

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

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18 **Abstract**

19 Wetlands are important sources of methane emission. Methane anaerobic oxidation,
20 aerobic oxidation and production, and dissolved methane are important process of
21 methane metabolism. We studied methane metabolism and the soil influencing factors.
22 Potential soil methane production, anaerobic oxidation and aerobic oxidation rates,
23 and dissolved methane in soil porewater changed seasonally and the annual average
24 was $21.1 \pm 5.1 \mu\text{g g}^{-1}\text{d}^{-1}$, $11.0 \pm 3.9 \mu\text{g g}^{-1}\text{d}^{-1}$, $20.9 \pm 5.8 \mu\text{g g}^{-1}\text{d}^{-1}$, and $62.9 \pm 20.6 \mu\text{mol l}^{-1}$,
25 respectively. Potential soil methane production and anaerobic and aerobic oxidation
26 were positively correlated among them and with soil pH and negatively correlated
27 with soil redox potential (Eh). Potential soil methane production and aerobic and
28 anaerobic oxidation rates were negatively related to pore soil methane concentration.
29 Thus, the more water saturated the soil (the lower Eh), the higher its capacity to
30 methane production was, but even higher was soil potential capacity to methane
31 oxidation both in the same anaerobic circumstances and when the soil was suddenly
32 submitted to aerobic conditions. All these results suggested a buffer effect in the
33 methane balance in wetland areas, the environmental circumstances favoring methane
34 production are also favorable to methane anaerobic oxidation.

35 **Keywords** Methane production · methane anaerobic oxidation · methane aerobic
36 oxidation · dissolved methane · Minjiang River estuarine wetland

37

38 **Introduction**

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

51 Methane metabolism is in several phases: production, oxidation, dissolved
52 methane, transport and emission (Buckley et al. 2008). Methane production, oxidation
53 and dissolved methane in water have great impacts on the ultimate reduction of
54 methane emissions in wetlands (Singh 2011). In the 21st century, methane anaerobic
55 oxidation has become the core and hot issues (Raghoebarsing et al. 2006; Kniermeyer
56 et al. 2007). However, the reports about methane anaerobic oxidation in coastal
57 estuarine wetlands are few. Furthermore, the relationships between potential soil
58 methane anaerobic oxidation capacity with potential soil methane production and with
59 potential soil methane aerobic oxidation capacity are uncertain in wetland areas.

60 Whether relationships exist between the soil capacity to produce methane and the soil
61 capacity to oxidize methane when the soil is in anaerobic conditions and whether
62 between production and oxidation in wet conditions in dry-aerobic periods are two
63 important questions that warrant research.

64 Methane metabolism in wetlands is strongly influenced by environmental factors
65 that vary both spatially and temporally (Datta et al. 2013). The availability of electron
66 acceptors and donors in soils plays a key role in regulating CH₄ production and
67 consumption (Moran et al. 2008; Ettwig et al. 2010; Ro et al. 2011) and thereby
68 controlling dissolved methane and the emission. Electron acceptors (e.g. Fe³⁺, NO₃⁻,
69 and sulfate) are reduced during wet periods, but regenerated (oxidized) during dry
70 periods (Neubauer et al. 2007). Soils can also provide carbon substrates to microbes
71 for mediating CH₄ production and enhancing plant growth that in turn governs more
72 than 90% of CH₄ transport (Le Mer and Roger 2001). Other environmental variables,
73 include soil temperature, pH, redox potential (Eh) and salinity also influences CH₄
74 metabolism (Song et al. 2008; Wang et al. 2017). Better knowledge and
75 characterization of CH₄ metabolism and transport of CH₄, are essential for better
76 understanding and characterizing of GHG emissions from wetlands advancing in the
77 knowledge of soil and soil pore water circumstances can favor the production and
78 oxidation processes in soil media and thus, the final methane contents in
79 soil-atmosphere interface and emission can give clues to choose between soil and
80 plant community management strategies to diminish as much as possible the methane
81 emissions. This information can also provide clues to improve the models and

82 projections of methane production and emissions on regional and global scale.

83 China has a coastline of 18,000 km in length, with numerous estuaries and bays
84 and a diversity of coastal wetlands which are important component of China's
85 wetlands, as well as the world's wetlands. Minjiang River estuarine wetland is the
86 main natural wetland of southeast coast of China. The wetland of Minjiang River
87 estuary is rich in biological species and abundant in waterbird resources (Liu et al.,
88 2006). Moreover, the Minjiang River estuary is an important tidal wetland ecosystem
89 due to its unique location at the transition between central and southern subtropical
90 climatic zones (Zheng et al. 2006). The tidal wetlands are rich in animal and plant
91 biodiversity (Zhou et al. 2006) being an excellent site representing the wide coastal
92 wetland areas of this part of China coast-

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

100

101 **Materials and Methods**

102 **Study area**

103 This study was conducted in the Shanyutan wetland (26°01'46"N; 119°37'31"E,

104 Fig. 1), the largest tidal estuarine wetland (approximately 3120 ha) in the estuary of
105 the Minjiang River.

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

113 *C. malaccensis* is one of the two dominant species of plants in this estuarine wetland.

114 *C. malaccensis* is a native plant typically found in the upper (mid to high) portions of
115 mudflats that grow between April and October, the highest population height is about
116 1.5 m and the density is about 1000 m⁻². Below-ground rhizomes are creeping growth
117 in the topsoil layers.

118

119 **Experimental design**

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

126 samples (one wetland type × one soil layer × twelve months' × three replicate plots)
127 were thus collected.

128

129 **Measurements of potential methane production**

130 In each sampled soil, potential soil methane production rate was determined by
131 placing 30 g of the fresh soil sample in a 120 ml incubation bottle and injecting 30 ml
132 of distilled water (water: soil ratio was thus 1:1) (Wang et al. 2010; Bergman et al.
133 2000). The incubation bottles were filled with oxygen-free nitrogen through a small
134 hole in the bottle stopper to eliminate the possibility of methane consumption caused
135 by carrying oxygen during the sampling process and slowly equilibrated with the
136 atmospheric pressure for 24 h to consume the residual oxygen in incubation bottles,
137 which ensures the soil sample is in a strictly anaerobic environment (Smemo and
138 Yavitt 2007; Wrede et al. 2012). Three replicates were set and placed in an anaerobic
139 incubator (YQX-II, Shanghai Yuejin Medical Equipment Factory) in the dark place
140 using the average soil temperature in situ. Then the gas samples were taken at 0, 24,
141 48, 72, and 96 h, and the sample incubation bottles were gently swirled for 1-2 min
142 before gas sampling. Each extraction was 2 ml and supplemented with the
143 corresponding volume of oxygen-free nitrogen. Methane concentration was
144 determined by a GC-2010 gas chromatograph (Shimadzu Scientific Instruments,
145 Kyoto, Japan). The potential methane production rate was calculated by the methane
146 concentration increment during the incubation time in the incubation bottles.

