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.

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 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.

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.

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 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 (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).'

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 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.

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).

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 (*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 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).

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 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 min after chamber closure' in most cases. 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.'

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.

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 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).

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.

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 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 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 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.

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 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 $\delta^{13}C$ 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 $\delta^{13}C$ or high δD values apart from the convergence values ($\delta^{13}C \cong -50‰$, $\delta D \cong -408‰$) 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 $\delta^{13}C$ 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.

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.

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.

Results

See the Major point 3.

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

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

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."

P7, L13 Take out "Interestingly"

Reply: We have taken out "Interestingly."

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).

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 analyzing the background samples, we obtained 2.0–4.3 ppm for CH_4 concentration, −53‰ to −45‰ for $\delta^{13}C$, 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.

P7, L26 similarly "to what?"

Reply: We intended to mention that the range of $\delta^{13}C$ of dissolved CH_4 was similar among surface water, 10 cm depth, and 20 cm depth. We have taken out "similarly" from the sentence.

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.

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

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).

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}) 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.

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".

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).

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

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."

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.

Reply: We have revised the figure accordingly.

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

Reply: The $\delta^{13}C$ and δD data are individual values from each incubation syringe and each day. Nevertheless, all 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 $\delta^{13}C$ = −6.6‰ and δD = +507‰ in our previous manuscript).

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."

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.

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

Ryo Shingubara[1,a], Atsuko Sugimoto[2,3,4], Jun Murase[5], Go Iwahana[2,6], Shunsuke Tei[2,3], Maochang Liang[1,b], Shinya Takano[1], Tomoki Morozumi[1], Trofim C. Maximov[7,8]

[1]Graduate School of Environmental Science, Hokkaido University, Sapporo, 060-0810, Japan
[2]Arctic Research Center, Hokkaido University, Sapporo, 001-0021, Japan.
[3]Faculty of Environmental Earth Science, Hokkaido University, Sapporo, 060-0810, Japan.
[4]Global Station for Arctic Research, Global Institution for Collaborative Research and Education, Hokkaido University, Sapporo, 060-0808, Japan.
[5]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan.
[6]International Arctic Research Center, University of Alaska Fairbanks, Fairbanks, 99775-7340, USA.
[7]Institute for Biological Problems of Cryolithozone, Siberian Branch, Russian Academy of Sciences, Yakutsk, 677890, Russia.
[8]Institute of Natural Sciences, North-Eastern Federal University, Yakutsk, 677000, Russia.
[a]Currently at: Graduate School of Environmental Studies, Nagoya University, Nagoya, 464-8601, Japan.
[b]Currently at: College of Horticulture and Gardening, Yangtze University, Jingzhou, 434023, China.

Correspondence to: Atsuko Sugimoto (atsukos@ees.hokudai.ac.jp)

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. Thaw depth became deeper from 2011 to 2013, which may partly explain the increase in CH_4 emission. Moreover, dissolved CH_4 concentration rose sharply 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 less significance of CH_4 oxidation relative to the larger pool of dissolved CH_4. 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 emission) 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).

削除: .
削除: and c
削除: in
削除: s
削除: as
削除: ssion
削除: flux
削除: flux
削除: large
書式変更: 蛍光ペン
コメント [shingu1]: Inserted in the Correction iteration
コメント [shingu2]: Inserted in the Correction iteration
削除: Concurrently
削除: less
コメント [shingu3]: Inserted in the Correction iteration
コメント [shingu4]: Inserted in the Correction iteration
削除: depression
削除: after
削除: ,
削除: which
削除: s
削除: greatest
削除: attributed observed high growth rates in atmospheric CH_4 concentration in 2007–2008 to high CH_4 emissions from natural wetlands that resulted from anomalous high temperatures in the Arctic and greater than average precipitation in the tropics
削除: since
削除: to
削除: s
削除: emissions
削除: as an end product of organic matter decomposition
削除: of soil
削除: with O_2

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 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 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 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 $\delta^{13}C$ 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).

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; 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.

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, $\delta^{13}C$, 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 CH_4 flux and other underlying processes to this unusual wetting event.

削除: reductive
削除: a
削除: in
削除: ,
削除: whereas
削除: in
削除: in
削除: has
削除: of
削除: in
削除: s
削除: high and dry microsites
削除: of
削除: sites
削除: microsite
削除: constantly
削除: microsite
削除: constantly
削除: microsite
削除: microsite
削除: and
削除: in
削除: to understand relationships between CH_4 flux and environmental factors
削除: prominent
削除: those
削除: the
削除: .

