Response to interactive comments from Anonymous Referee #1 (bg-2018-456)
We would like to express our sincere gratitude to Anonymous Referee #1 for helpful comments and corrections. Our responses to specific comments (reprinted in bold) are given below.

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The methodology is sound, but in particular the field methods need further clarification (detailed below). The conclusions drawn from this paper are justified, but I would have liked to see some support from field measurements of redox potentials; the field methodology description suggest that these measurements have been taken. The same holds for active layer thickness measurements. The authors conclude that long term
10 **water saturation is the cause of the enhanced CH$_4$ emission, which is made plausible. However, a more extensive discussion of alternative explanations, such as increase of active layer thickness, or change of vegetation is necessary. If, for instance, the active layer measurements also suggest an increase in active layer thickness over the years following the extreme precipitation, the conclusions of the authors about the effects of water saturation should be adapted.**
15 **I have no doubt that the authors should be able to accommodate the remarks above. I recommend publication with moderate revision. In particular the Methods section needs improvement and a more in-depth discussion of the alternative causes of the long term changes in CH$_4$ emission after extreme precipitation is necessary.**

20 *Reply:* We truly appreciate your positive suggestions.

1) Redox potential
We do not have redox potential data that can be compared among 2011 (wetting event), 2012, and 2013. We found a large artifact in redox potential value after installing the ORP electrode into soil, which lets the
25 atmospheric O$_2$ intrude to the soil and can increase the redox potential value. It took from several to 10 days for the redox potential to decrease and stabilize in anoxic soil.
In 2012 and 2013, we monitored temporal changes in redox potential for days or weeks after electrode installations, and obtained some redox potential data after stabilization (added as Table S6). As we have added to Sect. 4.2 in our manuscript, 'in this period, we observed redox potential values lower than −100 mV in wet areas
30 (Table S6), which are well below the upper limit for CH$_4$ production in soil (Conrad, 2007; Street et al., 2016). Methane production at a potential higher than −100 mV can also occur, because soil is heterogeneous and can have more reducing microsites than the rest of the bulk soil, where redox potential can be measured (Teh et al., 2005; Teh and Silver, 2006).'

35 2) Alternative explanations for the long term changes in CH$_4$ emission after the extreme precipitation
We have added data of interannual variation in thaw depth to the supplement as Table S1. In wet areas, we found increase in thaw depth from 2011 (22 ± 4 cm) to 2012 (25 ± 8 cm) and 2013 (35 ± 7 cm) in observations made during mid-July. We have added this information to Sect. 3.1 in the main text.
Indeed, it appears that the extreme precipitation in 2011 (Fig. 2) led to the thaw depth increase. In
40 addition, we did not evaluate vegetation cover quantitatively. Although we did not find drastic change in vegetation cover in the observed wet areas, abundance of sedges might have increased after the wetting event.
We have added these alternative explanations to the end of Sect. 4.2 and mentioned them in the concluding remarks in the main text.

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Detailed comments:

P. 3, Line 27-29: poorly readable sentence, reformulate.

Reply: We have rewritten the sentence in a simpler way. For further clarity, we have also added short explanations on each name of the observation points shown in Table 1 (such as sedge_K).

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P. 3, Line 30: How is 'predominantly' determined? Did you do any vegetation cover analysis?

Reply: No, this work did not analyze vegetation cover. We named a micro-relief covered by sphagnum mosses (*Sphagnum squarrosum*) as sphagnum_K, and micro-reliefs covered by sedges, especially by some cotton-sedges

10 (*Eriophorum angustifolium*) as sedge_V, sedge_K, sedge_B. We have taken out 'predominantly' from the manuscript and corrected Table 1 accordingly.

On the other hand, Morozumi et al. (in review) observed specific altitude, soil moisture, and plant species along a 50 m transect in site K. They defined four vegetation classes (tree, shrub, sphagnum, and cotton-sedge), and found cotton-sedges at the lowest and wettest areas. From clustering analysis of plant species composition in

15 site K and a local scale (10 km × 10 km) including site K, they identified these four vegetation classes as different clusters. The vegetation types in this work, i.e. tree mound, sphagnum (wet area), and sedge (wet area) correspond to their vegetation classes (tree, sphagnum, and cotton-sedge, respectively).

20 **P. 3, Line 31: What is meant by 'snapshot' measurements? What was the measurement frequency?**

Reply: We observed volumetric water content in surface soil for three to six times at each observation point in July 2011 (Table 1). We meant by 'snapshot' that these measurements had not been conducted continuously from early July to late July but only on 1 to 3 days in July for each observation point (please refer to Table S2 for detailed observation dates). We have deleted the word 'snapshot' from the main text and added this information

25 to the footnote of Table 1.

P. 4, Line 8: What is meant with 'principally closed'?

Reply: We mean that 'the chamber was closed for 30 min and headspace gas was sampled at 0 min, 15 min, and

30 30 min after chamber closure' <u>in most cases</u>. We have reformulated the sentence as follows; 'The chamber was closed for 15–30 min and headspace gas was sampled for two to three times after chamber closure. In most cases, chamber was closed for 30 min and headspace gas was collected at 0 min, 15 min, and 30 min after the closure.'

35 **P. 4, Line 13: Can you give an explanation on the detection limit of your chamber measurements, for low magnitude fluxes, e.g. negative CH_4 fluxes?**

Reply: The detection limit of CH_4 flux was 0.8–2.4 mg CH_4 m^{-2} day^{-1}, depending on the height of chamber headspace and conditions of the gas chromatograph in CH_4 concentration analyses (mentioned in Sect. 2.4). This limit also applies to negative CH_4 fluxes.

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P. 4, Line 18: Which atmosphere? Was this ambient air or some prepared gas mixture, and what was its composition? Please clarify.

Reply: This atmosphere was collected in Chokurdakh village or our observation sites, and filtered by Molecular

45 Sieves 5A (1/16 pellets, FUJIFILM Wako Pure Chemical Corporation, Japan) to remove contaminants such as ammonia and n-alkanes from ethane to n-butane (carbon dioxide and water vapor are also removed significantly).

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The filtered atmosphere was preserved in a 10 L aluminum bag. Its methane concentration was also analyzed by gas chromatography and found to be 2.0–4.3 ppm. We have corrected our manuscript accordingly.

P. 4, Line 23: An ORP electrode appears to have been used for temperature measurements, but I miss the redox potential data in this article. Why have these data not been used?
Reply: As we described above, we found large positive biases in redox potential after installing ORP electrodes. We obtained some redox potential data after stabilization in 2012 and 2013, and we have added these data to our manuscript as Table S6.

P. 4, Line 26: How do you define ground surface in a sphagnum cover?
Reply: In the wet area of sphagnum moss (sphagnum_K), we defined the moss surface as the origin of height, and measured water level relative to this moss surface. Thaw depth values and the depths shown with soil temperature measurements, dissolved CH_4 analyses, soil incubation experiments also mean the depth relative to the moss surface if the ground surface was covered by moss. We have reformulated the sentence in our manuscript as follows; 'The water level was expressed in height relative to the ground surface or the moss surface.'

P. 5, Line 24: Calculation of the chamber fluxes: two to three values are used for calculation of the fluxes, while the field methods section says that three samples have been taken from the chambers. If in some cases only two values have been used, some of the analysis results apparently have been rejected. Clarify the reasons for rejection of samples.
Reply: We are sorry for our misleading explanation. From one chamber observation, two to three samples were collected, and no result of sample analyses was rejected. The field methods in our manuscript have been corrected accordingly.

P. 6, Line 15. The Chokurdagh weather station appears to be at some distance from the sites, in particular site V. Please indicate the distance.
Reply: The distance between Chokurdakh weather station and site V is approximately 45 km (Fig. 1). We have added this information to Sect. 2.5 in the main text.

P. 10, Line 21. In the methodology section, it is suggested that redox potential measurments were taken, however, they are not mentioned in the article. At this point, it would be very interesting to know the redox conditions over the years.
Reply: As we described above, we do not have enough reliable data for comparing redox conditions among 2011 (wetting event), 2012, and 2013. But we partly obtained some redox potential data without biases in 2012 and 2013, and we have added them to the manuscript as Table S6. We found potential values lower than −100 mV in wet areas in 2012 and 2013, which is well below the upper limit for CH_4 production (Conrad, 2007; Street et al., 2016).

P. 11, Line 4. The 16S rRNA gene sequencing was not introduced in the Methodology section.
Reply: We have added a description of 16S rRNA gene sequencing to the methodology section (Sect. 2.3).

Response to interactive comments from Anonymous Referee #2 (bg-2018-456)

We would like to express our sincere gratitude to Anonymous Referee #2 for helpful comments and corrections. Our responses to specific comments (reprinted in bold) are given below.

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Major points

1. If the authors want to prove that the increase of CH_4 emission in 2012 and 2013 was due to reduced condition after high precipitation in 2011, the authors should show the precipitation data in the preceding years before 2009 (e.g. 2007 and 2008, if possible) to prove that low CH_4 emission in 2009 and 2010 was

10 **observed under long lasting oxic condition (although there is no GWL data). By showing it, readers can convince more easily the authors' hypothesis.**

Reply: The precipitation and air temperature data for 2007 and 2008 have been added to the manuscript (Fig. 2) from the same data source as 2009–2013 (WMO weather station 21946, GHCN-Daily). Annual precipitation was persistently low at 162–173 mm from 2007 to 2009, compared to 211–421 mm from 2010 to 2013 (in

15 hydrological year, i.e. from October in the previous year to September in the current year). This suggests dry soil conditions during our flux observations from 2009 to 2010, considering characteristically high air temperature and low precipitation in July 2010. We have revised Sect. 3.1 in our manuscript accordingly.

As we have added to the section, "Parmentier et al. (2011) reported that water level was lower in summer 2009 than the previous two summers at a tundra research station (Kytalyk) in the vicinity, approximately 30 km

20 to northwest of Chokurdakh." In addition, although we did not observe water level from 2009 to 2010 in our study area, we saw a drastic change in soil wetness conditions from 2010 to 2011, especially in sedge_V. We found no surface water even in the wettest area (sedge_V, containing some amount of cotton-sedge cover as will be described below in relation to the definition of wet area) in 2010, and we observed a high water level (10–14 cm) above the ground surface in 2011.

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2. In Figure 4 and 5, isotopic data of CH_4 are shown in different colors for different year (not for each sampling site). Therefore, readers cannot see the spatial difference of these isotopic values. Please revise the figures (in the same manner as Figure S1). By doing so, the reader can judge if the difference in dD is

30 **due to spatial difference or not. In addition, are there any temporal changes in dD values at 10 cm in 2011? If there is any relationships between higher dD values and environmental factors (i.e. drop with GWL or**

precipitation in summer), this can be important information to understand the effect of CH$_4$ oxidation or diffusion on variation in dD.

Reply: We have added spatial information to Fig. 4 and 5 (though we also wonder if you meant corrections of Fig. 5 and 6, we hope readers can see spatial variations in δD and δ^{13}C of dissolved CH$_4$ from Fig. 5, and that in dissolved CH$_4$ concentration from Fig. 4). In summer 2011, three of all the four wet areas (sphagnum_K, sedge_V, and sedge_B) showed low δ^{13}C or high δD values apart from the convergence values (δ^{13}C ≅ −50‰, δD ≅ −408‰) seen in deep soil layer or under high dissolved CH$_4$ concentration (Fig. 5). In this way, it does not appear that the large variations in δ^{13}C and δD of dissolved CH$_4$ in 2011 were limited to one special location.

We have added individual values of water level and δD of dissolved CH$_4$ observed on each date in 2011 at 10 cm depth in wet areas to the supplement (Table S4). We found increases in water level during summer 2011. However, we could not find clear temporal change in the δD, although we only have δD data for late July in 2011. We did not find clear temporal change in the delta values of dissolved CH$_4$ in 2012 and 2013, either.

Truly, it would be our important future task to conduct detailed investigation of the temporal variation in CH$_4$ dynamics regarding precipitation and water drainage within one summer, although this study found large interannual variations in CH$_4$ flux and disolved CH$_4$ concentration, and those in isotope ratios of dissolved CH$_4$ to some extent.

3. Results of phylogenic composition should be presented in the main text and as a main figure.

Reply: We have added results of phylogenic composition to Sect. 3.4 in the main text, and moved the data figure from the supplement to the main manuscript (Fig. 8).

Minor points

Abstract

P1, L23 "soil" incubation "emitted" CH$_4$

Reply: We appreciate your corrections. We have added "soil" before "incubation" to the sentence. Instead of "emitted", we have inserted "dissolved", because we do not show any data of isotopic compositions of the emitted CH$_4$ to the atmosphere but only those of dissolved CH$_4$ for in situ observation.

P1, L25 & L26 CH$_4$ "emission"

Reply: We have corrected our manuscript accordingly.

P1, L28 "in 2011" see Major point 2

Reply: As we described above, we found no clear spatial variation and no clear temporal variation in isotopic compositions of dissolved CH$_4$.

Introduction

P2, L5, Rewrite the sentence.

Reply: We have rewritten the sentence.

P2, L9-14 Referencing in the manuscript is incomplete.

Reply: We have corrected our manuscript accordingly.

P3, L8 "soil" incubation

Reply: We have corrected our manuscript accordingly.

Methods

P4, L25 When was GWL measurement conducted in each year? After every sampling? Or just one time?

