concentrations were log-transformed to reach their normal distribution. We used the “nlme” (Pinheiro et al. 2016) and “lme4” (Bates et al. 2015) R packages with the “lme” and “lmer” functions to conduct the mixed linear models. We chose the best model for each dependent variable using Akaike information criteria. We used the MuMIn (Barton 2012) R package in the mixed models to estimate the percentage of variance explained by the model. We presented in significant relationships the total variance explained by the model including the fixed and random factors (R$^2_c$) and also the variability explained by only the fixed factor (R$^2_m$).

We used Principal component analyses (PCA) to assess the multiple correlations among total potential methane production, anaerobic oxidation, aerobic oxidation and dissolved methane and environmental factor and the analyzed soil variables and their relative importance in the separation of soil samples from different seasons. The PCA were performed using Statistica 6.0 (StatSoft, Inc. Tule, Oklahoma,
Results

Potential soil methane production, methane anaerobic oxidation, methane aerobic oxidation, and dissolved methane along the year

Potential soil methane production rates changed seasonally in the Shanyutan wetland of Minjiang River estuary (Figs. 2, 3), with a maximum value of 57.4±7.7 μg g⁻¹ d⁻¹ in January 2013 and a minimum value of 4.85±1.1 μg g⁻¹ d⁻¹ in August 2012. The annual average value was 21.1±5.1 μg g⁻¹ d⁻¹. In general, potential soil methane production rate was significantly higher in winter than that of the summer (Fig. 3, P<0.05). However, there were not significantly different among other seasons (Fig. 3, P>0.05).

Potential soil methane anaerobic oxidation rates changed seasonally in the Shanyutan wetland of Minjiang River estuary (Figs. 2, 3), with a maximum value of 41.8±13.4 μg g⁻¹ d⁻¹ in January 2013 and a minimum value of 3.46±0.97 μg g⁻¹ d⁻¹ in August 2012. The annual average value was 11.0±3.9 μg g⁻¹ d⁻¹. In general, potential soil methane anaerobic oxidation production rate was significantly higher in winter than those of spring and autumn (Fig. 3, P<0.05). However, there were not significantly different among other seasons (Fig. 3, P>0.05).

Potential soil methane aerobic oxidation rates changed seasonally in the Shanyutan wetland of Minjiang River estuary (Figs. 2, 3), with a maximum value of 70.2±24.5 μg g⁻¹ d⁻¹ in January 2013 and a minimum value of 6.55±1.42 μg g⁻¹ d⁻¹ in
May 2012. The annual average value was 20.9±5.8 μg g\(^{-1}\) d\(^{-1}\). In general, potential soil methane aerobic oxidation production rate was not significantly different among seasons (Fig. 3, \(P>0.05\)).

Dissolved methane in soil porewater changed seasonally in the Shanyutan wetland of Minjiang River estuary (Figs. 2, 3), with a maximum value of 261±39 μmol l\(^{-1}\) in August 2012 and a minimum value of 7.52±0.37 μmol l\(^{-1}\) in January 2012. The annual average was 62.9±20.6 μmol l\(^{-1}\). In general, dissolved methane in soil porewater was significantly higher in summer than other seasons (Fig. 3, \(P<0.05\)). However, there were not significantly different among other seasons (Fig. 3, \(P>0.05\)).

**Relationship among potential soil methane production, methane anaerobic oxidation, methane aerobic oxidation and dissolved methane along the year**

The linear mixed models showed that soil potential soil methane anaerobic oxidation, potential soil methane production and potential soil methane aerobic oxidation rates were positively related to each other (Table 1). The statistical models of the corresponding three relationships (including plots and time as random factors) had very high total significance (\(R^2_c=0.99, P<0.0001\)) (Table 1). Methane concentrations in soil porewater were negatively correlated with potential soil anaerobic oxidation rates (\(R^2_m=0.15, R^2_c=0.91\)), potential soil methane production rates (\(R^2_m=0.24, R^2_c=0.99\)) and potential soil aerobic methane oxidation (\(R^2_m=0.13, R^2_c=0.94\)) (Table 1).
**Seasonality in environment variables**

Soil temperature changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 31.5±0.1 °C in September 2012 and a minimum value of 12.4±0.6 °C in February 2013. The annual average was 21.4±2.0 °C.