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

削除: and
削除: observations
削除: s
削除: study
削除: occur from
削除: to
削除: is
削除: to
削除: from
削除: up
削除: patchily
削除: Larch trees are found with shrubs (willows or dwarf birches) in dry areas with relatively high levels of micro-relief, where green mosses cover the surface (hereafter termed tree mounds).
削除: ground with relatively low levels of micro-relief form wetlands covered by sphagnum mosses and sedges
削除: In this study,
削除: observation points
削除: predominantly
削除: sphagnums
削除: are
削除: with site code K
削除: those
削除: with
書式変更: フォント：斜体
削除: are
削除: with one of the site codes V, K, or B (Table 1)
削除: Snapshot m
削除: as
削除: .
削除: by
削除: in
削除: s
削除: principally
書式変更: フォント：(英) +見出しのフォント (Times New Roman)
削除: at 0 min, 15 min, and 30 min

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 $\delta^{13}C$ 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 $\delta^{13}C$ 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

削除: Table S1
削除: at
削除: to
書式変更: フォント : 10 pt
削除: is
削除: later
書式変更: フォント : 10 pt
削除: S
削除: and background air samples
削除: ere
削除: and 100–300 mL glass bottle with O-ring stopcocks, respectively
削除: by
削除: W
削除:
削除: since 2011
削除: with a scale,
削除: and
削除: in
削除: .
書式変更: フォント : (日) MS 明朝, (言語
削除: .
削除: I
書式変更: フォント : (日) MS 明朝
削除: I
削除: in
削除: from
削除: point
削除: About
削除: s
削除: about
削除: the syringes

sampled twice at the start and the end of incubation, and dissolved CH_4 was extracted using the headspace 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

削除: by

削除: at

削除: to

削除: DNA was extracted as described in the caption of Figure S3.

書式変更: フォント：(英) +見出しのフォント (Times New Roman)

削除: S

削除: each

削除: (about 40 mL)

削除: that

削除: the

削除: on day 0, day 4 and day 8 into

削除: .

削除: .

削除: on

削除: in

削除: increment

削除: in situ

削除: in

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 $\delta^{13}C$ and δD, respectively. When calculating $\delta^{13}C$ and δD of dissolved CH_4, the 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:

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

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

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, 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 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 between Jul 3 and Aug 9).

書式変更: フォント：(英) +見出しのフォント (Times New Roman)
削除: from
削除: .
削除: s
書式変更: フォント：(英) +見出しのフォント (Times New Roman)
削除: available
削除: .
削除: from early July to early August
書式変更: フォント：(英) +見出しのフォント (Times New Roman)
削除: 78

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 $\delta^{13}C$ and δD of in situ dissolved CH_4

Variability of both $\delta^{13}C$ and δD of dissolved CH_4 was smaller in deeper layers, showing different patterns between $\delta^{13}C$ and δD, and across years (Fig. 5). The $\delta^{13}C$ 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, $\delta^{13}C$ 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 $\delta^{13}C$ and δD of dissolved

削除: During the observation period (2009–2013), monthly mean air temperature in July showed large interannual variation compared to other months, ranging from 8.5 to 15.5 °C (Fig. 2). Monthly mean air temperature was highest in July 2010, while the monthly precipitation was low (8 mm), showing dry weather conditions in this period.
削除: er
削除: causing
削除: this
削除: to
削除: ing
削除: .
削除: showed
削除: Table S1
削除: was
削除: combination
削除: As
削除: flux
削除: including
削除: the
削除: active
書式変更: 蛍光ペン
削除: Interestingly,
書式変更: 蛍光ペン
削除: became even higher
削除: from 2011 and 2012 to 2013
削除: and
削除: rise
削除: by
削除: while
削除: in the concentration
削除: .
削除: in situ
削除: ,
削除: to
削除: about
削除: along
削除: its