Reply: Water level was measured after most of the CH$_4$ flux observations in wet areas from 2011 to 2013. Detailed observation dates of water level are shown in Table S2. We have corrected the sentence accordingly.

P5, L3 How many soil incubation samples are prepared for each sampling point and for each initial and final measurement? Please clarify.

Reply: We prepared three soil samples for each sampling point. We collected dissolved CH_4 samples twice from each soil sample, and prepared three dissolved CH_4 samples for each of the initial and final measurements. Only for sedge_K, we prepared three replicate soil samples multiplied by four treatments of incubation (12 soil samples in total) to assess vertical variation and effect of incubation temperature. These incubation treatments were 10 cm depth at 5 °C, 10 cm depth at 10 °C, 20 cm depth at 5 °C, and 30 cm depth at 5 °C. We have added all this information to Sect. 2.3 in our manuscript.

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P5, L9-L12 If the analysis method of phylogenic composition is shown in Methods section, data (figure) should be shown as main figure (not as supplement)

Reply: We have moved the data figure from the supplement to the main manuscript (as Fig. 8). We have also added detailed method of the phylogenic composition analysis to Sect. 2.3 in the main text.

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P5, L15 Were the samples prepared in quadruplicate for each day of sampling? Or one sample was measured for each location and each sampling day? Please clarify.

Reply: We measured four replicate samples for each location and each sampling day. First, we prepared four replicate soil samples for each of the two sampling locations (sphagnum_K and sedge_K). Second, we collected headspace gas sample for three times (day 0, day 4 and day 8) from each incubated soil sample. We have added all this information to Sect. 2.3 in our manuscript.

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Results

See the Major point 3.

Reply: As we described above, we have added results of phylogenic compositions to Sect. 3.4.

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P6, L21, Please clarify the definition of "wet area" in this manuscript.

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Reply: The definition is based on vegetation. We defined "wet area" as micro-reliefs with wetland vegetation, namely micro-reliefs covered by sphagnum mosses (*Sphagnum squarrosum*) and those by sedges, especially by some amount of cotton-sedges (*Eriophorum angustifolium*). Because wetland vegetation can be identified visually, "wet area" can be identified easily based on this definition. We found that spatial distribution of the wetland vegetation corresponded to lower elevation in microtopography and higher soil moisture from transect observation (Morozumi et al., in review). We could also confirm from Table 1 in this study that "wet areas" had higher soil moisture than tree mounds. We have rewritten the definition of wet area in Sect. 2.1 accordingly.

P6, L25, Please show the thaw depth of each observation year, in addition to the averaged value.

Reply: We have shown the thaw depth of each observation year as Table S1. In wet area, the thaw depth observed during mid-July became deeper from 2011 (22 ± 4 cm) to 2012 (25 ± 8 cm) and 2013 (35 ± 7 cm). We have added this information to Sect. 3.1, and mentioned it in Sect. 4.2 and the concluding remarks as an alternative explanation of the multi-year effect of wetting on CH_4 emission.

P6, L26- See Major comment 2, please show the environmental data of several years prior to flux measurement in 2009 and 2010.

Reply: We have added precipitation and air temperature data for 2007 and 2008 to Fig. 2, and rewritten Sect. 3.1 accordingly.

P7, L2, Again, when was GWL measurement conducted in each year? After every sampling? Or just one time? If the authors measured GWL after every sampling, it can be useful information to understand the CH_4 production and oxidation processes. It may be especially true for summer 2011 when the dynamic GWL change must occur with precipitation.

Reply: As we described above, water level was measured after most of the CH_4 flux observations in wet areas from 2011 to 2013 (each observation date of water level is shown in Table S2), and we have added individual values of water level observed in wet areas on each date in 2011 to the supplement (Table S4). We found increases in water level during July 2011. However, we could not find clear temporal changes in the isotopic compositions of dissolved CH_4.

P7, L11 Take out "active"

Reply: We have taken out "active."

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P7, L13 Take out "Interestingly"

Reply: We have taken out "Interestingly."

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Section 3.3 See the Major comment 2. Please show the spatial (and temporal) variations of isotopic values.

Reply: As described above, we have added spatial information to Fig. 4 and 5. We have added data for the temporal variation of delta values at 10 cm within 2011 to the supplement (Table S4).

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P7, L25 Please show the ranges of concentrations and dD and d13C values of CH$_4$ in ambient air using for "in situ" dilution.

Reply: We wonder if you mean the air we used for extracting dissolved CH$_4$ from water samples by headspace method. We preserved this air as a background sample for each day of dissolved CH$_4$ sampling. As a result of
20   analyzing the background samples, we obtained 2.0–4.3 ppm for CH$_4$ concentration, −53‰ to −45‰ for δ^{13}C, and −168‰ to −78‰ for δD. We corrected delta values of dissolved CH$_4$ for the bias from background CH$_4$ based on mass balance. We have added these ranges to Sect. 2.2 in our manuscript.

25   **P7, L26 similarly "to what?"**

Reply: We intended to mention that the range of δ^{13}C of dissolved CH$_4$ was similar among surface water, 10 cm depth, and 20 cm depth. We have taken out "similarly" from the sentence.

30   **P8, L9, L10, Please show statistics.**

Reply: With regards to the sampling depths, CH_4 production rate was 0.66 ± 0.15 μmol day^{-1}, 0.33 ± 0.06 μmol day^{-1}, 0.003 ± 0.004 μmol day^{-1} for 10 cm, 20 cm, and 30 cm depths, respectively ($n = 3$ for all the depths). Difference in the rate values among the depths were significant based on Welch's ANOVA test ($p < 0.01$). Regarding the incubation temperature, production rate was 0.66 ± 0.15 μmol day^{-1} gdw^{-1} and 0.74 ± 0.14 μmol day^{-1} gdw^{-1} for 5 °C and 10 °C, respectively ($n = 3$ for both temperatures). Difference in these rate values was not significant based on *t*-test ($p > 0.5$). All these rates here were obtained for sedge_K. We have added all this information to the sentence in our manuscript.

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P8, L20- Please add figures showing change of d13C and dD in Figure S2.

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Reply: We have added plots of $\delta^{13}C$ and δD to Fig. S2. As seen in these plots, both $\delta^{13}C$ and δD increased along incubation day. Two headspace CH_4 samples from day 8 could not be analyzed for delta values, because the CH_4 concentration was low (< 10 ppm).

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Discussion

P8, L30, L31 Please show the ranges of CH_4 flux both in this site and in the some literature.

Reply: We have reformulated the sentence as follows; "our CH_4 flux in wet areas (36–140 mg CH_4 m^{-2} day^{-1})

20 was comparable to that reported for wet tundras (32–101 mg CH_4 m^{-2} day^{-1}) or permafrost fens (42–147 mg CH_4 m^{-2} day^{-1}) in a database across permafrost zones complied by Olefeldt et al. (2013)."

Section 4.2 Need more reference.

25 *Reply:* We have added references (Woo, 2012; Nassif and Wilson, 1975) to three sentences about hydrological processes in Sect. 4.2.

P9, L15, If the authors do not show the ORP data, take out "remarkably".

30 *Reply:* We have taken out "remarkably".

P9, L26, Again, please check if these higher dD values are not associated with sampling point and sampling time.

Reply: As we described above, we found no clear spatial variation and no clear temporal variation (Fig. 5, Table S4).

P9, L32, Here, I recommend showing the equilibrium concentration of dissolved CH_4 with atmospheric CH_4, to exclude the possibility that CH_4 exchange can effect on isotopic values.

Reply: We have added the following after the sentence in our manuscript. "The effect of CH_4 exchange between surface dissolved CH_4 and atmospheric CH_4 can be excluded, because all the dissolved CH_4 observed in this study was highly oversaturated (> 0.3 µmol L^{-1}, Fig. 4) compared to the equilibrium concentration of atmospheric CH_4 (4-5 nmol L^{-1}, assuming $1-10$ °C water temperature and 2 ppm atmospheric CH_4 concentration; Yamamoto et al., 1976)."

P10, L1 In addition, heavy precipitation may supply O_2 to surface layer of wet area.

Reply: We have included this thought to the sentence; "shallow layers are provided with O_2 from the atmosphere and precipitation."

Section 4.3 See the Major point 3. I think that the results of microbial analysis agree well with isotopic variation and, therefore, are should be shown in main text.

Reply: I truly appreciate your positive comment. We have moved the data figure of microbial analysis from the supplement to the main manuscript (as Fig. 8), and added description of the results to Sect. 3.4 in the main text. We have also modified Sect. 4.3 accordingly.

Being more confident with the interpretation, we have added the following sentence to the abstract; "delayed activation of acetoclastic methanogenesis following soil reduction could have also contributed to the enhancement of CH_4 production."

Concluding remarks

P11, L18-19 Add reference.

Reply: We have added references (Sugimoto and Wada, 1993; McCalley et al., 2014; Itoh et al., 2015).

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Figure 2 Please show the precipitation and temperature data in the preceding years before 2009. GWL data of sphagnum moss in 2013 seems missing.

10 *Reply:* We have added precipitation and temperature data for 2007 and 2008 to the figure. As we have added to the figure caption, "water level was very low (< -12 cm) in the wet area of sphagnum in 2013, and could not be measured."

15 **Figure 3, Add statistical information (yearly difference) in the figure.**

Reply: We have added statistical information to the figure.

Figure 7, Please represent the symbols for different sampling site by different colors.

20 *Reply:* We have revised the figure accordingly.

Figure 8, Are the d13C & dD data averaged value? Please clarify.

Reply: The δ^{13}C and δD data are individual values from each incubation syringe and each day. Nevertheless, all

25 the data points were plotted on one line. We have corrected the figure caption acccordingly (Figure 9 in our revised manuscript).

We have also corrected the ranges of both axes in the figure to include all the data points (we missed one data point with δ^{13}C $= -6.6$‰ and δD $= +507$‰ in our previous manuscript).

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P25, L5 "in the bottom left corner"? Please rewrite.

Reply: We have rewritten the sentence as follows; "initial isotopic compositions of the headspace CH_4 were −66‰ to −65‰ for $\delta^{13}C$ and −167‰ to −162‰ for δD."

5 **Figure S2, Please add figures showing change in d13C and dD.**

Reply: We have added the figures.

Table S2, Please show isotopic values and number of samples.

10 *Reply:* We have added isotopic values and number of samples to the table (Table S3 in our revised manuscript).

Multi-year effect of wetting on CH$_4$ flux at taiga-tundra boundary in northeastern Siberia deduced from stable isotope ratios of CH$_4$

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Abstract. The response of CH_4 emission from natural wetlands due to meteorological conditions is important because of its strong greenhouse effect. To understand the relationship between CH_4 flux and wetting, we observed interannual variations in chamber CH_4 flux, as well as the concentration, $\delta^{13}C$, and δD of dissolved CH_4 during the summer from 2009 to 2013 at the taiga-tundra boundary in the vicinity of Chokurdakh (70° 37′ N, 147° 55′ E), located on the lowlands of the Indigirka River in northeastern Siberia. We also conducted soil incubation experiments to interpret $\delta^{13}C$ and δD of dissolved CH_4 and to investigate variations in CH_4 production and oxidation processes. Methane flux showed large interannual variations in wet areas of sphagnum mosses and sedges (36–140 mg CH_4 m^{-2} day^{-1} emitted). Increased CH_4 emission was recorded in the summer of 2011 when a wetting event with extreme precipitation occurred. Although water level decreased from 2011 to 2013, CH_4 emission remained relatively high in 2012, and increased further in 2013. Concurrently, dissolved CH_4 concentration rose by one order of magnitude from 2011 to 2012, and increased further from 2012 to 2013. Large variations in $\delta^{13}C$ and δD of dissolved CH_4 were observed in 2011, and smaller variations were seen in 2012 and 2013, suggesting both enhancement of CH_4 production and depression of CH_4 oxidation. These multi-year effects of wetting on CH_4 dynamics may have been caused by continued soil reduction across multiple years following the wetting. Delayed activation of acetoclastic methanogenesis following soil reduction could also have contributed to the enhancement of CH_4 production. These processes suggest that duration of water saturation in the active layer can be important for predicting CH_4 emission following a wetting event in permafrost ecosystem.

1 Introduction

Atmospheric CH_4 has an important greenhouse effect (Myhre et al., 2013). The largest source of atmospheric CH_4 is the emission from natural wetlands, which is considered to be the main driver of interannual variations in the global CH_4 emission, depending on meteorological conditions such as air temperature and precipitation (Ciais et al., 2013). For instance, Dlugokencky et al. (2009) reported that high temperatures in the Arctic and high precipitation in the tropics led to high CH_4 emissions from natural wetlands, which caused the observed large growth rates in atmospheric CH_4 concentration during 2007 and 2008. Atmospheric CH_4 has been increasing from 2007 through the present (Nisbet et al., 2014).

Methane flux from wetland soil to the atmosphere (we define a positive flux value as CH_4 emissions) is determined by three processes: CH_4 production, oxidation, and transport (Lai, 2009). Methane is produced by strictly anaerobic *Archaea* (methanogens) mainly via hydrogenotrophic methanogenesis ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) or acetoclastic methanogenesis ($CH_3COOH \rightarrow CH_4 + CO_2$) as an end product of organic matter decomposition (Lai, 2009). In the soil's aerobic zone, CH_4 is oxidized to CO_2 by methanotrophic bacteria utilizing O_2, which reduces CH_4 emission to the atmosphere (Lai, 2009). Underground CH_4 is transported to the atmosphere via bubble ebullition, diffusion through soil layers and surface water, and via aerenchyma of vascular plants (Lai, 2009).