147

148 **Measurements of potential methane anaerobic oxidation**

149 In each collected soil sample, potential soil methane anaerobic oxidation rate was

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

170 **Measurements of potential methane aerobic oxidation**

171 In each soil sampled potential soil methane aerobic oxidation rate was determined by
172 Krüger et al. (2002) and Supparattanapan et al. (2009), by placing 30 g of fresh soil
173 sample in a 120 ml incubation bottle and injecting 30 ml of distilled water into it, the
174 water: soil ratio was 1:1 (Wang et al. 2010; Bergman et al. 2000). Then, pure methane

175 standard gas was injected into each incubation bottles so that the concentration of
176 methane in the incubation bottles was $10000 \mu\text{mol mol}^{-1}$, and three replicates were set
177 and incubation in the dark place using the average soil temperature in situ. Then the
178 gas samples were taken at 0, 24, 48, 72, and 96 h, and the sampled incubation bottles
179 were gently swirled for 1-2 min before gas sampling. Each extraction was 2 ml and
180 supplemented with the corresponding volume of oxygen-nitrogen. Methane
181 concentration was determined by a GC-2010 gas chromatograph (Shimadzu Scientific
182 Instruments, Kyoto, Japan). Potential soil methane anaerobic oxidation rate was
183 calculated by the methane concentration decrement as the incubation time in the
184 incubation bottles.

185

186 **Measurement (in situ) of porewater dissolved CH₄ concentration**

187 Porewater was sampled *in situ* once each month. Three specially designed stainless
188 steel tubes (2.0 cm inner diameter) were installed to a depth of 30 cm in each plot.
189 Porewater samples were collected immediately after the measurements of CH₄
190 emission using 50-ml syringes to inject it into pre-evacuated vials (20 ml) and stored
191 in a cooling box in the field. After transporting to the laboratory, the samples in the
192 vials were stored at $-20 \text{ }^{\circ}\text{C}$ until the analysis of CH₄ concentration. Before analysis,
193 the vials were first thawed at room temperature and were then vigorously shaken for 5
194 min to equilibrate the CH₄ concentrations between the porewater and the headspace.
195 The gas samples were taken from the headspace of the vials and analyzed for CH₄
196 concentration with the above gas chromatograph (Ding et al. 2003).

197

198 **Determination of methane concentrations**

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

207

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

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

212
$$P = \frac{dc}{dt} \cdot \frac{V_H}{W_s} \cdot \frac{MW}{MV} \cdot \frac{T_{st}}{T_{st} + T}$$

213

214 where P is the potential rate of methane anaerobic oxidation, aerobic oxidation and
215 production ($\mu\text{g}^{-1} \text{g}^{-1} \text{d}^{-1}$), dc/dt is the recorded change in the mixing ratio of C
216 (methane) in the headspace over time ($\text{mmol mol}^{-1} \text{d}^{-1}$), V_H is the volume of the
217 headspace (L), W_s is the dry weight of the soil (g), MW is the molecular weight of
218 methane (g), MV is the molecular volume (L), T is the temperature (K) and T_{st} is the
219 standard temperature (°K).

220 The concentration of CH₄ dissolved in the porewater was calculated following
221 (Ding et al. 2003):

$$222 \quad C = \frac{Ch \cdot Vh}{22.4 \cdot Vp}$$

223

224 where *Ch* is the CH₄ concentration (μl l⁻¹) in the air sample from the vials, *Vh* is the
225 volume of air in the bottle (ml), and *Vp* is the volume of the porewater in the bottle
226 (ml).

227

228 **Measurements of soil properties**

229 Total soil porewater (collected by centrifugation at 5000 r min⁻¹) dissolved organic-C
230 (DOC) concentration was measured using a TOC-V CPH total carbon analyzer
231 (Shimadzu Scientific Instruments, Kyoto, Japan). Porewater (collected by
232 centrifugation at 5000 r min⁻¹) NO₃⁻, SO₄²⁻ and Cl⁻ concentrations were determined by
233 ICS2100 ion chromatography (American Dionex Production, Sunnyvale, USA). Soil
234 temperature, Eh and pH were measured with an Eh/pH/temperature meter (IQ Scientific
235 Instruments, Carlsbad, USA) and salinity was measured using a 2265FS EC Meter
236 (Spectrum Technologies Inc., Paxinos, USA). Total Fe content was determined by
237 digesting fresh soil samples with 1 mol HCl L⁻¹. Ferrous ions were extracted using
238 1,10-phenanthroline and measured spectrometrically (Wang et al. 2012). Ferric
239 concentration was calculated by subtracting the ferrous concentration from the total
240 Fe concentration.

241

242 **Statistical analyses**

243 The significance of the differences in potential methane production, anaerobic
244 oxidation, aerobic oxidation and dissolved methane, soil variables and other
245 properties among the seasonal variation were assessed by One-Way ANOVA. We
246 analyzed the relationships of the potentials of soil methane production, soil methane
247 anaerobic oxidation, soil methane aerobic oxidation and dissolved methane among
248 them and with soil DOC, soil temperature, Eh, pH, salinity, soil NO_3^- , SO_4^{2-} and Cl^-
249 concentrations and plant biomass. Plot and time of sampling (month) were introduced
250 into the models as random factors. If a variable was non-normally distributed we
251 transform it to normalize its distribution. In concrete soil nitrate and soil ferric
252 concentrations were log-transformed to reach their normal distribution. We used the
253 “nlme” (Pinheiro et al. 2016) and “lme4” (Bates et al. 2015) R packages with the
254 “lme” and “lmer” functions to conduct the mixed linear models. We chose the best
255 model for each dependent variable using Akaike information criteria. We used the
256 MuMIn (Barton 2012) R package in the mixed models to estimate the percentage of
257 variance explained by the model. We presented in significant relationships the total
258 variance explained by the model including the fixed and random factors (R^2c) and
259 also the variability explained by only the fixed factor (R^2m).