CH_4 from 2011 to 2013, both $\delta^{13}C$ 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 $\delta^{13}C$ and δD of dissolved CH_4 is associated with dissolved CH_4 concentrations. The $\delta^{13}C$ 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 ($\delta^{13}C = -50 \pm 5‰$ and $\delta D = -408 \pm 5‰$ 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 $\delta^{13}C$ and δD values of produced CH_4 were less variable irrespective of sampling location, sampling depth, or incubation temperature. The $\delta^{13}C$ 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 $\delta^{13}C$ 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 ($\delta^{13}C = -50 \pm 2‰$ at 30 cm depth and $\delta D = -408 \pm 5‰$ at 20–30 cm depth; Fig. 5c and d), although $\delta^{13}C$ 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 $\delta^{13}C$ values of produced CH_4 in the incubation were higher. In contrast, Methanosarcinales were fewer in proportion in sphagnum_K, where $\delta^{13}C$ 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 $\delta^{13}C$ of the remaining CH_4 increased with a linear relationship between them (Fig. 9, S2). Observed slope $\Delta(\delta D)/\Delta(\delta^{13}C)$ 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.

削除: was
書式変更: インデント：最初の行： 12.7 mm
削除: those
削除: at
削除:
削除: at high CH_4 concentrations ($\delta^{13}C = -50 \pm 5‰$ and $\delta D = -408 \pm 5‰$ at > 200 μmol CH_4 L^{-1})
削除: .
書式変更: フォント：（日）ＭＳ 明朝
削除: points
削除: sphagnum
削除: K
削除: sedge_
削除: V
削除: point
削除: the
削除: deep
書式変更: フォント：斜体
削除: two
書式変更: フォント：（英）+見出しのフォント（Times New Roman）
書式変更: フォント：（英）+見出しのフォント（Times New Roman）
書式変更: フォント：斜体
書式変更: フォント：斜体
削除: point
削除: in situ
書式変更: インデント：最初の行： 12.7 mm
削除: Fig. 8
削除: for
削除: Fig. 8
削除: wet areas
削除: .

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

書式変更: フォント：（日）ＭＳ 明朝
削除: a
削除: reductive
削除: from northern wetlands
削除: ,
削除: ; Turetsky et al., 2014).
削除: neither
削除: n
削除: the
削除: .
削除: **Responses of CH_4**
削除: which was
削除: up to
削除: of
削除: flux
削除: was
削除: soil
削除: tive
削除: Soil reductive
削除: been formed
削除: due to
削除: the
削除: s
削除: Remarkably, t
削除: soil
削除: tive
削除: be
削除: Moreover
削除: soil reductive conditions
削除: created
削除: was
削除: soil reductive
削除: soil reductive conditions

summer 2011 to summer 2013. This continuous soil reduction from 2011 to 2013 could have promoted CH_4 production and/or decreased 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, $\delta^{13}C$ 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 $\delta^{13}C$ value (−50‰) and a low δD value (−408‰; Fig. 5b). Considering that δD increased much more rapidly than $\delta^{13}C$ in our oxidation experiment (Fig. 9), δD can be considered as a sensitive indicator of CH_4 oxidation. In contrast, $\delta^{13}C$ 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 $\delta^{13}C$. 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 significant in the surface soil layer during the year of the wetting event (2011). In 2012 and 2013, CH_4 oxidation became insignificant relative to the larger pool of dissolved CH_4 (Fig. 4). In the CH_4 production incubation experiment, $\delta^{13}C$ and δD of produced CH_4 were less variable at higher production rates ($\delta^{13}C$ = −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 ($\delta^{13}C$ = −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 $\delta^{13}C$ and δD values of dissolved CH_4 at 10 cm depth observed in 2012 and 2013 ($\delta^{13}C$: 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

削除: development of soil reductive conditions
コメント [shingu5]: Modified in the Correction iteration
削除: pressed
書式変更: 蛍光ペン
削除: after
書式変更: インデント : 最初の行 : 12.7 mm
削除: reduced
削除: were
書式変更: 蛍光ペン
削除: Fig. 8
書式変更: フォント : (英) +見出しのフォント (Times New Roman)
削除: and
書式変更: 下付き
削除: close to
削除: atmospheric O_2
書式変更: 下付き
書式変更: フォント : (英) +見出しのフォント (Times New Roman)
書式変更: 上付き
削除: which were gathered
削除: still
削除: active
削除: and was subsequentl
コメント [shingu6]: Modified in the Correction iteration
削除: y depressed
削除: in 2012 and 2013
削除: in situ
削除: towards
削除: compared
削除: a
削除: in
削除: after

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 less significant 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).