High water levels can lead to development of reducing conditions in soil, which can promote CH_4 production or depress CH_4 oxidation, both leading to increases in CH_4 flux (Lai, 2009). This is reflected in the widely observed positive

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relationship between water level and CH$_4$ flux, found in a meta-analysis across the circum-Arctic permafrost zone (Olefeldt et al., 2013). Meanwhile, Desyatkin et al. (2014) observed increases in CH$_4$ flux during the second consecutive year of flooding at a thermokarst depression in boreal eastern Siberia. Treat et al. (2007) reported observations at a temperate fen in the northeastern USA showing that high water level coincided with high CH$_4$ flux in interannual variations. However, water

5   level correlated negatively with CH$_4$ flux over shorter timescales, namely as monthly means or individual measurements. These observational results imply that wetting is not directly related to CH$_4$ flux in wetlands. To understand the relationship between wetting and CH$_4$ flux, it is necessary to assess the underlying processes.

        Stable isotopes of CH$_4$ have been used to estimate production pathways of CH$_4$ (Sugimoto and Wada, 1993; Sugimoto and Wada, 1995; McCalley et al., 2014; Itoh et al., 2015), determine the fraction of oxidized CH$_4$ versus produced

10   CH$_4$ (Marik et al., 2002; Preuss et al., 2013) and to study mechanisms of CH$_4$ transport by plants (Chanton, 2005). When CH$_4$ in soil is lost by oxidation or diffusion, both δ^{13}C and δD of the remaining CH$_4$ increase. While the hydrogen isotope ratio increases more than that of carbon during oxidation, both ratios are considered to change to the same extent during diffusion. Thus it is useful to analyze both carbon and hydrogen isotopes of CH$_4$ to distinguish the effects of both of these processes (Chanton, 2005).

15   The taiga-tundra boundary ecosystem (or transition zone) contains vegetation types of both taiga and tundra ecosystems. Liang et al. (2014) reported that the distribution of vegetation types at the taiga-tundra boundary on the lowland of the Indigirka River in northeastern Siberia is controlled by soil moisture, which corresponds to microtopography. Larches, the dominant tree species in the taiga forests of eastern Siberia, grow on micro-reliefs with higher ground level and drier soil, while wetland vegetation such as sphagnum mosses and sedges, typically seen in wet tundra (van Huissteden et al., 2005;

20   van der Molen et al., 2007), dominates lower and wetter micro-reliefs. Thus, it is reasonable to assume that the taiga-tundra boundary ecosystem has various micro-reliefs in terms of interannual variation in soil wetness conditions: always wet micro-reliefs, always dry micro-reliefs, and micro-reliefs with large interannual wetness variations. Hence, this ecosystem is a suitable area to evaluate the processes controlling CH$_4$ flux in relation to soil wetting and/or drying on an interannual timescale.

25   In this study, to understand relationships between CH$_4$ flux and environmental factors, we observed interannual variations in chamber CH$_4$ flux, along with the concentration, δ^{13}C, and δD of dissolved CH$_4$ during the summer, from 2009 to 2013, at the taiga-tundra boundary located on Indigirka River lowlands in northeastern Siberia. We also conducted soil incubation experiments to investigate how δ values of CH$_4$ reflect CH$_4$ production and oxidation processes in this ecosystem. In 2011, a wetting event with a significant amount of precipitation occurred. We focused in particular on the responses of

30   CH$_4$ flux and other underlying processes to this unusual wetting event.

3

2 Methods

2.1 Study sites

The taiga-tundra boundary on the lowlands of the Indigirka River was selected as our study area. Observations and sampling were conducted at three sites (V: Verkhny Khatistakha, K: Kodac, and B: Boydom) in the vicinity of Chokurdakh (70° 37′ N, 147° 55′ E), Republic of Sakha (Yakutia), Russia (Fig. 1 and Table 1). The sites are located in the Russian Arctic with an annual mean air temperature of −13.9 °C and an annual mean precipitation of 208 mm for the period of 1950–2008, according to the Baseline Meteorological Data in Siberia Database (Yabuki et al., 2011). Sites V, K, and B are alongside the Indigirka River or its tributary, and tree density decreases from site V to site B.

These study sites are underlain by continuous permafrost (Iwahana et al., 2014). Normally, snowmelt and the start of active layer thawing begin in the latter half of May through the first half of June, and the growing season occurs from the end of June through the beginning of August. Air temperature and surface soil temperature (10 cm depth) peak in July, whereas the maximum thaw depth occurs from the latter half of August to the first half of September. The freezing of the active layer starts in the latter half of September to October and the whole active layer freezes from November to December.

Observations of CH_4 flux were conducted at seven points with three typical vegetation types, as summarized in Table 1. These vegetation types are distributed in patches, corresponding to microtopography and soil moisture (Liang et al., 2014). Micro-relief with a higher ground level is covered by green moss, larch trees, and shrubs of willows or dwarf birches. On the other hand, lower micro-relief is covered by wetland vegetation of sphagnum moss or sedges. In this study, the former vegetation type was termed 'tree mound', and the latter type was termed 'wet area'. Observation points in tree mounds were selected at each of the sites V, K, and B, and termed 'tree mound_V', 'tree mound_K', 'tree mound_B' (Table 1). For observation points of wet areas, a micro-relief covered by sphagnum moss in site K was termed 'sphagnum_K', and points covered by sedges including especially cotton-sedges (*Eriophorum angustifolium*) in sites V, K, and B were termed 'sedge_V', 'sedge_K', and 'sedge_B', respectively. Measurements of volumetric water content in the surface soil layer (0–20 cm) by TDR (time domain reflectometry; TDR-341F, Fujiwara Scientific Company, Japan) showed that tree mounds were drier than wet areas; this will be described in Sect. 3.1 (Table 1).

2.2 Field observations and samplings

Methane flux was observed using the chamber method in each of the typical vegetation types described in Sect. 2.1 during the summer from 2009 to 2013. A transparent cylindrical flux chamber (acrylic resin, base area 4.7×10^2 cm^2, height 25 cm) was installed on the ground. The headspace gas of the chamber (ca. 12 L) was circulated with a pump (ca. 1 L min^{-1}). The chamber was closed for 15–30 min and headspace gas was sampled two to three times after chamber closure. In most cases, the chamber was closed for 30 min and headspace gas was collected at 0 min, 15 min, and 30 min after closure. Samples were kept in pre-evacuated glass vials with butyl rubber septa. To minimize soil disturbance, we stepped on wooden boards at observation points. In 2009 and 2010, CH_4 flux measurements were conducted in the latter half of July, and from 2011 to

4

2013, observations were conducted continuously from early July to the end of July or early August. For all of these years, the observation period included the warmest season when CH$_4$ emission was expected to be the most active (Table S2).

For measurements of dissolved CH$_4$, surface water and soil pore water were sampled in wet areas from 2011 to 2013. Surface water was directly taken up by a 50 mL plastic syringe with a three-way cock attached to its tip, whereas soil pore water was sampled by a 50 mL syringe (with a three-way cock attached) through a plastic tube inserted in the soil. Soon after collecting water samples, dissolved CH$_4$ was extracted inside the syringes by the headspace method, after adding 15–35 mL of the atmosphere prepared in a 10 L aluminum bag. This atmosphere was collected beforehand at Chokurdakh village or our observation sites, and filtered by Molecular Sieves 5A (1/16 pellets, FUJIFILM Wako Pure Chemical Corporation, Japan). The atmosphere was analyzed later for CH$_4$ concentration and isotopic compositions as a background sample (2.0–4.3 ppm for CH$_4$ concentration, −53‰ to −45‰ for δ^{13}C of CH$_4$, and −168‰ to −78‰ for δD of CH$_4$). The syringes were vigorously shaken for one minute and left standing for five minutes to ensure equilibration. Finally, headspace gas in the syringes was preserved in 10–20 mL pre-evacuated glass vials with rubber septa.

Concurrently with each flux measurement, soil temperature around the flux chamber was measured with a temperature sensor in an ORP electrode (PST-2739C, DKK-TOA Corporation, Japan) with an ORP meter (RM-30P or RM-20P). After flux measurement samples were collected, thaw depth was observed on the same day around each chamber by inserting a steel rod into the ground. From 2011 on, water level was also measured after flux measurements around each chamber in wet areas using a scale. The water level was expressed as height relative to the ground surface or the moss surface. Observation dates of these environmental factors are shown in Table S2.

2.3 Soil incubation experiments and microbial community analysis

Soil incubation experiments were conducted to estimate δ^{13}C and δD of produced CH$_4$ and fractionation factors of CH$_4$ oxidation for carbon and hydrogen isotopes. For CH$_4$ production experiments, surface soil was sampled in all the wet areas in Table 1 (sedge_V, sphagnum_K, sedge_K, and sedge_B) during summer 2013. Samples were taken at 10 cm depth at each sampling location. To observe vertical variations in δ values of produced CH$_4$ within the thaw layer, we also collected samples from two additional depths (20 cm and 30 cm) at sedge_K, which is a location typical of the taiga-tundra boundary region. These samples were from organic layers, except for the samples from 30 cm, which were from the top of the mineral layer.

Approximately 10 mL of soil was directly transferred into each plastic syringe (60 mL maximum capacity) along with in situ water (approximately 50 mL) to prevent the sample from being oxidized by the atmosphere. Syringes were preserved in water to ensure no leakage and were immediately pre-incubated for 4–8 days, then incubated in triplicate for 8 days. Pre-incubation and incubation temperatures were set at 5 °C. We also incubated syringes at 10 °C for samples from 10 cm depth at sedge_K to investigate temperature dependence of δ values of produced CH$_4$. For each of these seven incubation treatments (sphagnum_K, sedge_V, sedge_K, and sphagnum_K, 10 cm depth, 5 °C; sedge_K, 20 cm and 30 cm depths, 5 °C; sedge_K, 10 cm depth, 10 °C), three replicate soil samples were prepared. Water in each incubation syringe was

5

sampled twice at the start and the end of incubation, and dissolved CH_4 was extracted using the headless method described in Sect. 2.2. As a consequence, dissolved CH_4 samples were collected in triplicate for each of the initial and final conditions of one incubation treatment.

To interpret CH_4 production in the incubation experiments (Sect. 2.3), phylogenic composition of methanogens in the surface soil was additionally analyzed in 2016 using 16S rRNA gene sequencing. In July 2016, soil samples from 10 cm depth were collected in 10 mL plastic tubes in triplicate in the same four wet areas as the anaerobic incubation experiments, and kept frozen until analysis. DNA was extracted from 3 g of the soil samples as described by Ikeda et al. (2004). Extracted DNA was purified using the OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Calif.) and quantified using the Quant-iT PicoGreen dsDNA assay Kit (Invitrogen, Carlsbad, Calif.). Amplicon sequencing was conducted targeting the V3/V4 regions of 16S rRNA genes (Caporaso et al. 2011). Sequences obtained were processed through the QIIME pipeline (Caporaso et al. 2010). A representative sequence was picked from each operational taxonomic unit (OTU), and the Greengenes reference database (version 13.8) was used to assign taxonomic information and calculate the relative abundance of methanogenic archaea present.

For CH_4 oxidation, surface organic layers (0–13 cm depth) were sampled at sphagnum_K and sedge_K in July 2012, and then kept in a refrigerator until the experiment (6 days). These soil samples were cut into small pieces and mixed well with air. Ten grams (about 40 mL) of soil sample were transferred into plastic syringes (maximum 120 mL) in quadruplicate for each sampling location. Approximately 80 mL of air and 0.2–2 mL of 25% CH_4 gas were added to each syringe so the total volume in each syringe was 120 mL and the headspace CH_4 concentration was 5.0×10^2–4.8×10^3 ppm. Syringes were preserved in water and incubated at 8 °C for 8 days. Headspace gas was sampled on day 0, day 4, and day 8 from each syringe into 20 mL pre-evacuated glass vials with rubber septa. Consequently, quadruplicate gas samples were collected for each location and each sampling day.

2.4 Sample analysis and data processing

Methane concentrations in air samples were analyzed using a gas chromatograph (HP6890 series G1530A, Hewlett Packard, USA) equipped with a flame ionization detector and a CP-carboplot capillary column (Varian, USA). Methane flux was calculated from CH_4 concentration in chamber headspace by a linear regression of two to three concentration values against the time elapsed since chamber closure. The detection limit of CH_4 flux for each observation was calculated as 0.8–2.4 mg CH_4 m^{-2} day^{-1}, based on whether the change of chamber CH_4 concentration during the observation was significant relative to the precision of CH_4 concentration analysis. Regression r^2 was calculated (formally) as ≥ 0.87, when the flux value was larger than 2 mg CH_4 m^{-2} day^{-1}. Dissolved CH_4 concentrations were obtained from calculation of the headspace method where equilibrations of CH_4 between gas and water phases are described by the Bunsen absorption coefficient of CH_4 (Yamamoto et al., 1976).

