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Biogeosciences Discussions

Interactive comment on "Influence of niche differentiation on the abundance of methanogenic archaea and methane production potential in natural wetland ecosystems across China" *by* D. Liu et al.

D. Liu et al.

wxding@issas.ac.cn

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The response to reviewers' constructive comments and suggestions about the manuscript "Influence of niche differentiation on the abundance of methanogenic archaea and methane production potential in natural wetland ecosystems across China"

We would like to express our hearted gratitude to two anonymous reviewers for their constructive comments and suggestions that could really improve our manuscript greatly. We would like carefully revise the manuscript according to constructive com-

ments and suggestions of two reviewers. The responses are as following:

Comments by referee #1: 1. In correlating the methane production potential with the abundance of methanogens, a RNA-based approach, i.e., mcrA transcript gene abundance (normalized to 16S rRNA gene) should be more meaningful. It is kind of expected that the DNA approach does not show any correlation with the methane production rates. I realize that it's too late to redo the experiments using the reverse transcription qPCR (RT-qPCR), but at least the authors should discuss possible pitfalls of the DNA approach and offer alternative explanations for the lack of any correlation.

Answer: This is an excellent comment and suggestion. We will revise the part of Discussion according to above suggestion. We palned to add the sentence "a further study is required to understand the role of functional methanogenic archaea and their relationship with the production of CH_4 from different wetlands." At this moment, we are doing to evaluate the functional methogenic archaea using the ^{13}C stable isotope technique as above suggestions.

Comments by referee #1: 2. qPCR: I think qPCR of the functional gene of methanogens (for example, mcrA) would be more appropriate to link the gene abundance to methane production potential. I wonder why the authors did not consider the mcrA gene. Usually considering DNA extraction efficiency etc., a gene ratio, such as mcrA gene/16sRNA gene, should be used to correlate with methane production potential.

Answer: The measurement of mcrA gene is a good functional gene technique for detecting the abundance of methanogens. The primers used in this study as well as in pervious studies targeted methanogenic archaeal 16S rRNA genes (Watanable et al., 2006). As one of a series of studies, the aim of this study was to evaluate the possible relation between the abundance of methanogenic archaea and methane production potential, and understand the underlying factors affecting methane production in natural wetland ecosystems across China. At present, we are measuring the mcrA

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gene using the stable isotope probing (SIP) combined with nucleic acid-based techniques. We hope we can understand the relationship between the population of active methanogenic archaea and methane production potential in different wetlands in the future.

Comments by referee #1: 3. When correlating methane production potential with environmental parameters, temperature maybe one parameter, but there are other important ones. So it may not be surprising to see a scatter plot (Fig. 3). The authors should try a multiple-regression analysis to include pH, SOC, TN, DOC simultaneously etc.

Answer: Thanks greatly for the reviewer's suggestions. We have done the multiple-regression analysis relating pH, SOC, DOC and incubation temperature to CH_4 production potential. We will revise the sentence on page 7638 according to above suggestions in the revised manuscript.

Comments by referee #1: Specific comments:

Page 7640: Line5-10: this is repeat of the results.

Answer: We will revise these sentences to avoid the repeat as shown in the part of Results in the revised manuscript.

Comments by referee #1: Page 7643: line 3-4: I think it's premature to conclude that the abundance of methanogens is not an effective index for predicting the CH_4 production potential. This sentence should be qualified by stating that DNA-based, total methanogen abundance is not a good indicator, but if the authors measure active methanogen abundance, it maybe a good indicator.

Answer: This is a great comment. We planed to change this sentence into "and this indicates that DNA-based, total methanogen abundance might be not an effective indicator, for predicting the CH_4 production potential in wetlands."

Comments by referee #1: Figure 6: Can the authors also plot methane production

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potential vs. environmental factors such as substrate availability (DOC etc)?

Answer: We will add a new plot showing relationship between CH_4 production potential and DOC in Figure 6 in the revised manuscript.

Comments by referee #1: Fig.7: Are there any error bars associated with this graph?

Answer: We will add the standard error bars in Figure 7 in the revised manuscript.

Comments by referee #2: Specific comments

1. Please be consistent with using CH_4 /methane.

Answer: We will carefully revise the manuscript as this suggestion of the reviewer.

Comments by referee #2:

2. Title

I would recommend a shorter title such as: Relation between methanogenic archaea and methane production potential in natural wetland ecosystems across China.

Answer: This is a rather good suggestion. Before submission, we once sincerely considered the title. In the manuscript, we will improve the title as above suggestion.

Comments by referee #2:

3. Abstract

The aim of this paper is not very clear formulated within the first paragraph of the paper. Please state which are the main objectives before getting into the details of site description and methodology.

Answer: We will improve the sentences and try to make the main objectives of this study more clearly in the revised manuscript.