書式変更: フォント：（英）+見出しのフォント（Times New Roman）

書式変更: フォント：（英）+見出しのフォント（Times New Roman）

コメント [shingu7]: Modified in the Correction iteration

書式変更: 下付き

書式変更: 下付き

削除: .

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

削除: e

削除: in

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 less significance 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.

削除: ly

削除: anoxia

削除: .
Microbial community analysis by amplicon sequence of 16S rRNA gene was applied to soil samples at 10 cm depth at the same locations as the CH_4 production incubation experiment (Fig. S3, Table S3). Soils with high rates of CH_4 production and high $\delta^{13}C$ of CH_4 produced in incubation (sedge_K and sedge_B as in Fig. 7) had higher proportion of acetoclastic methanogens in the order Methanosarcinales than those with low CH_4 production rates and low $\delta^{13}C$ of produced CH_4 (sphagnum_K and sedge_V). This supports the interpretation that the ratio of acetoclastic to hydrogenotrophic methanogenesis controlled the $\delta^{13}C$ of produced CH_4 in incubation. .

削除: promotion

コメント [shingu8]: Modified in the Correction iteration

削除: depression

削除: in comparison

削除: promotion

削除: reducti

削除: on

書式変更: 下付き

削除: Additionally,

削除: ing

削除:

削除: would allow discussion of the response of CH_4 transport to wettings

削除: .

削除: A w

削除: microsites

削除: (sphagnum_K and sedge_V)

削除: in situ

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgments

This research was supported by JSPS Grant-in-Aid for Scientific Research (KAKENHI) numbers 21403011 and 16H05618, a Grant-in-Aid from the Global COE Program 'Establishment of Center for Integrated Field Environmental Science' (IFES-GCOE) funded by the Ministry of Education, Culture, Sports, Science and Technology-Japan (MEXT), JST (Strategic International Collaborative Research Program: SICORP)-EU cooperative research project 'Dynamics of permafrost and methane emission in Arctic terrestrial ecosystem in Eastern Siberia', the Green Network of Excellence (GRENE) Program funded by MEXT, and the COPERA (C budget of ecosystems and cities and villages on permafrost in eastern Russian Arctic) project funded by the Belmont Forum through JST. We sincerely thank A. Kononov, R. Petrov, E. Starostin, A. Alexeeva and other members of the Institute for Biological Problems of Cryolithozone SB RAS, and T. Stryukova, S. Ianygin and other staff at the Allikhovsky Ulus Inspectorate of Nature Protection for supporting our fieldwork in the vicinity of Chokurdakh. We also wish to acknowledge help from Y. Hoshino, S. Nunohashi, K. Tanaka, K. Saito, H. Kudo, and R. Shakhmatov in our research group at Hokkaido University.

削除: (SICORP).

References

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. and Knight, R.: QIIME allows analysis of high-throughput community sequencing data, Nature Methods, 7(5), 335–336, doi:10.1038/nmeth.f.303, 2010.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N. and Knight, R.: Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample, PNAS, 108(Supplement 1), 4516–4522, doi:10.1073/pnas.1000080107, 2011.

Chanton, J. P.: The effect of gas transport on the isotope signature of methane in wetlands, Organic Geochemistry, 36(5), 753–768, doi:10.1016/j.orggeochem.2004.10.007, 2005.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R. B., Piao, S. and Thornton, P.: Carbon and other biogeochemical cycles, in Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of

the Intergovernmental Panel on Climate Change, pp. 465–570, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA., 2014.

Conrad, R.: Microbial ecology of methanogens and methanotrophs, in Advances in Agronomy, vol. 96, pp. 1–63, Academic Press., 2007.

Desyatkin, A. R., Takakai, F. and Hatano, R.: Flood effect on CH_4 emission from the alas in Central Yakutia, East Siberia, Soil Science and Plant Nutrition, 60(2), 242–253, doi:10.1080/00380768.2014.883486, 2014.

Dlugokencky, E. J., Bruhwiler, L., White, J. W. C., Emmons, L. K., Novelli, P. C., Montzka, S. A., Masarie, K. A., Lang, P. M., Crotwell, A. M., Miller, J. B. and Gatti, L. V.: Observational constraints on recent increases in the atmospheric CH_4 burden, Geophysical Research Letters, 36(18), L18803, doi:10.1029/2009GL039780, 2009.