Carbon and hydrogen isotope ratios of in situ dissolved CH_4 and CH_4 samples from both incubation experiments were analyzed on a GC/GC/C/IRMS (modified after Sugimoto, 1996) —which is a continuous flow system consisting of two

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gas chromatographs, a combustion reactor, and an isotope ratio mass spectrometer (MAT253, Thermo Fisher Scientific, USA) —and on a GC/GC/P/IRMS (P: pyrolysis in a HTC reactor of GC IsoLink, Thermo Fisher Scientific), respectively. Carbon and hydrogen isotope ratios obtained were represented relative to VPDB and VSMOW, respectively. Precisions of the analyses were ±0.2‰ and ±2‰ for δ^{13}C and δD, respectively. When calculating δ^{13}C and δD of dissolved CH_4, the

5 effect of CH_4 in background air was removed based on the mass balance. In the aerobic incubation experiments, the fractionation factors of CH_4 oxidation for carbon and hydrogen were calculated using the following Rayleigh distillation equation:

$$\ln \frac{R_t}{R_0} = \left(\frac{1}{\alpha_{ox}} - 1\right) \ln \frac{[CH_4]_t}{[CH_4]_0}, \tag{1}$$

where R_0 and R_t represent isotope ratios under initial conditions and at time t, respectively; α_{ox} is the fractionation factor for

10 CH_4 oxidation (defined so that $\alpha_{ox} > 1$); and $[CH_4]_0$ and $[CH_4]_t$ are CH_4 concentrations under initial conditions and at time t, respectively.

All statistical tests for detecting differences in CH_4 fluxes or dissolved CH_4 concentrations were conducted using R software (version 3.3.3). Mann-Whitney's U test was applied to compare magnitudes between two years of data and Steel-Dwass's multiple comparison test was used to compare magnitudes among three years or more of data.

15 **2.5 Meteorological data**

Air temperature and precipitation observed at a weather station in Chokurdakh (WMO station 21946) were used to investigate interannual variations in meteorological conditions during our observation period of CH_4 flux (2009–2013) and during the preceding two years (2007–2008). The distance between the weather station and our farthest observation site (site V) is approximately 45 km (Fig. 1). These data were obtained from GHCN-Daily, a NOAA database (Menne et al., 2012a,

20 2012b).

3 Results

3.1 Environmental factors

Soil wetness conditions and thaw depth differed among vegetation types (Table 1). Tree mounds had lower surface water content (2.1–17%) than wet areas (42–48%). Among the two types of wet areas, the water level was lower in wet areas of

25 sphagnum mosses than those of sedges (Fig. 2). Wet areas of sedges experienced water levels higher than the ground surface (defined as 0 cm), reaching more than 10 cm above the ground surface. Corresponding with soil water content, the thaw depth was shallower at dry tree mounds (20–23 cm), and deeper in wet areas (31–56 cm). In wet areas, thaw depth became deeper from 2011 (22 ± 4 cm) to 2012 (25 ± 8 cm) and 2013 (35 ± 7 cm) in observations made during mid-July (Table S1). The overall average thaw depth observed on days when flux measurements were taken was 31 ± 12 cm (n = 77, 9–58 cm

30 between Jul 3 and Aug 9).

7

Figure 2 shows persistently low annual precipitation (162–173 mm) from 2007 to 2009. In 2010, July air temperature was characteristically high (15.5 °C) accompanying low monthly precipitation (8 mm). These show dry conditions during our flux observations in 2009 and 2010. Parmentier et al. (2011) reported that water level was lower in summer 2009 than the previous two summers at a tundra research station (Kytalyk) in the vicinity, approximately 30 km to northwest of Chokurdakh. In contrast, precipitation in July 2011 was extremely high (94 mm) with a relatively mild temperature (13.0 °C), which caused an unusual wetting. High precipitation continued in August (74 mm) and September (67 mm) of the same year. Corresponding with this heavy rainfall, water levels were also high in 2011, and subsequent observations show a clear decrease from 2011 to 2013 in wet areas of sedges ($p < 0.005$). Water levels also declined in wet area of sphagnum mosses, with values of −9 cm, −10 cm, and less than −12 cm in 2011, 2012, and 2013, respectively.

3.2 CH$_4$ flux and dissolved CH$_4$ concentration

Obtained CH$_4$ flux shows clear spatial and interannual variations (Fig. 3). Individual flux measurements ($n = 143$ in total) are summarized as mean values for the main summer seasons. From 2011 to 2013, continuous flux observations (Table S2) were conducted in concert with dissolved CH$_4$ analysis, and the interannual variation during this period will be discussed in detail.

With regards to the spatial variation of CH$_4$ flux, tree mounds had consistently small values around the detection limit for all measurements (−4.9 to 1.9 mg CH$_4$ m^{-2} day^{-1}), while wet areas showed CH$_4$ emissions. From 2009 to 2013, the CH$_4$ flux in wet areas showed large interannual variations ranging from 36 to 140 mg CH$_4$ m^{-2} day^{-1}. The flux increased in 2011 when the wetting event occurred, then remained relatively large in 2012 (compared to 2009 and 2010). Moreover, the flux increased again from 2011/2012 to 2013 ($p < 0.05$). No statistically significant correlation was found when CH$_4$ flux was plotted against soil temperature (10 cm depth), thaw depth, or water level using all the data from wet areas (Fig. S1).

In addition to CH$_4$ flux, dissolved CH$_4$ concentration increased after the wetting event in 2011 (Fig. 4). From 2011 to 2012, CH$_4$ concentration in soil pore water at 10 cm depth (Fig. 4b) exhibited a sharp increase of one order of magnitude ($p < 0.005$). It remained high from 2012 to 2013, and the concentrations in surface water and that at 20 cm depth (Fig. 4a and c) also increased significantly over the same period ($p < 0.05$). No significant difference in concentration was observed at 30 cm depth between 2012 and 2013. In terms of vertical variation, the concentration in surface water was lower than that in soil pore water (10, 20, and 30 cm depth).

3.3 δ^{13}C and δD of in situ dissolved CH$_4$

Variability of both δ^{13}C and δD of dissolved CH$_4$ was smaller in deeper layers, showing different patterns between δ^{13}C and δD, and across years (Fig. 5). The δ^{13}C of dissolved CH$_4$ had similarly large ranges (−68‰ to −40‰) in surface water and at 10 cm and 20 cm depths, compared to a small range (−53‰ to −46‰) at 30 cm depth. The δD of dissolved CH$_4$ was variable only in surface water (−415‰ to −308‰) and at 10 cm depth (−417‰ to −341‰), whereas it had a constant value of around −408‰ at 20 cm and 30 cm depths. Additionally, δ^{13}C values approached a relatively high value (approximately −50‰) at depth, while δD values converged to almost their lowest value. In terms of interannual variations in δ^{13}C and δD of dissolved

8

CH$_4$ from 2011 to 2013, both δ^{13}C and δD values in surface soil pore water (10 cm depth) were scattered more widely in 2011, showing standard deviations (SD) of 6.6‰ and 24‰, respectively, whereas their ranges were smaller in 2012 and 2013 (SD: 3.3‰ and 17‰ at maxima, respectively).

As shown in Fig. 6, convergence of δ^{13}C and δD of dissolved CH$_4$ is associated with dissolved CH$_4$ concentrations. The δ^{13}C and δD values of dissolved CH$_4$, including surface water and 10 cm depth, converged at high CH$_4$ concentrations to the values seen in deeper soil layers (δ^{13}C = −50 ± 5‰ and δD = −408 ± 5‰ at > 200 μmol CH$_4$ L^{-1}).

3.4 Soil incubation experiments and microbial community analysis

In the anaerobic incubation experiment, the CH$_4$ production rate was different among sampling locations (Fig. 7); the rate was higher for sedge_K and sedge_B (0.66 ± 0.15 μmol day^{-1} and 0.43 ± 0.09 μmol day^{-1}, respectively) than sedge_V and sphagnum_K (0.24 ± 0.02 μmol day^{-1} and 0.08 ± 0.01 μmol day^{-1}, respectively). In sedge_K, the sampling location tested in detail, production was more rapid for shallower soil layers among the 10 cm, 20 cm and 30 cm depths (0.66 ± 0.15 μmol day^{-1}, 0.33 ± 0.06 μmol day^{-1}, 0.003 ± 0.004 μmol day^{-1}, respectively; p < 0.01 in Welch's ANOVA test), while no difference in the rate was found between incubation temperatures (0.66 ± 0.15 μmol day^{-1} gdw^{-1} at 5 °C and 0.74 ± 0.14 μmol day^{-1} gdw^{-1} at 10 °C, p > 0.5 in t-test). When the CH$_4$ production rate was high, the δ^{13}C and δD values of produced CH$_4$ were less variable irrespective of sampling location, sampling depth, or incubation temperature. The δ^{13}C value of produced CH$_4$ at a high production rate (> 0.26 μmol day^{-1} gdw^{-1}) was −55 ± 4‰ (n = 12). Similarly, δD under rapid CH$_4$ production was −410 ± 9‰ (n = 12). These δ^{13}C and δD values of CH$_4$ obtained under rapid production were mostly comparable with the δ values of in situ dissolved CH$_4$ that converged in deep soil layers (δ^{13}C = −50 ± 2‰ at 30 cm depth and δD = −408 ± 5‰ at 20–30 cm depth; Fig. 5c and d), although δ^{13}C values in the incubation experiment were slightly lower than those in situ.

In the microbial community analysis using 16S rRNA gene sequencing (Fig. 8), soil with high rates of CH$_4$ production shown in the incubation experiment (sedge_K and sedge_B as in Fig. 7) had more abundant total methanogens within the detected archaea than that with slow CH$_4$ production rates (sphagnum_K and sedge_V). Acetoclastic methanogens in the order Methanosarcinales were higher in proportion among methanogens in sedge_K and sedge_B, where δ^{13}C values of produced CH$_4$ in the incubation were higher. In contrast, Methanosarcinales were fewer in proportion in sphagnum_K, where δ^{13}C of the produced CH$_4$ was lower.

In the CH$_4$ oxidation experiment, CH$_4$ concentration in headspace declined continuously in every sample (Fig. S2). As CH$_4$ oxidation proceeded, both δD and δ^{13}C of the remaining CH$_4$ increased with a linear relationship between them (Fig. 9, S2). Observed slope Δ(δD)/Δ(δ^{13}C) was 11, indicating a much larger fractionation of hydrogen than carbon, regardless of vegetation types in wet areas (sphagnum or sedge). The hydrogen isotope fractionation factors of CH$_4$ oxidation calculated from the data shown in Fig. 9 were 1.25 and 1.16 for wet areas of sphagnum and sedge, respectively, while carbon isotope fractionations were 1.021 and 1.015, respectively.

4 Discussion

4.1 CH$_4$ flux at tree mounds and wet areas at the taiga-tundra boundary on the Indigirka River lowlands

Methane flux observed in our study was clearly larger at wet areas than at dry tree mounds (Table 1, Fig. 3). Such differences in CH$_4$ flux between wetland vegetation and dry areas with trees or shrubs is generally observed (van Huissteden et al., 2005; van der Molen et al., 2007; Flessa et al., 2008) and is consistent with the fact that CH$_4$ production requires reducing conditions in soil (Conrad, 2007). Our CH$_4$ flux in wet areas (36–140 mg CH$_4$ m^{-2} day^{-1}) was comparable to that reported for wet tundras (32–101 mg CH$_4$ m^{-2} day^{-1}) or permafrost fens (42–147 mg CH$_4$ m^{-2} day^{-1}) in a database across permafrost zones complied by Olefeldt et al. (2013). In forests, many studies have observed CH$_4$ absorption instead of emission (King et al., 1997; Dutaur and Verchot, 2007; Flessa et al., 2008; Morishita et al., 2014). However, our observations at tree mounds rarely found CH$_4$ absorption or emission. In addition, CH$_4$ was not consumed even under O$_2$- and CH$_4$-rich conditions in incubation experiments of tree mound soil from site K (Murase et al., 2014), indicating that a lack of methanotrophic bacterial activity limited CH$_4$ absorption at this vegetation type. Our results show that CH$_4$ emission from wet areas is expected to make a greater contribution to ecosystem-scale CH$_4$ exchange at the taiga-tundra boundary on the Indigirka River lowlands.

4.2 Methane flux, production, and oxidation responses to the wetting event

In 2009 and 2010 the CH$_4$ emission in wet areas was low (Fig. 3), even at relatively high soil temperatures in 2010 (Fig. S1), under dry conditions that were not directly observed in this study. The wetting event in 2011 initiated the high CH$_4$ emission that continued through 2013 despite decreasing water levels (Fig. 2). Moreover, a further increase in flux was observed in 2013, accompanying a build-up of dissolved CH$_4$ (2011– 2013) as shown in Fig. 4.