Soil ferric concentration changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 87.0±9.9 mg g⁻¹ in June 2012. In September 2012 which is the lowest value was 12.2±2.3 mg g⁻¹, and annual average was 37.8±6.9 mg g⁻¹.

Soil pH changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 7.62±0.01 in January 2013 and a minimum value of 6.30±0.02 in November 2012. The annual average was 6.72±0.11.

Soil Eh changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 41.1±0.7mV in November 2012. In January 2013 that had the lowest value, was -36.5±0.7 mV and annual average was 16.3±6.5mV.

Soil salinity changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 4.21±0.71 mS cm⁻¹ in April 2012. In February 2012 the lowest value was 1.91±0.33 mS cm⁻¹ and annual average was 3.08±0.21 mS cm⁻¹.

Dissolved sulfate in soil porewater concentration changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 362±36 mg l⁻¹ in December 2012. In February 2013 the lowest value was 128±12 mg l⁻¹.
Dissolved nitrate in soil porewater concentration changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 2.46±0.44 mg l⁻¹ in December 2012. In October 2012 the lowest value were 0.106±0.039 mg l⁻¹ and annual average was 0.828±0.212 mg l⁻¹.

Dissolved chloridion in soil porewater concentration changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 4595±279 mg l⁻¹ in November 2012. In August 2012 the lowest value were 1412±92 mg l⁻¹, and annual average was 2821±283 mg l⁻¹.

Plant biomass changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 2313±657 g m⁻² in December 2012. April 2012 had the lowest value were 759±320 g m⁻², and annual average was 1462±198 g m⁻².

Air temperature changed seasonally in the Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of 35.5±0.0 °C in July 2012. February 2013 had the lowest value was 10.2±0.1 °C, and annual average was 23.0±2.4 °C.

Potential soil methane production, methane anaerobic oxidation, aerobic oxidation rates, dissolved methane, and their relationships with soil properties

The linear mixed models showed that potential soil methane production rates were positively related to soil pH ($R^2_m=0.15$, $R^2_c=0.90$) and negatively related to soil Eh ($R^2_m=0.15$, $R^2_c=0.90$) and with soil temperature ($R^2_m=0.13$, $R^2_c=0.90$) (Table 1).
Potential soil methane anaerobic oxidation rates were positively related to soil pH \((R^2_m=0.14, R^2_c=0.99)\) and negatively with soil Eh \((R^2_m=0.15, R^2_c=0.99)\) (Table 1). Finally, methane concentration in soil pore water was positively related to soil temperature \((R^2_m=0.32, R^2_c=0.99)\) (Table 1).

The PCA analysis was completely consistent with the previous commented univariant analyses. Soil samples collected in summer were located across the PC1 axis coinciding with higher porewater methane concentrations, higher soil and air temperature and soil Eh and lower soil pH and potential soil methane production and oxidation rates, both anaerobic and aerobic (Fig. 5). Just the contrary patterns were related to soil samples collected in winter that were placed in the other side of the PC1 axis (Fig. 5).

**Discussion**

**Seasonal variation of potential methane production, anaerobic oxidation, aerobic oxidation and dissolved methane**

Potential soil methane production rate was higher in winter than in summer. In winter there are lots of the plant litter input and thereby the soil carbon concentration which is the most important substrate for methane production, increases (Yagi and Minami 1990), thereby promoting the soil methane production (Van der Gon and Neue 1995). Moreover, the optimum temperature of methane production is about 20 C (Wagner and Pfeiffer 1997) and in our study, the average temperature was 14.4 and 29.2 C in winter and summer, respectively, thus more closely to the optimum for soil methane production in winter than in summer. Moreover, in summer, the plant growth was
higher than in other seasons, and more O₂ was released into the soil, generating soil redox conditions which were not proper for methane production. Furthermore, in Fujian province the acid rain was high and the summer was main rainy season, so the soil pH decreased in summer and thereby inhibiting methane production. In contrast, winter was the dry season and had relatively higher pH, favoring methane production. In our study, the linear mixed models showed that potential soil methane production rates were positively related to soil pH and negatively related to soil Eh and temperature.