Dutaur, L. and Verchot, L. V.: A global inventory of the soil CH_4 sink, Global Biogeochemical Cycles, 21(4), GB4013, doi:10.1029/2006GB002734, 2007.

Flessa, H., Rodionov, A., Guggenberger, G., Fuchs, H., Magdon, P., Shibistova, O., Zrazhevskaya, G., Mikheyeva, N., Kasansky, O. A. and Blodau, C.: Landscape controls of CH_4 fluxes in a catchment of the forest tundra ecotone in northern Siberia, Global Change Biology, 14(9), 2040–2056, doi:10.1111/j.1365-2486.2008.01633.x, 2008.

van Huissteden, J., Maximov, T. C. and Dolman, A. J.: High methane flux from an arctic floodplain (Indigirka lowlands, eastern Siberia): METHANE FLUX ARCTIC FLOODPLAIN SIBERIA, Journal of Geophysical Research: Biogeosciences, 110(G2), G02002, doi:10.1029/2005JG000010, 2005.

Iijima, Y., Nakamura, T., Park, H., Tachibana, Y. and Fedorov, A. N.: Enhancement of Arctic storm activity in relation to permafrost degradation in eastern Siberia, International Journal of Climatology, 36(13), 4265–4275, doi:10.1002/joc.4629, 2016.

Ikeda, S., Watanabe, K. N., Minamisawa, K. and Ytow, N.: Evaluation of Soil DNA from Arable Land in Japan Using a Modified Direct-extraction Method, Microbes Environ., 19(4), 301–309, doi:10.1264/jsme2.19.301, 2004.

Itoh, M., Shimamura, T., Ohte, N. and Takemon, Y.: Differences in hydrophyte life forms induce spatial heterogeneity of CH_4 production and its carbon isotopic signature in a temperate bog peatland: $\delta^{13}CH_4$ variations in hydrophyte species, Journal of Geophysical Research: Biogeosciences, 120(7), 1177–1195, doi:10.1002/2014JG002881, 2015.

Iwahana, G., Takano, S., Petrov, R. E., Tei, S., Shingubara, R., Maximov, T. C., Fedorov, A. N., Desyatkin, A. R., Nikolaev, A. N., Desyatkin, R. V. and Sugimoto, A.: Geocryological characteristics of the upper permafrost in a tundra-forest transition of the Indigirka River Valley, Russia, Polar Science, 8(2), 96–113, doi:10.1016/j.polar.2014.01.005, 2014.

King, G.: Responses of atmospheric methane consumption by soils to global climate change, Global Change Biology, 3(4), 351–362, doi:10.1046/j.1365-2486.1997.00090.x, 1997.

Kumagai K. and Konno Y.: Methane emissions from rice paddy fields after upland farming, Japanese Journal of Soil Science and Plant Nutrition, 69(4), 333–339, doi:10.20710/dojo.69.4_333, 1998 (in Japanese, with English abstract).

Lai, D. Y. F.: Methane dynamics in northern peatlands: A review, Pedosphere, 19(4), 409–421, doi:10.1016/S1002-0160(09)00003-4, 2009.

Liang, M., Sugimoto, A., Tei, S., Bragin, I. V., Takano, S., Morozumi, T., Shingubara, R., Maximov, T. C., Kiyashko, S. I., Velivetskaya, T. A. and Ignatiev, A. V.: Importance of soil moisture and N availability to larch growth and distribution in the Arctic taiga-tundra boundary ecosystem, northeastern Siberia, Polar Science, 8(4), 327–341, doi:10.1016/j.polar.2014.07.008, 2014.

Marik, T., Fischer, H., Conen, F. and Smith, K.: Seasonal variations in stable carbon and hydrogen isotope ratios in methane from rice fields: Seasonal variations in CH_4 isotope ratios from rice, Global Biogeochemical Cycles, 16(4), 1094, doi:10.1029/2001GB001428, 2002.

McCalley, C. K., Woodcroft, B. J., Hodgkins, S. B., Wehr, R. A., Kim, E.-H., Mondav, R., Crill, P. M., Chanton, J. P., Rich, V. I., Tyson, G. W. and Saleska, S. R.: Methane dynamics regulated by microbial community response to permafrost thaw, Nature, 514(7523), 478–481, doi:10.1038/nature13798, 2014.