These interannual variations from 2011 to 2013 could be caused by the development of reducing soil conditions over multiple years after the wetting event. Reducing soil conditions may have developed, to some extent, as a result of the extreme precipitation in the summer of 2011 (Fig. 2). The surface soil layer, particularly under high water levels, could eliminate O$_2$ from soil pore spaces due to water saturation. These reducing conditions may have been preserved by freezing of the soil throughout the following winter. Additionally, a surface soil layer saturated with ice could have prevented snowmelt water (rich in O$_2$) from infiltrating the soil during the spring thaw season of 2012 (Woo, 2012). These processes would have led to the continuation of reducing conditions in the soil, which were created in summer 2011, into 2012. Through further decomposition of soil organic matter with the consumption of O$_2$, reducing soil conditions may have been exacerbated in the water-saturated soil layer to a greater extent in summer 2012. Water in the saturated soil layer may be retained without exchange for a relatively long time in our study sites, because lateral runoff is assumed to be small in the flat floodplain that comprises the Indigirka River lowlands (Nassif and Wilson, 1975). In addition, deep percolation loss is prevented by the impermeable permafrost layer (Woo, 2012). From summer 2012 to summer 2013, reducing conditions in the soil may have been similarly prolonged, especially in the deep soil layer, despite the decrease in water level from

10

summer 2011 to summer 2013. This continuous soil reduction from 2011 to 2013 could have promoted CH$_4$ production and/or depressed CH$_4$ oxidation, which may explain the increase in dissolved CH$_4$ concentration and CH$_4$ flux in wet areas following the wetting event and continuing until 2013 (Fig. 3 and 4).

In 2011, δ^{13}C and δD of dissolved CH$_4$ (10 cm depth) were scattered broadly across a wide range, whereas in 2012 and 2013 the ranges were narrower and they clustered around a high δ^{13}C value (−50‰) and low δD value (−408‰; Fig. 5b). Considering that δD increased much more rapidly than δ^{13}C in our oxidation experiment (Fig. 9), δD can be considered as a sensitive indicator of CH$_4$ oxidation. In contrast, δ^{13}C is not a good indicator because its fractionation factor of CH$_4$ oxidation (1.015‑1.021) was similar to that of CH$_4$ diffusion (1.019; Chanton, 2005), thus the effects of CH$_4$ oxidation and diffusion cannot be discerned by δ^{13}C. Additionally, δD of dissolved CH$_4$ (Fig. 5) was clearly lower in deeper layers (20 cm and 30 cm depths) than in shallower layers (surface water and 10 cm depth), which indicates δD showed CH$_4$ oxidation in situ as well, because shallower layers are provided with O$_2$ from the atmosphere and precipitation. The effect of CH$_4$ exchange between surface dissolved CH$_4$ and atmospheric CH$_4$ can be excluded, because all the dissolved CH$_4$ observed in this study was highly oversaturated (> 0.3 μmol L^{-1}, Fig. 4) compared to the equilibrium concentration of atmospheric CH$_4$ (4-5 nmol L^{-1}, assuming 1–10 °C water temperature and 2 ppm atmospheric CH$_4$ concentration; Yamamoto et al., 1976). Thus, δD values at 10 cm in 2011 were scattered broadly compared with those in 2012 and 2013 that clustered around a low value, suggesting that CH$_4$ oxidation was still active in the surface soil layer during the year of the wetting event (2011) and was subsequently depressed in 2012 and 2013. In the CH$_4$ production incubation experiment, δ^{13}C and δD of produced CH$_4$ were less variable at higher production rates (δ^{13}C = −55 ± 4‰ and δD = −410 ± 9‰ as in Fig. 7). Analogously, those of in situ dissolved CH$_4$ converged at a high CH$_4$ concentration around similar values (δ^{13}C = −50 ± 5‰ and δD = −408 ± 5‰ in Fig. 6). This suggests that δ values of produced CH$_4$ became almost constant under rapid CH$_4$ production in situ and that the convergence of δ values of dissolved CH$_4$ observed in situ reflect rapid CH$_4$ production. Hence, the narrow ranges of δ^{13}C and δD values of dissolved CH$_4$ at 10 cm depth observed in 2012 and 2013 (δ^{13}C: around −50‰ and δD: around −408‰, Fig. 5b) suggest enhanced CH$_4$ production relative to the wetting year (2011).

Multi-year effects of wetting on CH$_4$ flux through soil reduction processes have been previously proposed by Kumagai and Konno (1998) and Desyatkin et al. (2014) as one possible factor for explaining the increase in CH$_4$ flux after wetting. Kumagai and Konno (1998) reported a CH$_4$ flux increase at a temperate rice field in Japan one year after the rice field was irrigated and restored from farmland that had been drained for eight years. Desyatkin et al. (2014) observed flux increases at a thermokarst depression in boreal eastern Siberia during the second consecutive year of flooding following large volumes of precipitation. On the other hand, studies at natural wetlands in the northeastern USA (Smemo and Yavitt, 2006; Treat et al., 2007; Olson et al., 2013) and southern Canada (Moore et al., 2011) reported that interannual variations of CH$_4$ flux correspond with those of water level and/or precipitation in the current year. In our study area, multi-year soil reduction may be important because soil temperature is generally lower than 11 °C (10 cm to 30 cm depth; Fig. S1a and Iwahana et al., 2014) due to a shallow active layer underlain by permafrost. Therefore, decomposition of organic matter can

11

be slow (Treat et al., 2015), which would slowly decrease soil redox potential, allowing it to remain relatively high in the first year of wetting.

In the following two years (2012–2013), we observed redox potential values lower than −100 mV in wet areas (Table S6), which are well below the upper limit for CH_4 production in soil (Conrad, 2007; Street et al., 2016). Methane production at a potential higher than −100 mV can also occur, because soil is heterogeneous and can have more reducing microsites than the rest of the bulk soil, where redox potential can be measured (Teh et al., 2005; Teh and Silver, 2006).

In addition to the multi-year soil reduction, it appears that the wetting event led to the thaw depth increase in wet areas from 2011 to 2013 (Table S1). Although thaw depth increased, summer air temperatures decreased from 2011 (7.7, 13.0 °C as June and July mean temperatures, respectively) to 2012 (7.4, 9.2 °C) and 2013 (6.6, 10.5 °C) as shown in Fig. 2. The wetting event may have led to the CH_4 flux increase from 2011 to 2013 (Fig. 3) partly through the thaw depth increase, by thickening the soil layer where CH_4 production occurs (Nakano et al., 2000; van Huissteden et al., 2005). However, the clear increase in dissolved CH_4 concentration (Fig. 4) and the enhanced CH_4 production and depressed CH_4 oxidation reflected in our isotopic data (Fig. 5b) cannot be explained by the thaw depth increase. Additionally, in the incubation experiment of CH_4 production (Fig. 7), the CH_4 production rate under anaerobic conditions was slower in the deeper layer, especially at 30 cm depth (mineral soil) compared to 10 cm and 20 cm depths (organic soil) in sedge_K. Treat et al. (2015) also reported, from a pan-Arctic synthesis of anaerobic incubations, that difference in soil types (organic/mineral) and that in substrate quality along depth are important controls on CH_4 production rate. Our results from the incubations suggests that the deep layer comprised of mineral soil, where CH_4 production becomes active when thaw depth increase, is not the main region for CH_4 production.

This study did not evaluate vegetation cover quantitatively, and the wetting event might have also led to some vegetation change (such as increase of sedges), although no drastic changes were found visually in the observed wet areas. Increase in cover by sedges might have raised CH_4 flux partly by providing labile organic substrate for CH_4 production or conduits for the CH_4 transport from the soil to the atmosphere (Chanton, 2005; Lai, 2009; Ström et al., 2015).

4.3 Process behind CH$_4$ production response

When CH_4 production is initiated after the onset of anoxia in rice paddy soil, it first occurs via hydrogenotrophic methanogenesis, and then by both hydrogenotrophic and acetoclastic methanogenesis, which increases CH_4 production rate (Conrad, 2007). Afterwards, the ratio of acetoclastic to hydrogenotrophic methanogenesis can stabilize (Roy et al., 1997). Considering that this ratio is an important control on isotopic compositions of produced CH_4, stabilization of production pathways might explain the convergence in δ values of dissolved CH_4 at our study sites under high CH_4 concentration (Fig. 6), and the reduced variability of δ values of produced CH_4 in our experiment under rapid production conditions (Fig. 7). As acetoclastic methanogenesis leads to higher $\delta^{13}C$ in produced CH_4 than hydrogenotrophic methanogenesis (Sugimoto and Wada, 1993), acetoclastic methanogenesis may have been activated when dissolved CH_4 concentration or CH_4 production rate were high during our study. This interpretation is supported by the microbial community analysis (Fig. 8), where

12

acetoclastic methanogens of Methanosarcinales were more abundant in wet areas, with a higher $\delta^{13}C$ of produced CH_4 in the incubation. Therefore, the high and less-variable $\delta^{13}C$ values observed at 10 cm depth in 2012 and 2013 (Fig. 5b) suggest a greater contribution from acetoclastic methanogenesis compared to the wetting year (2011). Similar to findings from rice paddy soil (Conrad, 2007), acetoclastic methanogenesis may have experienced delayed activation after anoxic conditions began in 2011, which could also have promoted CH_4 production in 2012 and 2013.

5 Concluding remarks

At the taiga-tundra boundary on the Indigirka River lowlands, we observed an increase in CH_4 flux in wet areas following the wetting event in 2011, and a further increase in flux in 2013. Our results show interannual variations in $\delta^{13}C$ and δD of dissolved CH_4, and when compared with our incubation experiments, suggest both enhancement of CH_4 production and depression of CH_4 oxidation in 2012 and 2013 compared to 2011. This enhancement of production could be partly caused by activation of acetoclastic methanogenesis following the development of reducing soil conditions after the wetting event. Analyses of isotopic compositions of CH_4 both in situ and in incubation experiments can be combined to investigate the effects of CH_4 production and oxidation on these isotopic compositions, and to clarify the relationship between CH_4 flux and wetting. In the future, measuring the $\delta^{13}C$ of dissolved CO_2 would be useful to further validate activation of acetoclastic methanogenesis (Sugimoto and Wada, 1993; McCalley et al., 2014; Itoh et al., 2015). Outside of these processes, the wetting event might have affected CH_4 flux partly via the thaw depth increase or some amount of vegetation change. It would be useful to analyze $\delta^{13}C$ and δD values of emitted CH_4 in order to assess changes in CH_4 transport (such as by increase of sedge cover) and to investigate the relationship between dissolved CH_4 concentration and CH_4 flux in detail (Chanton, 2005).

In recent years, strong storm activity and wetting events in terrestrial ecosystems have been observed in northern regions (Iijima et al., 2016). A wetting event at the taiga-tundra boundary can switch micro-reliefs with large interannual variations in soil wetness conditions to significant CH_4 sources; we observed clear increases in CH_4 flux at wet areas after the wetting event. In order to predict CH_4 flux following a wetting event in a permafrost ecosystem, our results show the multi-year process of soil reduction affected by the duration of water saturation in the active layer.

Author contribution

Ryo Shingubara and Atsuko Sugimoto designed the experiments and Ryo Shingubara carried them out. Go Iwahana, Shunsuke Tei, Liang Maochang, Shinya Takano, Tomoki Morozumi, and Trofim C. Maximov helped with sampling, in situ data collection, and preparing resources for the fieldwork. Jun Murase contributed to the laboratory analysis. Ryo Shingubara prepared the manuscript with contributions from all co-authors.

13

Competing interests

The authors declare that they have no conflict of interest.

削除: (SICORP).

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Table 1. Observation points of chamber CH$_4$ flux. Concentration and isotopic compositions of dissolved CH$_4$ were also observed in the following wet areas.

Site	Landscape	Observation points and surface conditions	Dominant vegetation	Volumetric water content (%)[b]	Thaw depth (cm)[c]
V (Verkhny Khatistakha) 70° 15' N 147° 28' E	Larch forest and wetland	tree mound_V	Green moss, *Larix gmelinii*	17 ± 5 (*n* = 3)	23 ± 3 (*n* = 5)
		sedge_V (wet area)	*Carex spp.*, *Eriophorum angustifolium*	48 ± 4 (*n* = 3)	56 ± 3 (*n* = 4)
K (Kodac)[a] 70° 34' N 148° 16' E	Typical taiga-tundra boundary	tree mound_K	Green moss, *Larix gmelinii*	2.1 ± 0.6 (*n* = 4)	23 ± 4 (*n* = 9)
		sphagnum_K (wet area)	*Sphagnum squarrosum*	42 ± 5 (*n* = 6)	31 ± 8 (*n* = 15)
		sedge_K (wet area)	*Eriophorum angustifolium*	44 ± 4 (*n* = 6)	32 ± 13 (*n* = 28)
B (Boydom) 70° 38' N 148° 09' E	Low-centered polygon	tree mound_B	Green moss, *Larix gmelinii*	6 ± 2 (*n* = 5)	20 ± 4 (*n* = 8)
		sedge_B (wet area)	*Eriophorum angustifolium*	46 ± 2 (*n* = 5)	36 ± 9 (*n* = 8)

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[a] Site K was previously named as Kryvaya (Iwahana et al., 2014) or Kodak (Liang et al., 2014).

[b] Observed for the surface soil layer down to 20 cm on 1 to 3 days in July 2011 at each observation point (see Table S2 for detailed observation dates). Standard deviations are shown.

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[c] Observed from early July to early August during 2010–2013 (see Table S1 for the interannual variation and Table S2 for detailed observation dates). Standard deviations are shown.