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

265 USA).

266

267 **Results**

268

269 **Potential soil methane production, methane anaerobic oxidation, methane** 270 **aerobic oxidation, and dissolved methane along the year**

271 Potential soil methane production rates changed seasonally in the Shanyutan wetland
272 of Minjiang River estuary (Figs. 2, 3), with a maximum value of $57.4 \pm 7.7 \mu\text{g g}^{-1} \text{d}^{-1}$ in
273 January 2013 and a minimum value of $4.85 \pm 1.1 \mu\text{g g}^{-1} \text{d}^{-1}$ in August 2012. The annual
274 average value was $21.1 \pm 5.1 \mu\text{g g}^{-1} \text{d}^{-1}$. In general, potential soil methane production
275 rate was significantly higher in winter than that of the summer (Fig. 3, $P < 0.05$).
276 However, there were not significantly different among other seasons (Fig. 3, $P > 0.05$).

277 Potential soil methane anaerobic oxidation rates changed seasonally in the
278 Shanyutan wetland of Minjiang River estuary (Figs. 2, 3), with a maximum value of
279 $41.8 \pm 13.4 \mu\text{g g}^{-1} \text{d}^{-1}$ in January 2013 and a minimum value of $3.46 \pm 0.97 \mu\text{g g}^{-1} \text{d}^{-1}$ in
280 August 2012. The annual average value was $11.0 \pm 3.9 \mu\text{g g}^{-1} \text{d}^{-1}$. In general, potential
281 soil methane anaerobic oxidation production rate was significantly higher in winter
282 than those of spring and autumn (Fig. 3, $P < 0.05$). However, there were not
283 significantly different among other seasons (Fig. 3, $P > 0.05$).

284 Potential soil methane aerobic oxidation rates changed seasonally in the
285 Shanyutan wetland of Minjiang River estuary (Figs. 2, 3), with a maximum value of
286 $70.2 \pm 24.5 \mu\text{g g}^{-1} \text{d}^{-1}$ in January 2013 and a minimum value of $6.55 \pm 1.42 \mu\text{g g}^{-1} \text{d}^{-1}$ in

287 May 2012. The annual average value was $20.9 \pm 5.8 \mu\text{g g}^{-1} \text{d}^{-1}$. In general, potential soil
288 methane aerobic oxidation production rate was not significantly different among
289 seasons (Fig. 3, $P > 0.05$).

290 Dissolved methane in soil porewater changed seasonally in the Shanyutan
291 wetland of Minjiang River estuary (Figs. 2, 3), with a maximum value of 261 ± 39
292 $\mu\text{mol l}^{-1}$ in August 2012 and a minimum value of $7.52 \pm 0.37 \mu\text{mol l}^{-1}$ in January 2012.
293 The annual average was $62.9 \pm 20.6 \mu\text{mol l}^{-1}$. In general, dissolved methane in soil
294 porewater was significantly higher in summer than other seasons (Fig. 3, $P < 0.05$).
295 However, there were not significantly different among other seasons (Fig. 3, $P > 0.05$).

296

297 **Relationship among potential soil methane production, methane anaerobic** 298 **oxidation, methane aerobic oxidation and dissolved methane along the year**

299

300 The linear mixed models showed that soil potential soil methane anaerobic oxidation,
301 potential soil methane production and potential soil methane aerobic oxidation rates
302 were positively related to each other (Table 1). The statistical models of the
303 corresponding three relationships (including plots and time as random factors) had
304 very high total significance ($R^2\text{c}=0.99$, $P < 0.0001$) (Table 1). Methane concentrations
305 in soil porewater were negatively correlated with potential soil anaerobic oxidation
306 rates ($R^2\text{m}=0.15$, $R^2\text{c}=0.91$), potential soil methane production rates ($R^2\text{m}=0.24$,
307 $R^2\text{c}=0.99$) and potential soil aerobic methane oxidation ($R^2\text{m}=0.13$, $R^2\text{c}=0.94$) (Table
308 1).

309

310 **Seasonality in environment variables**

311 Soil temperature changed seasonally in the Shanyutan wetland of Minjiang River
312 estuary (Fig. 4), with a maximum value of 31.5 ± 0.1 °C in September 2012 And a
313 minimum value of 12.4 ± 0.6 °C in February 2013. The annual average was
314 21.4 ± 2.0 °C.

315 Soil ferric concentration changed seasonally in the Shanyutan wetland of
316 Minjiang River estuary (Fig. 4), with a maximum value of 87.0 ± 9.9 mg g⁻¹ in June
317 2012. In September 2012 which is the lowest value was 12.2 ± 2.3 mg g⁻¹, and annual
318 average was 37.8 ± 6.9 mg g⁻¹.

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

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

325 Soil salinity changed seasonally in the Shanyutan wetland of Minjiang River estuary
326 (Fig. 4), with a maximum value of 4.21 ± 0.71 mS cm⁻¹ in April 2012. In February
327 2012 the lowest value was 1.91 ± 0.33 mS cm⁻¹ and annual average was 3.08 ± 0.21 mS
328 cm⁻¹.

329 Dissolved sulfate in soil porewater concentration changed seasonally in the
330 Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of
331 362 ± 36 mg l⁻¹ in December 2012. In February 2013 the lowest value was 128 ± 12 mg

332 l⁻¹ and annual average was 227±25 mg l⁻¹.

333 Dissolved nitrate in soil porewater concentration changed seasonally in the
334 Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of
335 2.46±0.44 mg l⁻¹ in December 2012. In October 2012 the lowest value were
336 0.106±0.039 mg l⁻¹ and annual average was 0.828±0.212 mg l⁻¹.

337 Dissolved chloridion in soil porewater concentration changed seasonally in the
338 Shanyutan wetland of Minjiang River estuary (Fig. 4), with a maximum value of
339 4595±279 mg l⁻¹ in November 2012. In August 2012 the lowest value were 1412±92
340 mg l⁻¹, and annual average was 2821±283 mg l⁻¹.

341 Plant biomass changed seasonally in the Shanyutan wetland of Minjiang River
342 estuary (Fig. 4), with a maximum value of 2313±657 g m⁻² in December 2012. April
343 2012 had the lowest value were 759±320 g m⁻², and annual average was 1462±198 g
344 m⁻².

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

348

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

351

352 The linear mixed models showed that potential soil methane production rates were
353 positively related to soil pH ($R^2_m=0.15$, $R^2_c=0.90$) and negatively related to soil Eh
354 ($R^2_m=0.15$, $R^2_c=0.90$) and with soil temperature ($R^2_m=0.13$, $R^2_c=0.90$) (Table 1).

355 Potential soil methane anaerobic oxidation rates were positively related to soil pH
356 ($R^2_m=0.14$, $R^2_c=0.99$) and negatively with soil Eh ($R^2_m=0.15$, $R^2_c=0.99$) (Table 1).
357 Finally, methane concentration in soil pore water was positively related to soil
358 temperature ($R^2_m=0.32$, $R^2_c=0.99$) (Table 1).