Comments by referee #2: The last two paragraphs are too descriptive, rewrite a shorter and clear conclusion and main findings of the study: we conclude that CH_4

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production potential. . .

Answer: We will change the last two sentences in the art of the Abstract into "We conclude that CH_4 production potential in the freshwater wetlands of Eastern China is affected by the supply of methanogenic substrates rather than temperature. 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 methangoens with low CH_4 production potential, which in turn suppresses CH_4 production."

Comments by referee #2: Page 7630 Line 1-2: spatial variability in which context, related to what factors?

Answer: We will change the first two sentences into "Methane (CH_4) emissions from natural wetland ecosystems exhibit great uncertainty in the magnitude and distribution on regional, national, and continental scales related to temperature, substrates, water table and methanogenic archaea etc."

Comments by referee #2: First two paragraphs: you mention after each wetland site the climate zone, which one belongs to the Qinghai-Tibetan Plateau? Due to the altitude of the plateau would be classified perhaps as desert boreal/arctic zone?

Answer: The Qinghai-Tibetan Plateau is generally classified as the alpine climate zone. This is an excellent comment. We will add this information in the revised manuscript.

Comments by referee #2: Line 5: vertical soil profiles

Line24: rather than temperature

Line 24: is mainly affected (be consistent with using the present or pasttense)

Line25: stability of the water table.

Answer: We will carefully revise these sentences in the revised manuscript according to the reviewer's suggestion.

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Comments by referee #2:

4. Introduction

The introduction is generally well structured. I would recommend a more detailed reference list especially for the lines 5-10 "up to date, many studie. . . ". The following references and related articles (with references therein) which focused on CH_4 emissions from wetlands might be of interest: T.R.Christensen et al 1993, 1996, 2003 etc; P.M.Crill et al., 1988; Walter and Heimann 2000, 2001a,b; Reeburgh and Whalen, 1992; Petrescu et al., 2008, 2010; Cavicchioli 2006; Frolking, 2007; Rivkina et al., 2007 etc.

Line 11: please have a look in Walter and Heimann 2000 for a very detailed overview on factors influencing the CH_4 emissions.

Answer: Thanks greatly for this suggestion. We will rewritten some sentences in the part of Introduction in the revised manuscript according to the above suggestion.

Comments by referee #2: Page 7631 Lines 5-10: The following sentence: "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". I would reformulate: "However, there is great uncertainty in the magnitude and distribution of methane sources from regional to global scales because of the large spatial and temporal variations in emissions across individual wetland types".

Page 7632 line 11: "We have examined. . . potential and methanogenic population. . . "

Answer: We will revise these sentences in the revised manuscript as suggested by the reviewer.

Comments by referee #2:

5. Material and methods:

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Good experimental approach and description of the methodology. Please have a look at Yurova et al., 2008 for DOC.

Pages 7632-7633: Add after m (meters) a.s.l. in line 23 and 11, 16 respectively.

Page 7633: Lines 1-5: "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-reformulate sentence as: Vegetation in the C. lasiocarpa marsh is composed of 90% C. lasiocarpa and 10% Glyceria spiculosa. The soil profile is composed of standing water, root layer, peat layer and grey soil layer".

Line 10: "Ruoergai highlight is, however, up to 3400 m, resulting in the little annual . . . "change with: Ruoergai highland riches 3400m a.s.l. and shows little annual. . . "

Line 28: Soil samples. . .

Answer: We will revise these sentences in the revised manuscript according to above comment and suggestion.

Line 13: You talk about low and high average monthly temperature but you don't mention the year when these were taken. Is it 2009 same as the sampling?

Answer: The data given are the multi-year average temperature, and we will add the reference in the revised manuscript.

Comments by referee #2:

6. Results

Page 7636 line 15: "Soil properties of different natural wetlands are shown in Table 1". From this sentence one would understand that you talk about all wetlands in general. Please refer to the ones present in your study. You may say: In Table 1 we present the site characteristics and soil properties of the 5 wetlands in study. Same thing for line 18: of other wetlands. . .

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Page 7637 Line 4: DOC concentration was measured in the. . .

Line 5: lowest in the..

Answer: We will have carefully revise these sentences according to the reviewer's suggestions.

Comments by referee #2: Point 3.2: You present figures for the top 30cm of CH_4 production as temperature related and in Figure 3 the relationship between CH_4 production potential of the top soil layer and incubation temperature. I would be very interested if you could also show a similar figure for the water table depth or even better showing how T, WT and CH_4 (and/or microbial populations) are correlated at each site.

Answer: We have done the statistical analysis according to the suggestion and will presente the plots in Fig. 3 in the revised manuscript.