Potential soil methane anaerobic oxidation rates were also higher in winter than those of spring and autumn, and also than those of summer, although, not significantly different. As commented in Fujian province acid rain is high and the summer was the main rainy season, so in summer the soil pH decrease and thereby inhibited the methane anaerobic oxidation microbial activity. In contrast, winter was the dry season with relatively higher pH which was favorable to the growth of microbes involved in methane anaerobic oxidation. In our study, supporting these comments, the linear mixed models showed that potential soil methane anaerobic oxidation rates were positively related to soil pH. Moreover, Nauhaus et al. (2002) showed that the optimum temperature value was between 4-16 °C for methane anaerobic oxidation. In our study, the average seasonal temperatures were 18.7, 29.2, 23.3 and 14.4 °C for spring, summer, autumn and winter respectively, so obviously, the winter was the most suitable season for methane anaerobic oxidation.

Potential soil methane aerobic oxidation rate was not significantly different
among seasons. However, there was still a trend to higher values in winter than in the
other seasons. The reason would be the same than for methane anaerobic oxidation,
that winter was the dry season, with relative higher soil pH. In our study, the linear
mixed models showed that potential soil methane aerobic oxidation rates were also
positively related to soil pH. Moreover, Dasselaar et al. (1998) found that temperature
promotion of the methane aerobic oxidation was higher when the temperature was
4~12℃ than when it was 12~18 ℃. In our study, the temperatures closer to 12℃ were
those of winter.

Dissolved methane in soil porewater was instead higher in summer than in the
other seasons, which had no significant differences among them. The dissolved
methane in soil porewater resulted from many factors, such as methane production,
oxidation and transportation, etc. The lower summer methane anaerobic and aerobic
oxidation were likely the most determinant factors of these higher values of dissolved
methane in summer.

The soils with highest soil pH and lowest Eh were those that showed the highest
potentials of methane production and anaerobic oxidation. But the most interesting
result was that soil samples with the highest soil pH and lowest Eh were also those
that showed the highest potentials of aerobic methane oxidation. Consistently, with
these results, Kettunen et al (1999) also observed that the maximum potential capacity
to methane aerobic oxidation was higher in soils below than above table level. Similar
results have also been observed in boreal pine fen areas (Saarino et al. 1998).

These results thus suggested a buffer effect in the methane balance in wetland
areas. Environmental and soil conditions favoring methane production are also more favorable for methane anaerobic oxidation during the same circumstances and also in drier periods, for aerobic methane oxidation. In fact alternation between wet-dry periods related to wetland source-sink of methane have been observed everywhere (Juutinen et al. 2003; Knorr et al. 2008; Brown et al. 2014; Goodrich et al. 2015). But the fact that as more favorable the conditions of soil are to produce methane higher is also its capacity to oxidize methane was observed in both flooded (anaerobic) and dry (aerobic) periods. This observation warrants future research to corroborate this possible general pattern.

Relationship among potential methane production, anaerobic oxidation, aerobic oxidation and dissolved methane