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

366

367 **Discussion**

368 **Seasonal variation of potential methane production, anaerobic oxidation, aerobic** 369 **oxidation and dissolved methane**

370 Potential soil methane production rate was higher in winter than in summer. In winter
371 there are lots of the plant litter input and thereby the soil carbon concentration which
372 is the most important substrate for methane production, increases (Yagi and Minami
373 1990), thereby promoting the soil methane production (Van der Gon and Neue 1995).
374 Moreover, the optimum temperature of methane production is about 20 C (Wagner
375 and Pfeiffer 1997) and in our study, the average temperature was 14.4 and 29.2 C in
376 winter and summer, respectively, thus more closely to the optimum for soil methane
377 production in winter than in summer. Moreover, in summer, the plant growth was

378 higher than in other seasons, and more O₂ was released into the soil, generating soil
379 redox conditions which were not proper for methane production. Furthermore, in
380 Fujian province the acid rain was high and the summer was main rainy season, so the
381 soil pH decreased in summer and thereby inhibiting methane production. In contrast,
382 winter was the dry season and had relatively higher pH, favoring methane production.
383 In our study, the linear mixed models showed that potential soil methane production
384 rates were positively related to soil pH and negatively related to soil Eh and
385 temperature.

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

399 Potential soil methane aerobic oxidation rate was not significantly different

400 among seasons. However, there was still a trend to higher values in winter than in the
401 other seasons. The reason would be the same than for methane anaerobic oxidation,
402 that winter was the dry season, with relative higher soil pH. In our study, the linear
403 mixed models showed that potential soil methane aerobic oxidation rates were also
404 positively related to soil pH. Moreover, Dasselaar et al. (1998) found that temperature
405 promotion of the methane aerobic oxidation was higher when the temperature was
406 4~12°C than when it was 12~18 °C. In our study, the temperatures closer to 12°C were
407 those of winter.

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

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

421 These results thus suggested a buffer effect in the methane balance in wetland

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

431

432 **Relationship among potential methane production, anaerobic oxidation, aerobic** 433 **oxidation and dissolved methane**

434

435 Potential methane anaerobic oxidation and potential methane production showed a
436 very significant positive correlation. This pattern has been previously observed in
437 tropical and boreal wet soils and peatlands where these two variables have also shown
438 to be significantly correlated, in agreement with our results (Smemo and Yavitt 2011;
439 Blazewicz et al. 2012). The relationship between methane anaerobic oxidation and
440 methane production was mainly related to the functional microbial association, where
441 anaerobic methanotroph (ANME) *archaea* was the main microorganism involved in
442 methane production and can also participate in the methane anaerobic oxidation
443 (Alperin and Hoehler 2009; Lloyd et al. 2011). Methane production *archaea* can

444 oxidize methane as observed in pure culture experiments (Moran et al. 2005; Joye and
445 Samarkin 2009; Roberts and Aharon 1994). In addition, there was a significant
446 positive correlation between methane anaerobic oxidation and methane aerobic
447 oxidation in this study. Recent studies have demonstrated that aerobic and anaerobic
448 methane oxidation bacteria can coexist in the same places, suggesting that the
449 proportion of different species can depend on the oxygen and methane availability and
450 also that diverse microbial activity was important to sustain methanotrophic activity
451 (Siniscalchi et al., 2017). Eller et al. (2005) observed the co-occurrence of methane
452 aerobic and anaerobic process in the same soil samples and water columns. Moreover,
453 potential methane aerobic oxidation and potential methane production processes
454 showed a very significant positive correlation as expected from methane being the
455 substrate of methane oxidation (Nesbit and Breitenbeck 1992). However, negative
456 relationships between methane concentrations in soil porewater and the studied
457 potential methane production rates and also potential soil methane oxidation, both in
458 anaerobic and aerobic conditions were then observed. These results suggest that
459 methane production was not the most determinant factor controlling the dissolved
460 methane in soil porewater. However, porewater dissolved methane was directly
461 limited by methane anaerobic oxidation and aerobic oxidation in Minjiang estuarine
462 wetland. These results were not in agreement with the fact that methane storage was
463 the key factor in the oxidation of methane in coastal sulphate-rich marine sediments
464 (Nauhaus et al. 2002; Treude et al. 2005; Orcutt et al. 2005). This possible
465 explanation was consistent with the linear mixed models showing the inverse

466 relationships between methane present in porewater and the soil potential capacity of
467 methane production and also of methane oxidation.

468

469 **Conclusions**

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

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

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

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

485

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494

495 **Conflicts of Interest**

496 The authors declare no conflicts of interest.

497

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691

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

model <- lme(Variable~fixed factor, data=dades, random=~1 plot/time,method="REML")			
Variable	Fixed factor	Fixed factor statistics	Model statistics (R^2_m =fixed factor, R^2_c =fixed + random factor)
Potential soil anaerobic CH ₄ oxidation	Potential soil CH ₄ production	Estimates=0.578 F=43.7 P<0001	R^2_m =0.56 R^2_c =0.99
Potential soil aerobic CH ₄ oxidation	Potential soil CH ₄ production	Estimates=0.707 F=21.2 P<0001	R^2_m =0.38 R^2_c =0.99
Potential soil aerobic CH ₄ oxidation	Potential soil anaerobic CH ₄ oxidation	Estimates=1.14 F=49.0 P<0001	R^2_m =0.58 R^2_c =0.99
CH ₄ pore-water soil concentration	Potential soil CH ₄ production	Estimates=-0.029 F=11.8 P=0.0016	R^2_m =0.24 R^2_c =0.91
CH ₄ pore-water soil concentration	Potential soil anaerobic CH ₄ oxidation	Estimates=-0.42 F=6.31 P=0.017	R^2_m =0.15 R^2_c =0.91
CH ₄ pore-water soil concentration	Potential soil aerobic CH ₄ oxidation	Estimates=-0.46 F=5.09 P=0.031	R^2_m =0.13 R^2_c =0.94
Potential soil anaerobic CH ₄ oxidation	Soil pH	Estimates=6.17 F=5.47 P=0.026	R^2_m =0.14 R^2_c =0.99
Potential soil anaerobic CH ₄ oxidation	Soil Eh	Estimates=-0.016 F=5.89 P=0.021	R^2_m =0.15 R^2_c =0.99
Potential soil CH ₄ production	Soil pH	Estimates=5.80 F=6.55 P=0.015	R^2_m =0.15 R^2_c =0.90
Potential soil CH ₄ production	Soil Temperature	Estimates=-0.045 F=5.87 P=0.021	R^2_m =0.13 R^2_c =0.90
Potential soil CH ₄ production	Soil Eh	Estimates=-0.015 F=6.76 P=0.014	R^2_m =0.15 R^2_c =0.90
CH ₄ pore-water soil concentration	Soil Temperature	Estimates=0.082 F=16.4 P<0001	R^2_m =0.32 R^2_c =0.99