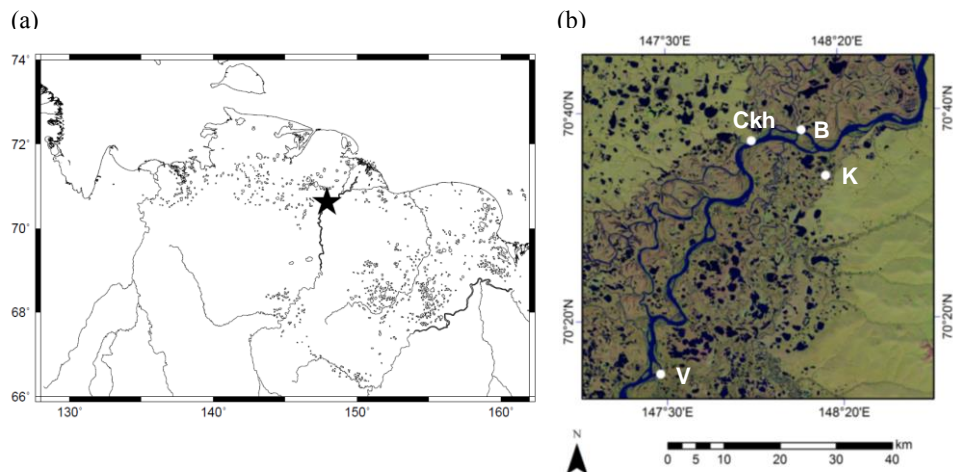削除: observation dates

(a)

(b)

Figure 1: Locations of study sites. (a) Location of study region in northeastern Siberia (the Generic Mapping Tools 5.0.0). (b) Satellite image of Indigirka River lowland around Chokurdakh village (Ckh: 70° 37' N, 147° 55' E) from Landsat 8. Observation sites (V, K, B) were selected in this region alongside the main stem and a tributary of the Indigirka River.
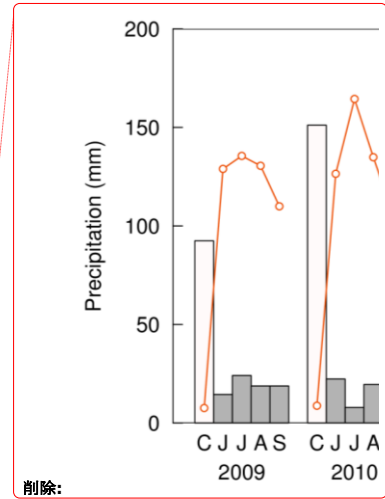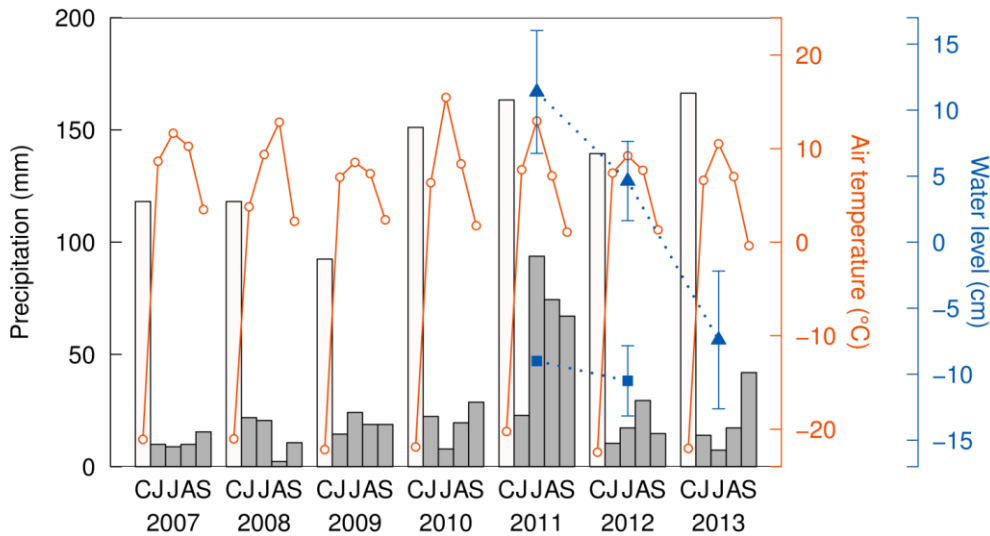
Figure 2: Interannual variations in precipitation (bars) and air temperature (solid lines) observed at a weather station at Chokurdakh for the cold season with snow cover (C: total from October in the previous year to May in the current year) and the warm season (JJAS), and water level (dotted lines) measured in wet areas of sedges (triangle) and sphagnum mosses (square). Water level was very low (< −12 cm) in the wet area of sphagnum in 2013, and could not be measured. Error bars represent standard deviations. Methane flux was observed during the main summers (early July to early August) from 2009 to 2013.
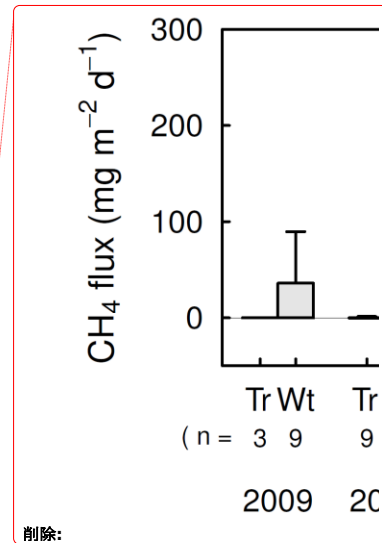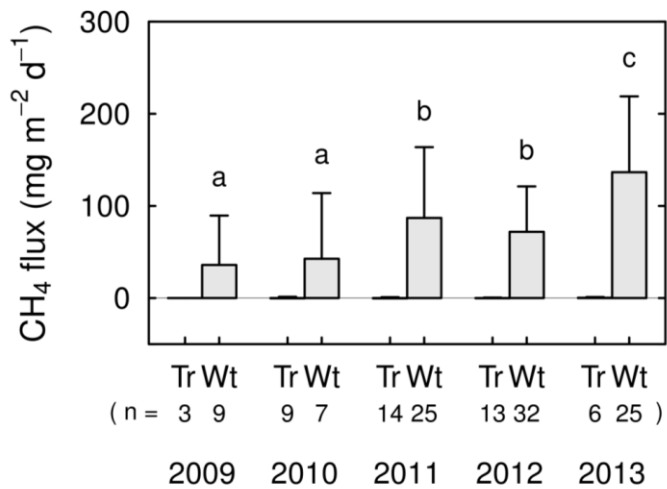
Figure 3: Interannual variations in averaged CH_4 flux in tree mounds (denoted as "Tr") and wet areas ("Wt") for main summer seasons from 2009 to 2013. Replication numbers ("n") are shown for each averaged flux value, and standard deviations are represented by error bars. Different letters show statistical interannual differences in the flux values for wet areas. See Table S2 for flux values at respective observation points.
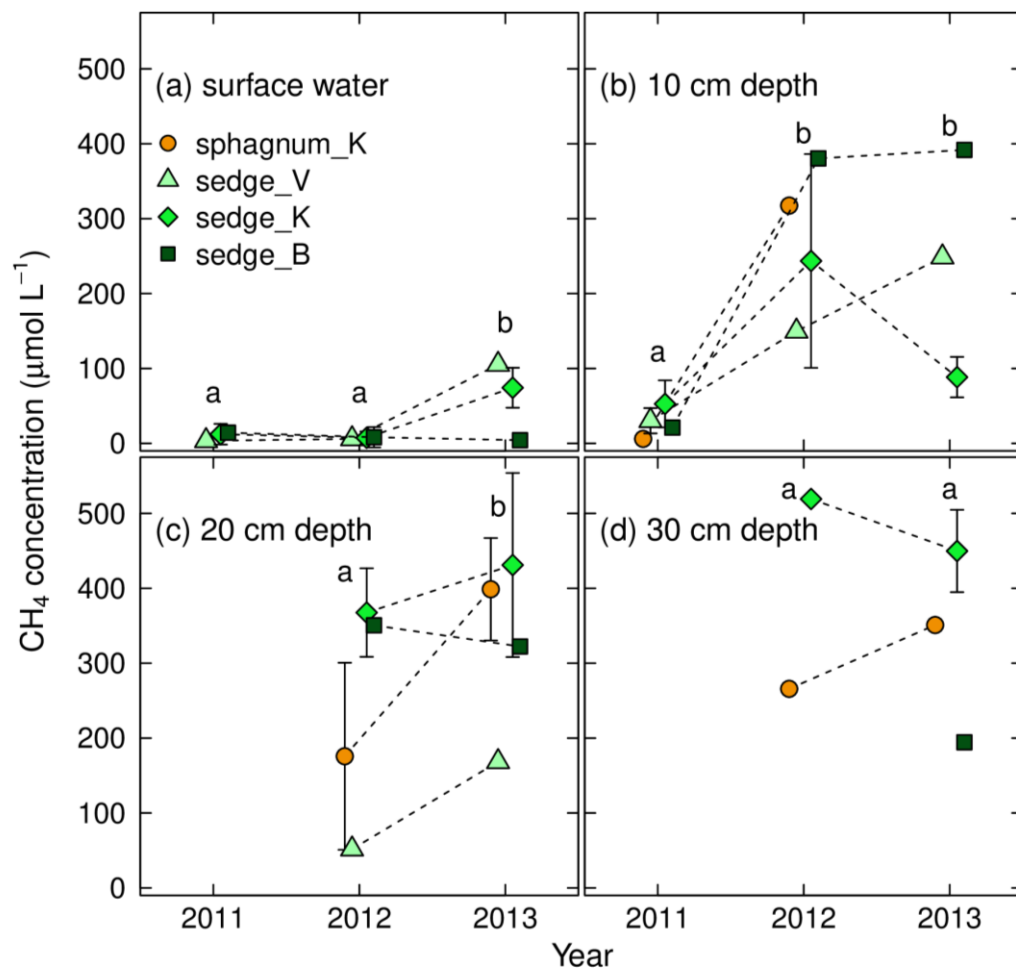
Figure 4: Interannual variation in dissolved CH_4 concentration in (a) surface water and soil pore water at (b) 10 cm, (c) 20 cm, and (d) 30 cm depths in wet areas from 2011 (wetting event) to 2013. Different letters in each panel denote statistical differences among years in averaged concentration across the four wet areas ($p < 0.05$). Error bars represent standard deviations. See Table S3 for numerical values of dissolved CH_4 concentrations.
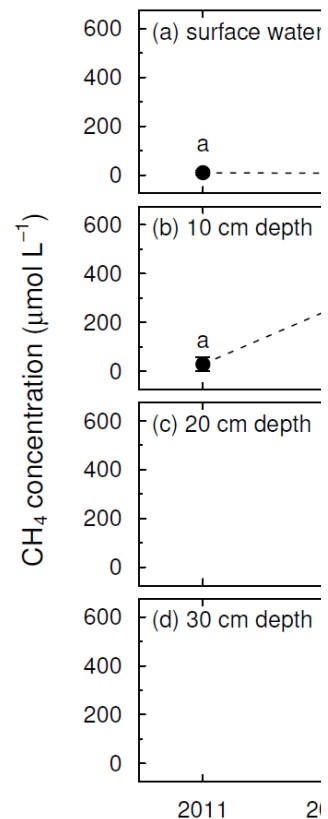
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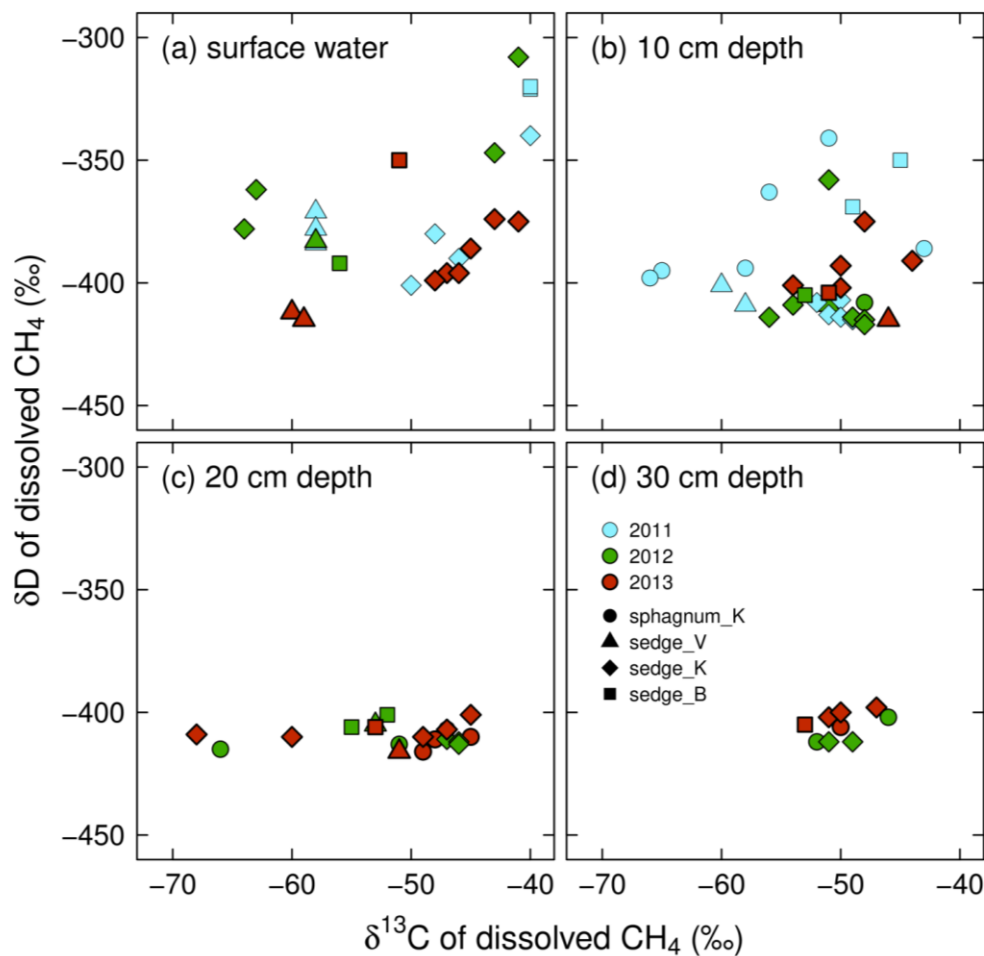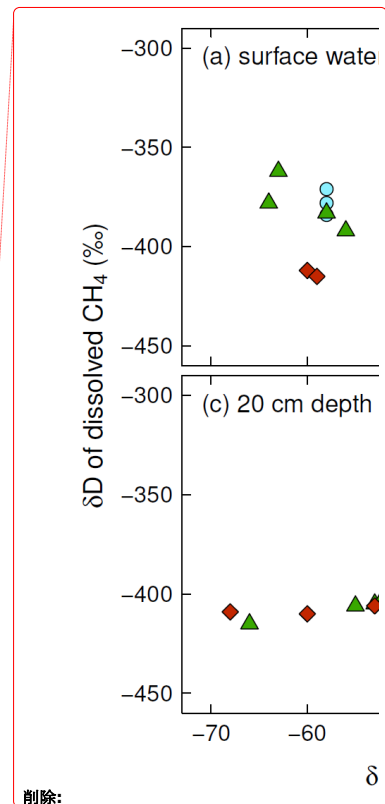削除: concentration values at respective observation points