Menne, M. J., Durre, I., Vose, R. S., Gleason, B. E. and Houston, T. G.: An overview of the Global Historical Climatology Network-Daily database, J. Atmos. Oceanic Technol., 29(7), 897–910, doi:10.1175/JTECH-D-11-00103.1, 2012a.

Menne, M. J., Durre, I., Korzeniewski, B., McNeal, S., Thomas, K., Yin, X., Anthony, S., Ray, R., Vose, R. S., Gleason, B. E. and Houston, T. G.: Global Historical Climatology Network - Daily (GHCN-Daily), Version 3.22, NOAA National Data Climatic Data Center, doi:10.7289/V5D21VHZ, 2012b.

van der Molen, M. K., van Huissteden, J., Parmentier, F. J. W., Petrescu, A. M. R., Dolman, A. J., Maximov, T. C., Kononov, A. V., Karsanaev, S. V. and Suzdalov, D. A.: The growing season greenhouse gas balance of a continental tundra site in the Indigirka lowlands, NE Siberia, Biogeosciences, 4(6), 985–1003, doi:10.5194/bg-4-985-2007, 2007.

Moore, T. R., De Young, A., Bubier, J. L., Humphreys, E. R., Lafleur, P. M. and Roulet, N. T.: A multi-year record of methane flux at the Mer Bleue Bog, southern Canada, Ecosystems, 14(4), 646, doi:10.1007/s10021-011-9435-9, 2011.

Morishita, T., Matsuura, Y., Kajimoto, T., Osawa, A., Zyryanova, O. A. and Prokushkin, A. S.: CH_4 and N_2O dynamics of a Larix gmelinii forest in a continuous permafrost region of central Siberia during the growing season, Polar Science, 8(2), 156–165, doi:10.1016/j.polar.2014.01.004, 2014.

Murase, J., Sugimoto, A., Shingubara, R. and Maximov, T. C.: Methane oxidation potential of Arctic wetland soil of a taiga-tundra ecotone in Northeastern Siberia, Yokohama, Japan., 2014.

Myhre, G., Shindell, D., Bréon, F.-M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.-F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Takemura, T. and Zhang, H.: Anthropogenic and natural radiative forcing, in Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, pp. 658–740, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA., 2013.

Nakano, T., Kuniyoshi, S. and Fukuda, M.: Temporal variation in methane emission from tundra wetlands in a permafrost area, northeastern Siberia, Atmospheric Environment, 34(8), 1205–1213, doi:10.1016/S1352-2310(99)00373-8, 2000.

Nassif, S. H. and Wilson, E. M.: The Influence of slope and rain intensity on runoff and infiltration, Hydrological Sciences Bulletin, 20(4), 539–553, doi:10.1080/02626667509491586, 1975.

Nisbet, E. G., Dlugokencky, E. J. and Bousquet, P.: Methane on the Rise—Again, Science, 343(6170), 493–495, doi:10.1126/science.1247828, 2014.

Olefeldt, D., Turetsky, M. R., Crill, P. M. and McGuire, A. D.: Environmental and physical controls on northern terrestrial methane emissions across permafrost zones, Global Change Biology, 19(2), 589–603, doi:10.1111/gcb.12071, 2013.

Olson, D. M., Griffis, T. J., Noormets, A., Kolka, R. and Chen, J.: Interannual, seasonal, and retrospective analysis of the methane and carbon dioxide budgets of a temperate peatland, Journal of Geophysical Research: Biogeosciences, 118(1), 226–238, doi:10.1002/jgrg.20031, 2013.

Parmentier, F. J. W., Huissteden, J. van, Molen, M. K. van der, Schaepman-Strub, G., Karsanaev, S. A., Maximov, T. C. and Dolman, A. J.: Spatial and temporal dynamics in eddy covariance observations of methane fluxes at a tundra site in northeastern Siberia, Journal of Geophysical Research: Biogeosciences, 116(G3), doi:10.1029/2010JG001637, 2011.

Preuss, I., Knoblauch, C., Gebert, J. and Pfeiffer, E.-M.: Improved quantification of microbial CH_4 oxidation efficiency in arctic wetland soils using carbon isotope fractionation, Biogeosciences, 10(4), 2539–2552, doi:https://doi.org/10.5194/bg-10-2539-2013, 2013.