696 **Figure legends**

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

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

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

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

707 **Fig. 5.** Principal component analyses (PCA) to observe the multiple correlations
708 among potential soil methane anaerobic oxidation, potential soil methane production,
709 potential soil methane aerobic methane oxidation and the environmental factors and
710 the analyzed soil variables. We represented the position of different cases (soil
711 samples) (a) and the loads of the commented variables (b) in the layout generated by
712 the two first PCA axes (explaining together a 52.0% of the total variance). Ait T^a =
713 Air temperature, Cl =concentration of Cl⁻ in soil, Eh = soil potential redox, Fe³⁺ =
714 Soil Fe³⁺ concentration, Maerox=Potential soil methane aerobic oxidation,
715 Manaox=Potential soil methane anaerobic oxidation, Mprod=Potential soil methane
716 production, Msoil=concentration of methane in soil porewater, pH = soilpH,
717 salinity=soil salinity, Soil T^a =soil temperature, sulfate=soil sulfate concentration, aut

718 = autumn, su = summer, sp = spring, wi = winter.

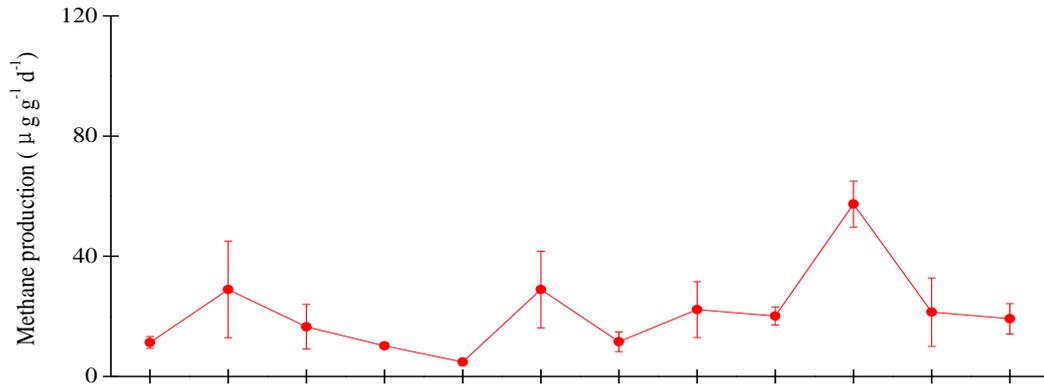
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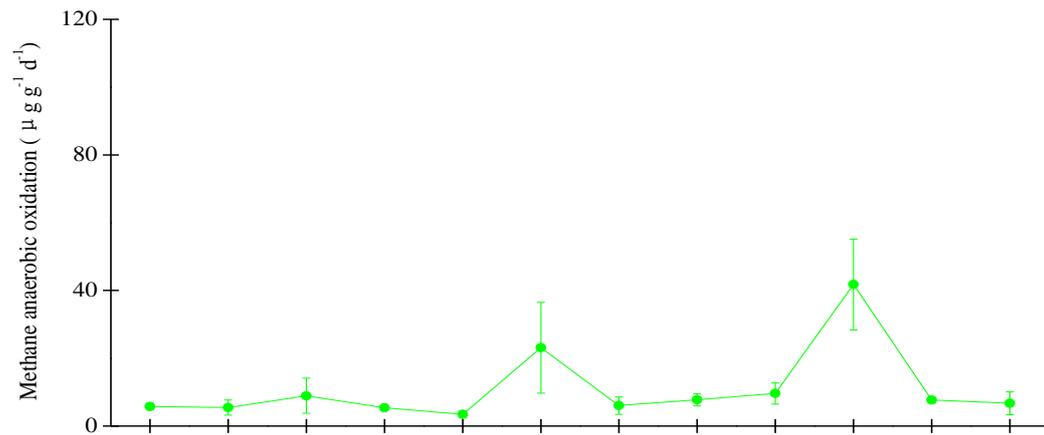
746 **Fig. 1.**