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Figure 5: In situ $\delta^{13}C$ versus δD of dissolved CH_4 in (a) surface water and soil pore water at (b) 10 cm depth, (c) 20 cm depth, and (d) 30 cm depth from the wet event in 2011 to 2013. Individual delta values are shown here. See Table S3 for averaged delta values for each observation point and each year.
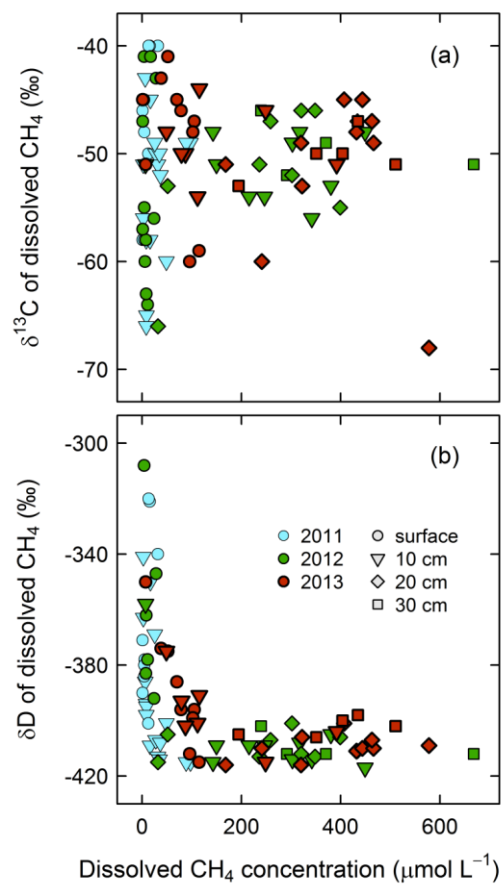
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Figure 6: In situ (a) δ^{13}C and (b) δD versus concentration of dissolved CH$_4$ at four depths (surface water, 10 cm, 20 cm, and 30 cm) in wet areas from 2011 to 2013.

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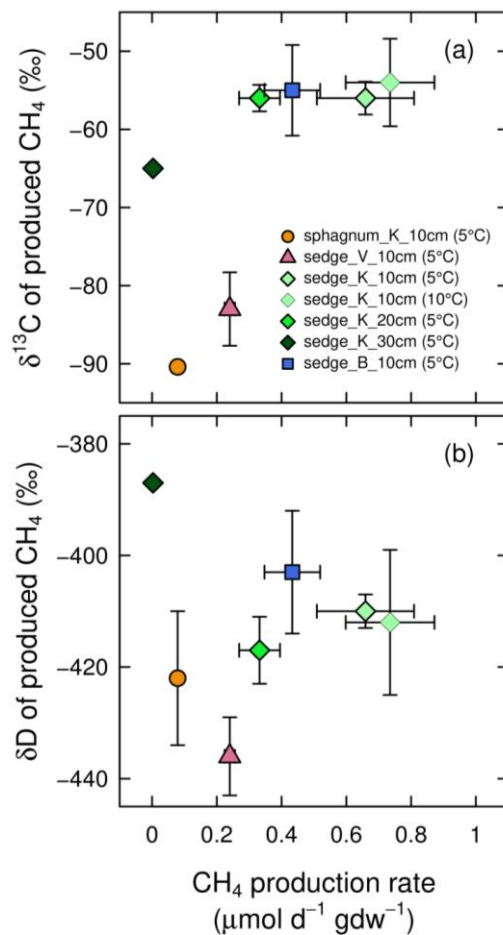
Figure 7: (a) $\delta^{13}C$ and (b) δD of produced CH_4 versus CH_4 production rate in the anaerobic soil incubation experiment. Production rates are shown in moles of produced CH_4 per day and per weight of dry soil in gram. Soil samples were collected at four observation points (sphagnum_K, sedge_V, sedge_K, and sedge_B) at three depths (10 cm, 20 cm, and 30 cm) and incubated at two temperatures (5 °C and 10 °C). These samples contain organic layers except for those collected at 30 cm. Error bars represent standard deviations.

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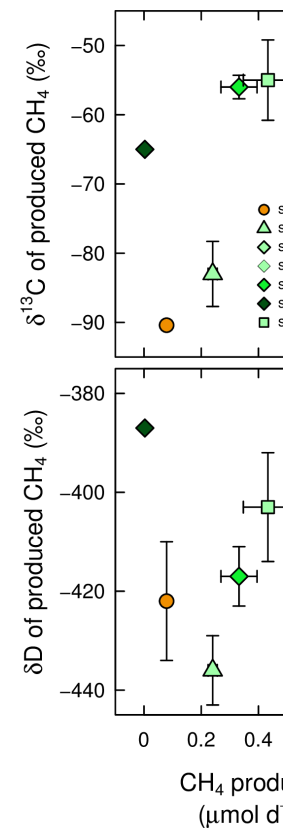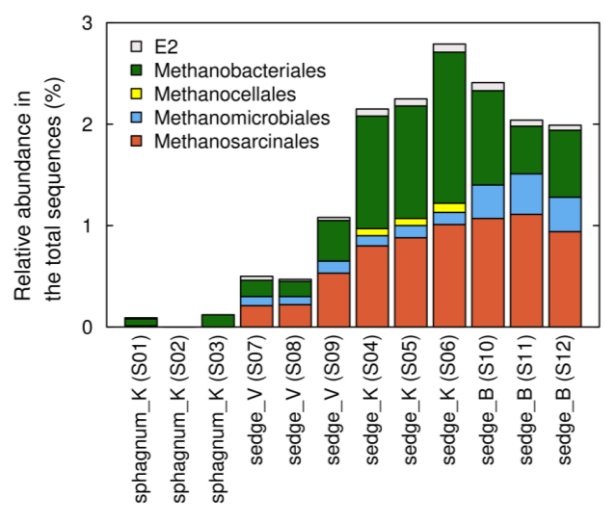
Figure 8: Phylogenetic compositions of methanogenic archaea by order in wet areas. Soil samples (organic layers) were taken in triplicate from 10 cm depth in each wet area in July 2016. See Table S5 for detailed results.

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$\delta D = 11 \times \delta^{13}C + 517$
$r^2 = 0.991$

δD of CH$_4$ (‰)

δ^{13}C of CH$_4$ (‰)
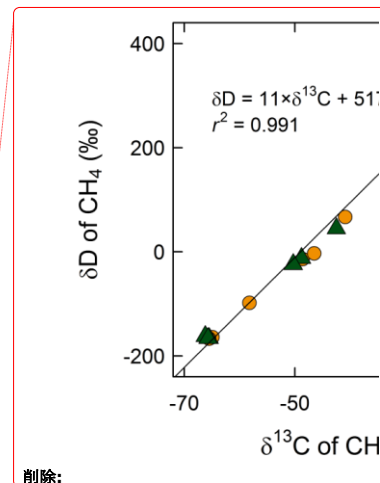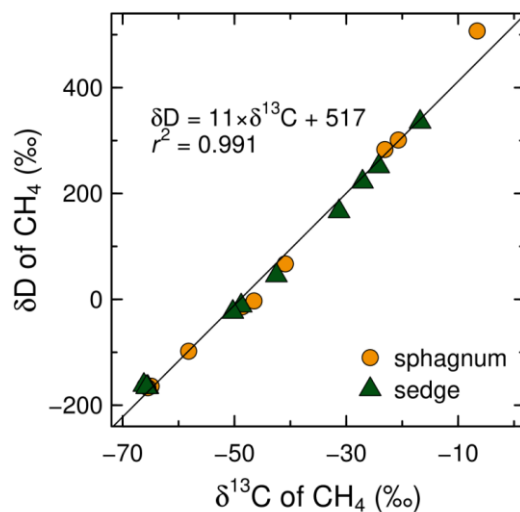
○ sphagnum
▲ sedge

Figure 9: Enrichment of D/H (CH$_4$) and ^{13}C/^{12}C (CH$_4$) through CH$_4$ oxidation during the aerobic incubation experiment of surface organic layers from wet areas of sphagnum mosses and sedges in site K. Individual delta values of the headspace CH$_4$ from each incubated syringe and each day are shown. Initial isotopic compositions of the headspace CH$_4$ were −66‰ to −65‰ for δ^{13}C and −167‰ to −162‰ for δD.
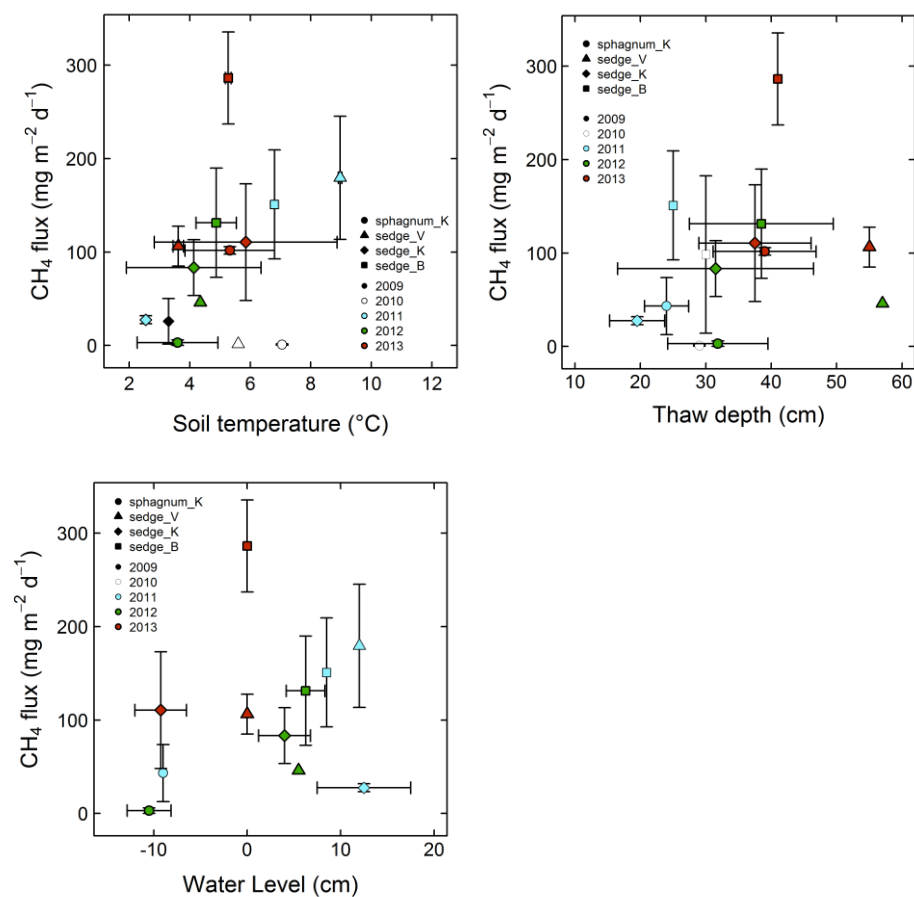
Figure S1. Averaged CH$_4$ flux over each wet area and each year plotted against (a) soil temperature (10 cm depth), (b) thaw depth, and (c) water level. Error bars represent standard deviations. Most observations for soil temperature and thaw depth were conducted at the same time of flux measurements, but not for all (Table S1).
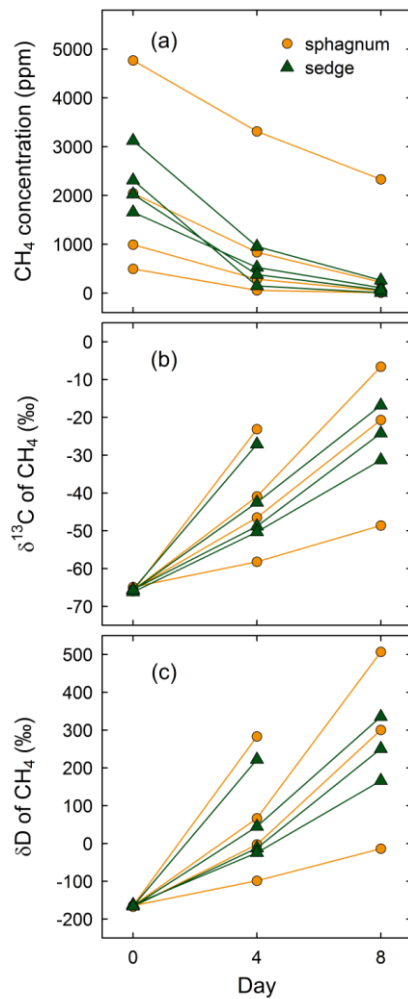
Figure S2. Temporal changes in (a) concentration, (b) $\delta^{13}C$, and (c) δD of the remaining headspace CH_4 in the soil incubation experiment for CH_4 production. Surface organic layers (0-13 cm) from wet areas (sphagnum_K and sedge_K) were incubated in quadruplicate at 8 °C.
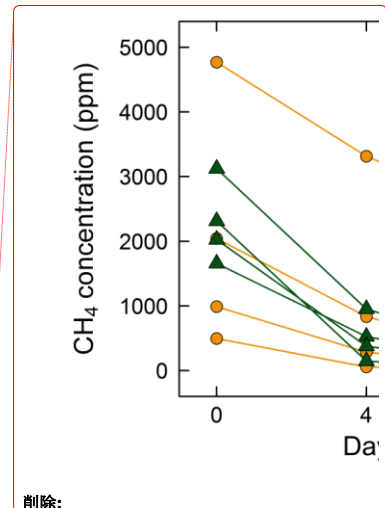
Table S1. Thaw depths in tree mound and wet area observed along with CH$_4$ flux from 2010 to 2013. Averaged values and ranges are shown for each vegetation type and each year. Standard deviations are represented when $n \geq 3$. See Table S2 for each observation date.