Potential methane anaerobic oxidation and potential methane production showed a very significant positive correlation. This pattern has been previously observed in tropical and boreal wet soils and peatlands where these two variables have also shown to be significantly correlated, in agreement with our results (Smemo and Yavitt 2011; Blazewicz et al. 2012). The relationship between methane anaerobic oxidation and methane production was mainly related to the functional microbial association, where anaerobic methanotroph (ANME) archaea was the main microorganism involved in methane production and can also participate in the methane anaerobic oxidation (Alperin and Hoehler 2009; Lloyd et al. 2011). Methane production archaea can
oxidize methane as observed in pure culture experiments (Moran et al. 2005; Joye and Samarkin 2009; Roberts and Aharon 1994). In addition, there was a significant positive correlation between methane anaerobic oxidation and methane aerobic oxidation in this study. Recent studies have demonstrated that aerobic and anaerobic methane oxidation bacteria can coexist in the same places, suggesting that the proportion of different species can depend on the oxygen and methane availability and also that diverse microbial activity was important to sustain methanotrophic activity (Siniscalchi et al., 2017). Eller et al. (2005) observed the co-occurrence of methane aerobic and anaerobic process in the same soil samples and water columns. Moreover, potential methane aerobic oxidation and potential methane production processes showed a very significant positive correlation as expected from methane being the substrate of methane oxidation (Nesbit and Breitenbeck 1992). However, negative relationships between methane concentrations in soil porewater and the studied potential methane production rates and also potential soil methane oxidation, both in anaerobic and aerobic conditions were then observed. These results suggest that methane production was not the most determinant factor controlling the dissolved methane in soil porewater. However, porewater dissolved methane was directly limited by methane anaerobic oxidation and aerobic oxidation in Minjiang estuarine wetland. These results were not in agreement with the fact that methane storage was the key factor in the oxidation of methane in coastal sulphate-rich marine sediments (Nauhaus et al. 2002; Treude et al. 2005; Orcutt et al. 2005). This possible explanation was consistent with the linear mixed models showing the inverse
relationships between methane present in porewater and the soil potential capacity of
methane production and also of methane oxidation.

Conclusions

1. Potential methane production, anaerobic oxidation and aerobic oxidation were all
shown to be higher in winter than other seasons, however, the dissolved methane in
soil porewater was higher in summer than other seasons.

2. The concentration of soil pH and Eh are the studied factors that had the stronger
relationships with potential soil methane production and anaerobic and aerobic
oxidation rates. This showed thus, strong relationships among the different soil
metabolic methane processes and the basic potential chemical activities of soils.

3. The positive correlation between methane production, methane anaerobic oxidation
and methane aerobic oxidation suggested that at least some of the soil conditions and
of the overall set of microorganisms communities that favor methane production also
favor its oxidation.

4. The negative relationships between methane concentrations in soil porewater with
the potential soil of methane production and oxidation in anaerobic and aerobic
conditions suggest that the higher the soil potential to produce methane, the higher the
potential soil capacity to oxidize methane in aerobic and anaerobic conditions.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Gauthier M, Bradley RL, Simek M (2013) Anaerobic methane oxidation may be more prevalent in surface soils than was originally thought//EGU General Assembly Conference Abstracts 15:14169


Juutinen S, Alm J, Larmola T, Huttunen JT, Moreno M, Saarnio S, Martikainen PJ,


Table 1. Significant observed relationships of the potentials of soil methane production, soil methane anaerobic oxidation and soil methane aerobic oxidation among them and with soil properties. Plot and time of sampling (month) were introduced in the models as random factors.

```r
model <- lme(Variable~fixed factor, data=dades, random=~1|plot/time,method="REML")
```