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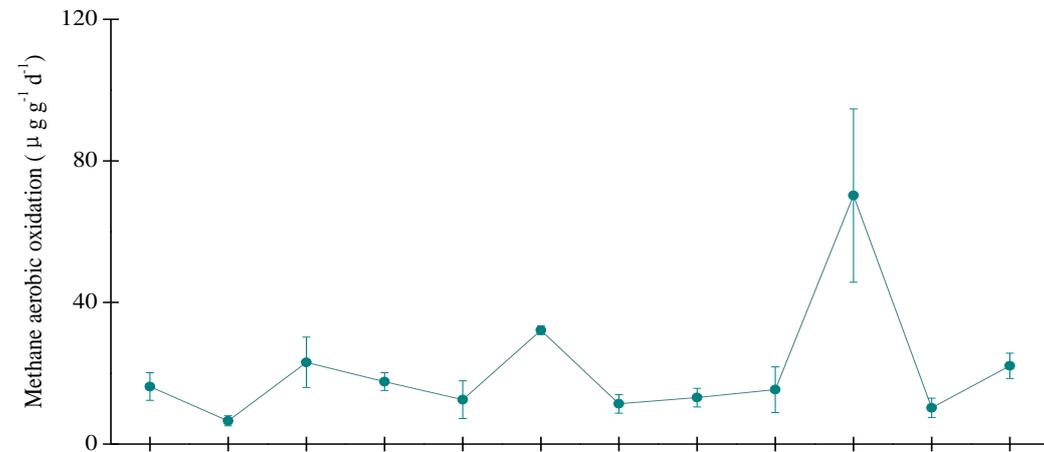
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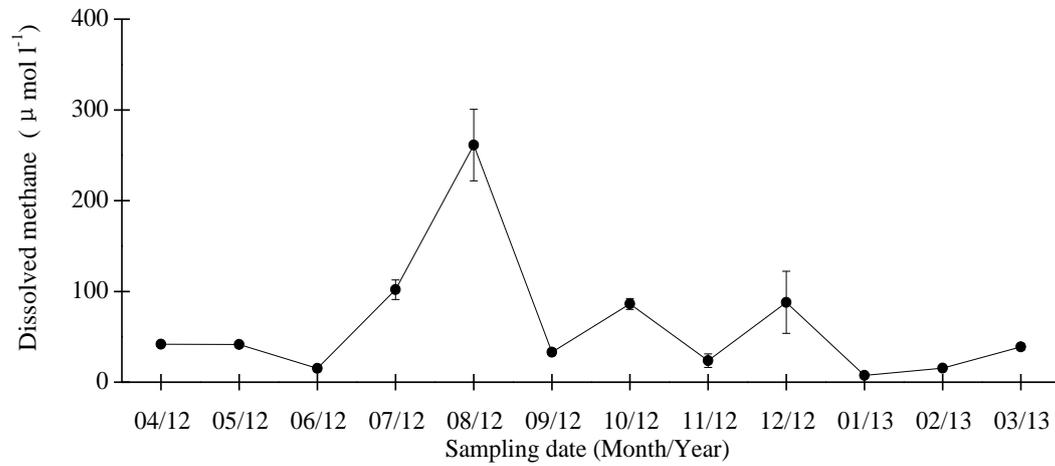
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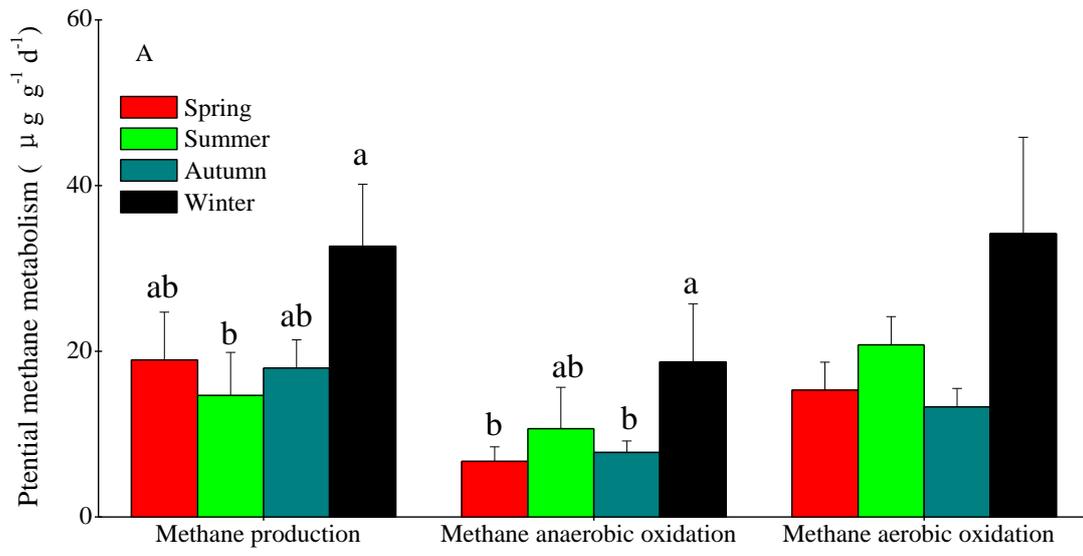
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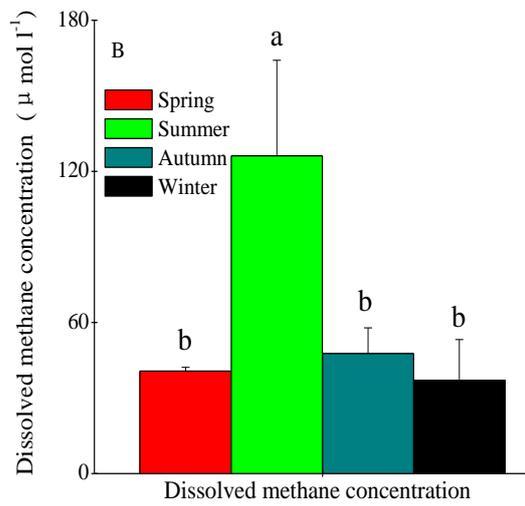
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753 **Fig. 2.**

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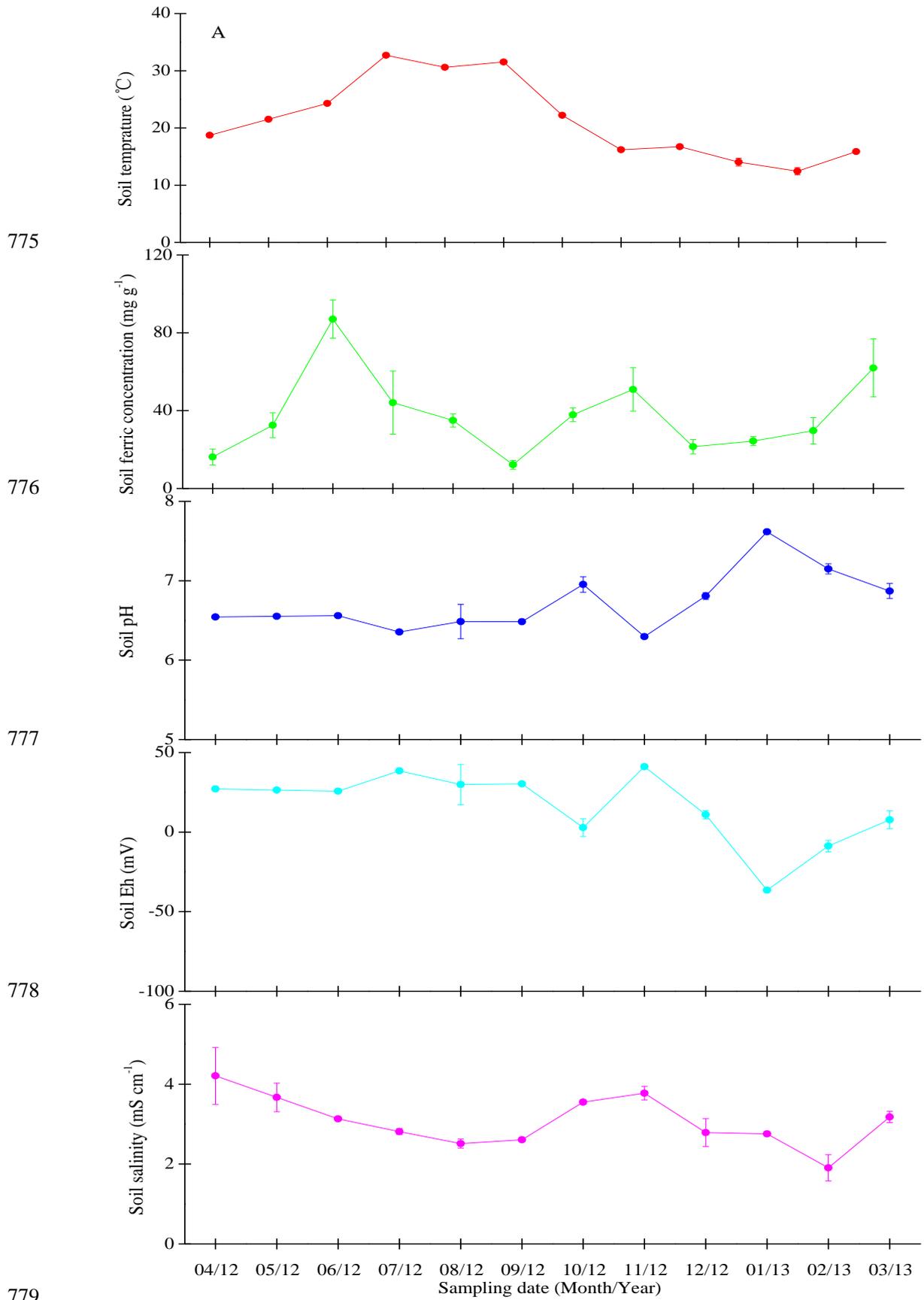