Comments by referee #2: Line 24: marsh, and was 665. . . "

Line 25: give the lowest value for the Ruoergai peatland.

Page 7638 Line 2: . . . to the other wetlands counting 8.29. . . weather the lowest was measured in the Poyang. . .

Line 21:...to the top layer for all the samples

Page 7639 Line 1: growing season

Line 2: You already jump to conclusions. Please reformulate: In this study we show that...

Answer: We will carefully revise these sentences.

Comments by referee #2: Line 5.You refer to Ding et al 2004 for similar results: how about comparisons with studies from US, Canada, Russia for the same type of vegetation wetland and climate?

Answer: This is a good suggestion. We once made a comparison with other studies on

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CH_4 emission from different freshwater marshes and peatlands in US, Canada and so on in Ding et al (2004). In that paper, we found that mean CH_4 flux over 2 years during the growing season from May to October in the *Carex lasiocarpa* marsh in Sanjiang plain was as high as 19.6 mg CH_4 m^{-2} h^{-1}. Such high emissions were also observed in Canada, Panama and USA (Delaune et al., 1983; Harriss et al., 1985; Keller, 1990; Bubier et al., 1993). These findings suggested that the freshwater marsh around the world is a substantial source of atmospheric CH_4.

Comments by referee #2: Page7640 Line 3: for R^2 be consistent throughout the paper how many decimals you use. . . I see 2, sometimes 3. . . I would suggest to keep in round with 2 decimals.

Page 7641 Line 16: oxidation, while C. . .

Answer: We will carefully revise figures in the manuscript and keep 2 decimals as suggested by the reviewer.

Comments by referee #2: Line 14-16: 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.

What might be the reason? Many studies have found that water table fluctuations between +1 and -10 cm in a short period of time enhance the CH_4 emissions. During the summer showers when the WT rises and floods occur, the wetlands become hot spots for CH_4 (high picks are observed). Is this the case for any of the 5 wetlands?

Answer: This is an excellent comment and suggestion. The fluctuation of the water table might enhance CH_4 emissions from peatland and freshwater marsh. In this study, Poyang lake wetland and Hongze lake wetland are greatly different from peatland in the Ruoergai plateau and freshwater marsh in the Sanjiang plain. No peat is found at all. In other words, the soil will expose to air when the water table drops in winter in Poyang wetland, due to the lowering of the Yangtze River water level. This drop will

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accelerate the decomposition of soil labile organic carbon due to high temperature in the subtropical zone. In red soil intermittently inundated by water, soil organic carbon concentration is rather low in China. Thereby there are low substrates for methanogens resulting in low CH_4 production. In contrast, although some rather than all of freshwater marshes are seasonally flooded in the Sanjiang plain, they have rich peat, which in turn provide enough substrates for methanogenesis.

Comments by referee #2:

7. Conclusions

You mention that the production potential increases with latitude. Please give examples of other studies which have a similar conclusion and explain why. What is the difference between a low-high latitude wetland? You also conclude about the position and stability of the water table. You did not discuss much on this topic, please refer to other studies and relate your findings to it.

Answer: In China, no measurement across China has been done in the same study. However, when analyzing data in different papers, we can find similar phenomenon. For example, Ding et al. (2004) found that the fluxes of CH_4 emitted from freshwater marsh in the Sanjiang plain of Northeast China were 19.65 $mg{\cdot}m^{-2}{\cdot}h^{-1}$, but 5.53 $mg{\cdot}m^{-2}{\cdot}h^{-1}$ in the wetlands of the Southeast China (Tong et al., 2009; Zeng et al., 2010). The difference might be attributed to the different type of wetlands, which shows distinct capacities to supply substrates for the production of CH_4. In China, freshwater marsh locates in the Northeast China and peatland mainly distributes in the alpine region, which is generally rich in soil organic carbon and dissolved organic carbon. While lake wetlands mostly appears in the low latitude of Southern China, where the content of soil organic carbon is relatively low.

In the revised manuscript, we will revise the part of Discussion according to above suggestion. For example, we planed to revise the sentences on page 7640 by adding “Altor and Mitsch (2006) observed that CH_4 emissions from intermittently-flooded wetlands

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were remarkably lower than those in permanently-inundated, natural and anthropogenic wetlands. They summarized the higher emissions were due to greater availability of methanogenic substrates and consistently low redox potential in continuously anoxic wetlands although there were opposite results were observed in some wetlands (Hargreaves et al., 2001; Cheng et al., 2007), we argue that. . . ".

Comments by referee #2: Line 7: . . . affected by temperature and depends on the supply of substrates. . .

Figure captions

Fig.1.Change: Location of the four study sites in China.

Answer: We will carefully revise these sentences according to above suggestions.

Interactive comment on Biogeosciences Discuss., 7, 7629, 2010.