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fixed factor</th>
<th>Fixed factor statistics</th>
<th>Model statistics ($R^2_m$=fixed factor, $R^2_c$=fixed + random factor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential soil anaerobic CH$_4$ oxidation</td>
<td>Potential soil CH$_4$ production</td>
<td>Estimates=0.578 F=43.7 P&lt;0001</td>
<td>$R^2_m$=0.56 $R^2_c$=0.99</td>
</tr>
<tr>
<td>Potential soil aerobic CH$_4$ oxidation</td>
<td>Potential soil CH$_4$ production</td>
<td>Estimates=0.707 F=21.2 P&lt;0001</td>
<td>$R^2_m$=0.38 $R^2_c$=0.99</td>
</tr>
<tr>
<td>Potential soil aerobic CH$_4$ oxidation</td>
<td>Potential soil anaerobic CH$_4$ oxidation</td>
<td>Estimates=1.14 F=49.0 P&lt;0001</td>
<td>$R^2_m$=0.58 $R^2_c$=0.99</td>
</tr>
<tr>
<td>CH$_4$ pore-water soil concentration</td>
<td>Potential soil CH$_4$ production</td>
<td>Estimates=-0.029 F=11.8 P&lt;0001</td>
<td>$R^2_m$=0.24 $R^2_c$=0.91</td>
</tr>
<tr>
<td>CH$_4$ pore-water soil concentration</td>
<td>Potential soil anaerobic CH$_4$ oxidation</td>
<td>Estimates=-0.42 F=6.31 P&lt;0.017</td>
<td>$R^2_m$=0.15 $R^2_c$=0.91</td>
</tr>
<tr>
<td>CH$_4$ pore-water soil concentration</td>
<td>Potential soil aerobic CH$_4$ oxidation</td>
<td>Estimates=-0.46 F=5.09 P&lt;0.031</td>
<td>$R^2_m$=0.13 $R^2_c$=0.94</td>
</tr>
<tr>
<td>Potential soil anaerobic CH$_4$ oxidation</td>
<td>Soil pH</td>
<td>Estimates=6.17 F=5.47 P=0.026</td>
<td>$R^2_m$=0.14 $R^2_c$=0.99</td>
</tr>
<tr>
<td>Potential soil anaerobic CH$_4$ oxidation</td>
<td>Soil Eh</td>
<td>Estimates=-0.016 F=5.89 P=0.021</td>
<td>$R^2_m$=0.15 $R^2_c$=0.99</td>
</tr>
<tr>
<td>Potential soil CH$_4$ production</td>
<td>Soil pH</td>
<td>Estimates=5.80 F=6.55 P=0.015</td>
<td>$R^2_m$=0.15 $R^2_c$=0.90</td>
</tr>
<tr>
<td>Potential soil CH$_4$ production</td>
<td>Soil Temperature</td>
<td>Estimates=-0.045 F=5.87 P=0.021</td>
<td>$R^2_m$=0.13 $R^2_c$=0.90</td>
</tr>
<tr>
<td>Potential soil CH$_4$ production</td>
<td>Soil Eh</td>
<td>Estimates=-0.015 F=6.76 P=0.014</td>
<td>$R^2_m$=0.15 $R^2_c$=0.90</td>
</tr>
<tr>
<td>CH$_4$ pore-water soil concentration</td>
<td>Soil Temperature</td>
<td>Estimates=0.082 F=16.4 P&lt;0001</td>
<td>$R^2_m$=0.32 $R^2_c$=0.99</td>
</tr>
</tbody>
</table>
**Figure legends**

**Fig. 1.** Study area and sampling site (▲) in southeastern China.

**Fig. 2.** Monthly variation of potential soil methane production rate, potential soil methane anaerobic oxidation, potential soil methane aerobic rate, and dissolved methane concentration in soil porewater.

**Fig. 3.** Seasonal values of potential soil methane production rate (A), potential soil methane anaerobic oxidation rate (A), potential soil methane aerobic oxidation rate (A), and dissolved methane concentration in soil porewater (B). Different letters indicate significantly different among seasons.

**Fig. 4.** Monthly variation of soil properties (A), porewater properties (B), plant biomass (C), and air temperature (D).

**Fig. 5.** Principal component analyses (PCA) to observe the multiple correlations among potential soil methane anaerobic oxidation, potential soil methane production, potential soil methane aerobic methane oxidation and the environmental factors and the analyzed soil variables. We represented the position of different cases (soil samples) (a) and the loads of the commented variables (b) in the layout generated by the two first PCA axes (explaining together a 52.0% of the total variance).  

\[ \text{Ait T} = \text{Air temperature, } \text{Cl} = \text{concentration of Cl- in soil, } \text{Eh} = \text{soil potential redox, } \text{Fe}^{3+} = \text{Soil } \text{Fe}^{3+} \text{ concentration, } \text{Maerox} = \text{Potential soil methane aerobic oxidation, } \text{Manaox} = \text{Potential soil methane anaerobic oxidation, } \text{Mprod} = \text{Potential soil methane production, } \text{Msoil} = \text{concentration of methane in soil porewater, } \text{pH} = \text{soilpH, salinity} = \text{soil salinity, Soil T} = \text{soil temperature, sulfate} = \text{soil sulfate concentration, aut} \]
= autumn, su = summer, sp = spring, wi = winter.
Fig. 1.
Methane production (μg g⁻¹ d⁻¹)

Methane anaerobic oxidation (μg g⁻¹ d⁻¹)

Methane aerobic oxidation (μg g⁻¹ d⁻¹)

Dissolved methane (μmol l⁻¹)

Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.