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Fig. 3.



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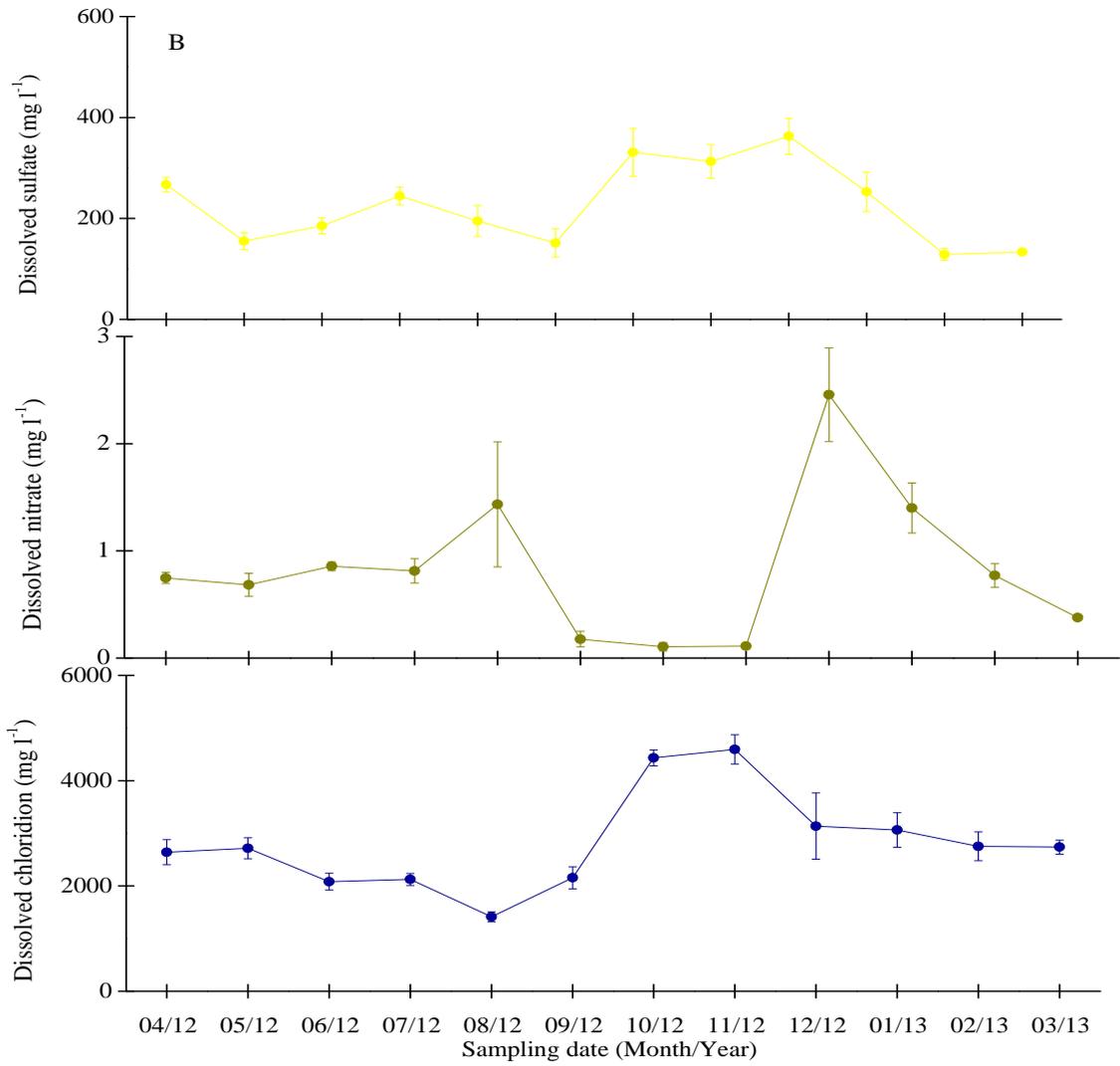
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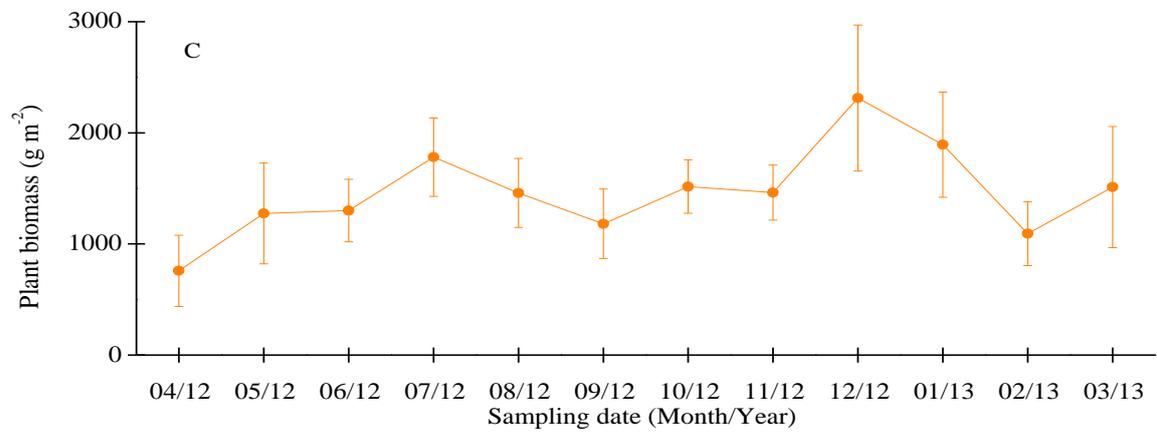
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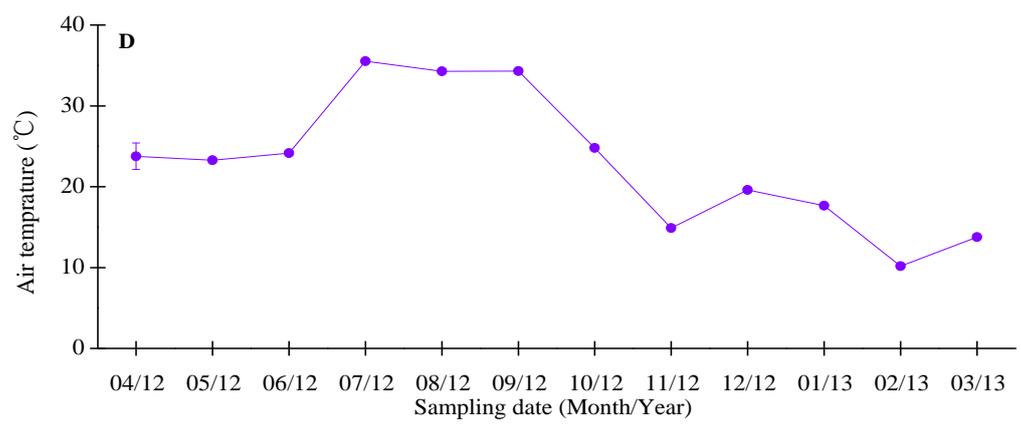
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Fig. 4.