Roy, R., Klüber, H. D. and Conrad, R.: Early initiation of methane production in anoxic rice soil despite the presence of oxidants, FEMS Microbiol Ecol, 24(4), 311–320, doi:10.1111/j.1574-6941.1997.tb00448.x, 1997.

Smemo, K. A. and Yavitt, J. B.: A multi-year perspective on methane cycling in a shallow peat fen in central New York State, USA, Wetlands, 26(1), 20–29, doi:10.1672/0277-5212(2006)26[20:AMPOMC]2.0.CO;2, 2006.

Street, L. E., Dean, J. F., Billett, M. F., Baxter, R., Dinsmore, K. J., Lessels, J. S., Subke, J.-A., Tetzlaff, D. and Wookey, P. A.: Redox dynamics in the active layer of an Arctic headwater catchment; examining the potential for transfer of dissolved methane from soils to stream water, Journal of Geophysical Research: Biogeosciences, 121(11), 2776–2792, doi:10.1002/2016JG003387, 2016.

Ström, L., Falk, J. M., Skov, K., Jackowicz-Korczynski, M., Mastepanov, M., Christensen, T. R., Lund, M. and Schmidt, N. M.: Controls of spatial and temporal variability in CH4 flux in a high arctic fen over three years, Biogeochemistry, 125(1), 21–35, doi:10.1007/s10533-015-0109-0, 2015.

Sugimoto, A.: GC/GC/C/IRMS system for carbo isotope measurement of low level methane concentration, Geochemical Journal, 30(3), 195–200, doi:10.2343/geochemj.30.195, 1996.

Sugimoto, A. and Wada, E.: Carbon isotopic composition of bacterial methane in a soil incubation experiment: Contributions of acetate and CO2/H2, Geochimica et Cosmochimica Acta, 57(16), 4015–4027, doi:10.1016/0016-7037(93)90350-6, 1993.

Sugimoto, A. and Wada, E.: Hydrogen isotopic composition of bacterial methane: CO_2/H_2 reduction and acetate fermentation, Geochimica et Cosmochimica Acta, 59(7), 1329–1337, doi:10.1016/0016-7037(95)00047-4, 1995.

Teh, Y. A. and Silver, W. L.: Effects of soil structure destruction on methane production and carbon partitioning between methanogenic pathways in tropical rain forest soils, Journal of Geophysical Research: Biogeosciences, 111(G1), G01003, doi:10.1029/2005JG000020, 2006.

Teh, Y. A., Silver, W. L. and Conrad, M. E.: Oxygen effects on methane production and oxidation in humid tropical forest soils, Global Change Biology, 11(8), 1283–1297, doi:10.1111/j.1365-2486.2005.00983.x, 2005.

Treat, C. C., Bubier, J. L., Varner, R. K. and Crill, P. M.: Timescale dependence of environmental and plant-mediated controls on CH_4 flux in a temperate fen, Journal of Geophysical Research, 112(G1), doi:10.1029/2006JG000210, 2007.

Treat, C. C., Natali, S. M., Ernakovich, J., Iversen, C. M., Lupascu, M., McGuire, A. D., Norby, R. J., Chowdhury, T. R., Richter, A., Šantrůčková, H., Schädel, C., Schuur, E. A. G., Sloan, V. L., Turetsky, M. R. and Waldrop, M. P.: A pan-Arctic synthesis of CH_4 and CO_2 production from anoxic soil incubations, Global Change Biology, 21(7), 2787–2803, doi:10.1111/gcb.12875, 2015.

Woo, M.-K.: Permafrost hydrology, Springer, Berlin, Heidelberg, Germany, https://doi.org/10.1007/978-3-642-23462-010.1007/978-3-642-23462-0, 2012.

Yabuki, H., Park, H., Suzuki, R. and Ohata, T.: Baseline Meteorological Data in Siberia (BMDS) Version 5.0, 5.00, Arctic Data archive System (ADS), Japan, [online] Available from: https://ads.nipr.ac.jp/dataset/A20131107-002, 2011.

Yamamoto, S., Alcauskas, J. B. and Crozler, T. E.: Solubility of methane in distilled water and seawater, Journal of Chemical and Engineering Data, 21(1), 78–80, doi:10.1021/je60068a029, 1976.