Year	Thaw depth (cm)			
	Tree mound		Wet area	
	mean	range	mean	range
2010	23 ($n = 2$)	21–25 (Jul 20–21)	30 ($n = 2$)	29–30 (Jul 20–21)
2011	23 ± 6 ($n = 6$)	14–30 (Jul 9–30)	22 ± 4 ($n = 9$)	15–28 (Jul 9–21)
2012	21 ± 4 ($n = 11$)	16–27 (Jul 3 – Aug 9)	35 ± 14 ($n = 24$)[a]	9–57 (Jul 3 – Aug 9)[a]
2013	20 ± 3 ($n = 3$)	18–23 (Jul 15 – Aug 2)	40 ± 9 ($n = 20$)[b]	23–58 (Jul 11 – Aug 2)[b]

[a] During Jul 8–20, 2012 in wet area, the mean value was 25 ± 8 cm, and the range was 9–37 cm.

[b] During Jul 11–18, 2013 in wet area, the mean value was 35 ± 7 cm, and the range was 23–46 cm.

Table S2. Averaged CH_4 flux (in mg CH_4 m^{-2} day^{-1}) over each observation point and each year (2009–2013). Standard deviations are shown in case of $n \geq 3$. Dates of the flux observation are indicated in parenthesis. Superscripts represent observed environmental variables on each day: a) soil temperature (2009–2013), b) thaw depth (2010–2013), c) water level (2011–2013), and d) volumetric water content in surface soil (2011).

Observation points	Year				
	2009	2010	2011	2012	2013
tree mound_V	–	0 (Jul 16)	−1 ± 2 (Jul 23[b], 29[abd])	0 (Aug 7[ab])	0 (Aug 2[ab])
sedge_V (wet area)	3 ± 3 (Jul 23)	2 (Jul 16[a])	179 ± 66 (Jul 23[c], 29[acd])	46 (Aug 7[abc])	106 ± 21 (Aug 2[abc])
tree mound_K	0 (Jul 22)	−1 ± 3 (Jul 21[ab])	0 (Jul 15[bd], 18[bd])	0 (Jul 3[ab], 8[a], 12[ab], 24[ab]; Aug 2[ab], 6[ab])	1 (Jul 15[ab])
sphagnum_K (wet area)	–	1 ± 1 (Jul 21[ab])	43 ± 31 (Jul 11[bd], 17[b], 18[bcd], 21[bcd])	3 ± 3 (Jul 3[abc], 8[abc], 12[abc], 24[abc]; Aug 2[abc], 6[abc])	102 ± 4 (Jul 11[ab], 18[ab], 25[ab], 31[ab])
sedge_K (wet area)	26 ± 24 (Jul 22[a])	–	28 ± 4 (Jul 11[bcd], 17[bc], 18[bcd], 21[abcd])	83 ± 30 (Jul 3[abc], 8[abc], 12[abc], 20[abc], 21[a], 24[abc]; Aug 2[abc], 6[abc])	111 ± 63 (Jul 11[abc], 18[abc], 25[abc], 31[abc])
tree mound_B	–	0 (Jul 20[b])	0 (Jul 9[bd], 30[bd])	−1 ± 1 (Jul 13[ab], Aug 9[ab])	0 (Jul 16[ab])
sedge_B (wet area)	79 ± 80 (Jul 25)	98 ± 84 (Jul 20[b])	151 ± 58 (Jul 9[bcd], 30[acd])	131 ± 58 (Jul 13[ab], Aug 9[abc])	286 ± 49 (Jul 16[abc])

Table S3. Concentration, δ¹³C, and δD of dissolved CH₄ in surface water and soil pore water averaged over each wet area and each year. Standard deviations are shown in case of $n \geq 3$.

Wet area	Depth	Concentration (μmol CH₄ L⁻¹) 2011	2012	2013	δ¹³C (‰) 2011	2012	2013	δD (‰) 2011	2012	2013
sphagnum_K	surface water	—	—	—	—	—	—	—	—	—
	10 cm	6 ± 3 (n = 6)	318 (n = 1)	—	−56 ± 9 (n = 6)	−48 (n = 1)	—	−380 ± 23 (n = 6)	−408 (n = 1)	—
	20 cm	—	176 ± 125 (n = 3)	399 ± 68 (n = 3)	—	−55 ± 10 (n = 3)	−47 ± 2 (n = 3)	—	−412 ± 4 (n = 3)	−412 ± 3 (n = 3)
	30 cm	—	266 (n = 2)	351 (n = 1)	—	−49 (n = 2)	−50 (n = 1)	—	−407 (n = 2)	−406 (n = 1)
sedge_V	surface water	3 ± 2 (n = 3)	6 (n = 2)	105 (n = 2)	−58.1 ± 0.2 (n = 3)	−56 (n = 2)	−60 (n = 2)	−378 ± 7 (n = 3)	−383 (n = 1)	−414 (n = 2)
	10 cm	30 ± 17 (n = 3)	150 (n = 1)	249 (n = 1)	−59 (n = 2)	−51 (n = 1)	−46 (n = 1)	−405 (n = 2)	−409 (n = 1)	−415 (n = 1)
	20 cm	—	52 (n = 1)	168 (n = 1)	—	−53 (n = 1)	−51 (n = 1)	—	−405 (n = 1)	−416 (n = 1)
	30 cm	—	—	—	—	—	—	—	—	—
sedge_K	surface water	12 ± 14 (n = 4)	8 ± 8 (n = 11)	74 ± 27 (n = 6)	−46 ± 4 (n = 4)	−51 ± 9 (n = 10)	−45 ± 3 (n = 6)	−378 ± 27 (n = 4)	−349 ± 30 (n = 4)	−388 ± 11 (n = 6)
	10 cm	53 ± 31 (n = 6)	244 ± 143 (n = 7)	88 ± 27 (n = 5)	−50 ± 1 (n = 6)	−52 ± 3 (n = 7)	−49 ± 4 (n = 5)	−412 ± 4 (n = 6)	−405 ± 21 (n = 7)	−392 ± 11 (n = 5)
	20 cm	—	368 ± 59 (n = 3)	431 ± 123 (n = 5)	—	−46 ± 1 (n = 3)	−54 ± 10 (n = 5)	—	−412 ± 1 (n = 3)	−407 ± 4 (n = 5)
	30 cm	—	519 (n = 2)	450 ± 55 (n = 3)	—	−50 (n = 2)	−49 ± 2 (n = 3)	—	−412 (n = 2)	−400 ± 2 (n = 3)
sedge_B	surface water	14 (n = 2)	8 ± 14 (n = 3)	4 (n = 2)	−40 (n = 2)	−56 (n = 1)	−48 (n = 2)	−320 (n = 2)	−392 (n = 1)	−350 (n = 1)
	10 cm	21 (n = 2)	380 (n = 1)	392 (n = 1)	−47 (n = 2)	−53 (n = 1)	−51 (n = 1)	−360 (n = 2)	−405 (n = 1)	−404 (n = 1)
	20 cm	—	351 (n = 2)	322 (n = 1)	—	−54 (n = 2)	−53 (n = 1)	—	−404 (n = 2)	−406 (n = 1)
	30 cm	—	—	194 (n = 1)	—	—	−53 (n = 1)	—	—	−405 (n = 1)

Table S4. Individual values of water level, dissolved CH_4 concentration (10 cm depth), and $\delta^{13}C$ and δD of dissolved CH_4 (10 cm depth) observed in each wet area on each date in 2011. Duplicated data are shown in some cases. Although water level increased during July 2011, clear temporal change was not found in the delta values.

Observation point	Date in 2011	Water level (cm)	Dissolved CH_4 concentration (μmol L^{-1})	$\delta^{13}C$ of dissolved CH_4 (‰)	δD of dissolved CH_4 (‰)
sphagnum_K	Jul 17	— (< 0)	6, 8	−43, −58	−386, −394
	Jul 18	−10	2, 2	−56, −51	−363, −341
	Jul 21	−8	8, 9	−66, −65	−398, −395
sedge_V	Jul 23	10	16	−58	−409
	Jul 29	14	49, 26	−60	−401
sedge_K	Jul 11	5	−	−	−
	Jul 17	15	27, 37	−50, −52	−407, −408
	Jul 18	15	32, 35	−51, −50	−413, −414
	Jul 21	15	88, 97	−49, −49	−415, −415
sedge_B	Jul 9	6	−	−	−
	Jul 30	11	17, 26	−45, −49	−350, −369

Table S5. Phylogenic composition of methanogenic archaea in wet areas. Soils (organic layers) were sampled in July 2016 from 10 cm depth in the same wet areas as the CH$_4$ production incubation experiment in triplicate. Then, microbial communities in the samples were analyzed by amplicon sequencing of 16S rRNA gene. See Fig. 8 for a plot by the level of orders.

Order	Family	Genus	Relative abundance in the total sequences (%)											
			sphagnum_K (S01)	sphagnum_K (S02)	sphagnum_K (S03)	sedge_V (S07)	sedge_V (S08)	sedge_V (S09)	sedge_K (S04)	sedge_K (S05)	sedge_K (S06)	sedge_B (S10)	sedge_B (S11)	sedge_B (S12)
Methanosarcinales	Methanosarcinaceae	*Methanosarcina*	0.01	0.00	0.00	0.21	0.09	0.47	0.06	0.08	0.08	0.25	0.20	0.16
	Methanosaetaceae	*Methanosaeta*	0.00	0.00	0.00	0.00	0.13	0.06	0.74	0.80	0.93	0.82	0.91	0.78
Methanomicrobiales	Methanoregulaceae	*Candidatus* Methanoregula	0.00	0.00	0.00	0.09	0.07	0.10	0.10	0.10	0.11	0.23	0.32	0.28
	Methanospirillaceae	*Methanospirillum*	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.01
	Methanoregulaceae	Other Methanoregulaceae	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.02	0.01	0.08	0.08	0.05
Methanocellales		Methanocellales	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.07	0.09	0.00	0.00	0.00
Methanobacteriales	Methanobacteriaceae	*Methanobacterium*	0.07	0.00	0.12	0.16	0.15	0.40	1.11	1.11	1.49	0.93	0.47	0.66
E2	[Methanomassiliicoccaceae]	[Methanomassiliicoccaceae]	0.01	0.00	0.00	0.04	0.02	0.03	0.07	0.07	0.08	0.08	0.06	0.05

Table S6. Redox potential observed in summers 2012 and 2013 by ORP meter (RM-20P or RM-30P, DKK-TOA Corporation, Japan) connected with an ORP electrode (PST-2739C). Measurement accuracy of the ORP meter is ±10 mV. Redox potential value was accepted when the potential stabilized after installing the ORP electrode into the soil.

Observation points	Depth (cm)	Redox potential (mV versus the normal hydrogen electrode)	
		2012	2013
tree mound_K	10	608 to 643	478 to 482
	20	627 to 631	—
sphagnum_K (wet area)	10	−183 to 814	547 to 617
	20	−129	—
sedge_K (wet area)	10	−177 to −121	−114 to −69
	20	−250 to −78	−223 to −194
	30	−152 to −118	—
sedge_B (wet area)	10	—	−113 to −102