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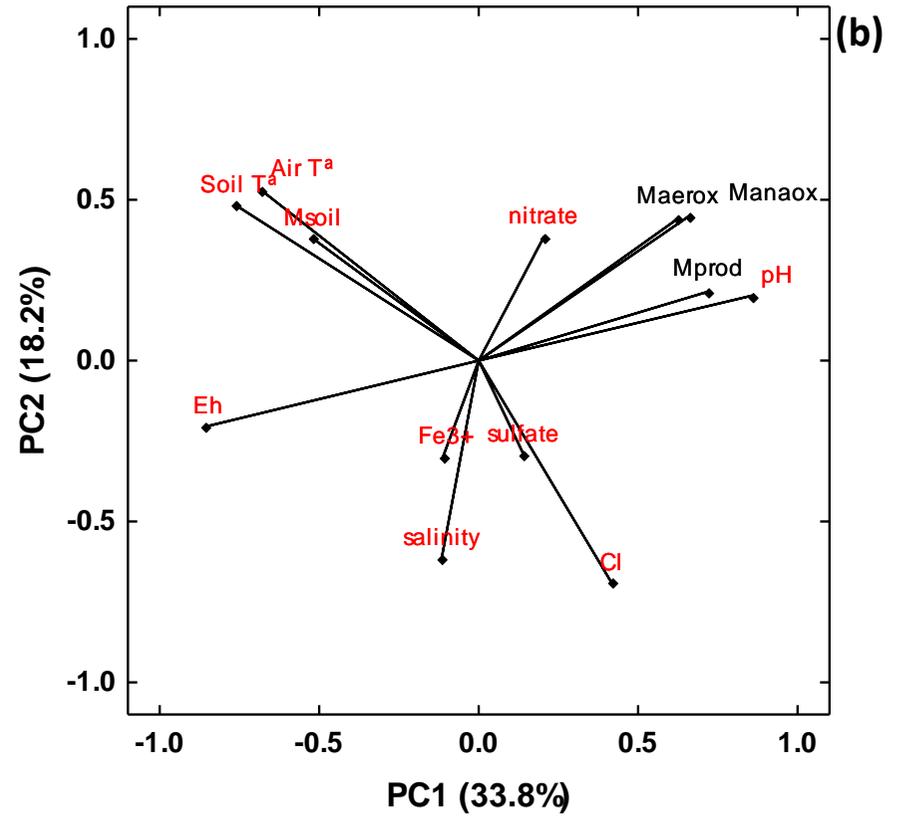
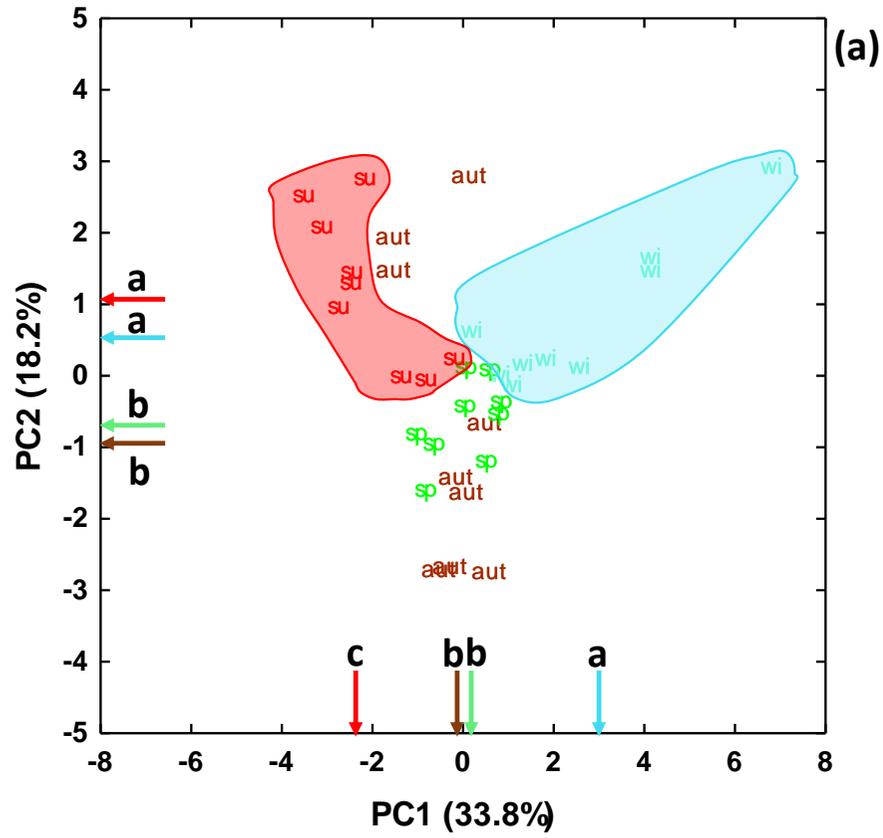
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815 Fig. 5.