削除: Turetsky, M. R., Kotowska, A., Bubier, J., Dise, N. B., Crill, P., Hornibrook, E. R. C., Minkkinen, K., Moore, T. R., Myers‐Smith, I. H., Nykänen, H., Olefeldt, D., Rinne, J., Saarnio, S., Shurpali, N., Tuittila, E.-S., Waddington, J. M., White, J. R., Wickland, K. P. and Wilmking, M.: A synthesis of methane emissions from 71 northern, temperate, and subtropical wetlands, Global Change Biology, 20(7), 2183–2197, doi:10.1111/gcb.12580, 2014.

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)

[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.

[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: *spp.*

削除: Table S1

削除: observation dates

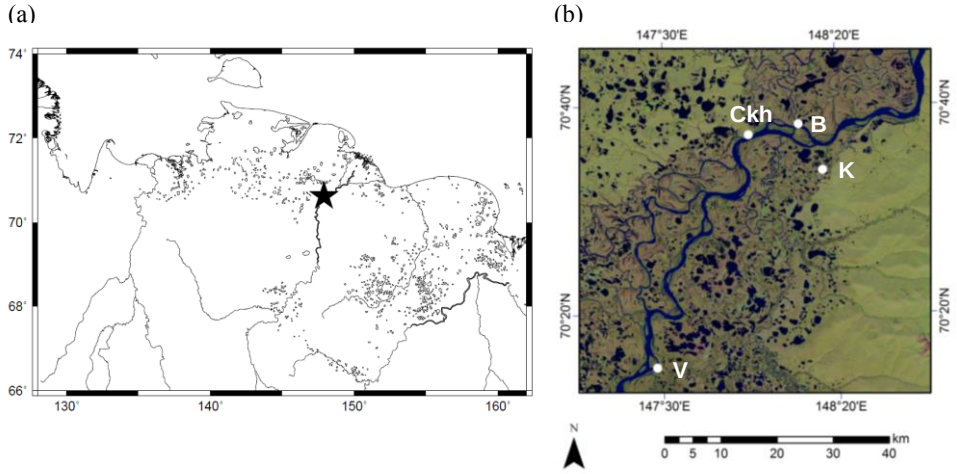
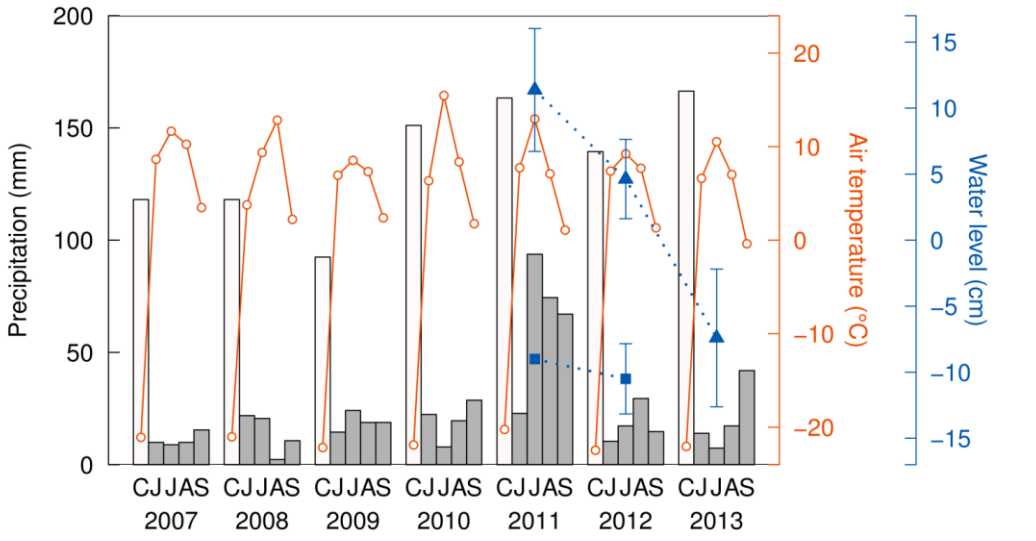


Figure 1: Locations of study sites. (a) Location of study region in northeastern Siberia (the Generic Mapping Tools 5.0.0). (b) Satellite image of Indigirka River lowland around Chokurdakh village (Ckh: 70° 37' N, 147° 55' E) from Landsat 8. Observation sites (V, K, B) were selected in this region alongside the main stem and a tributary of the Indigirka River.

削除: N



削除:

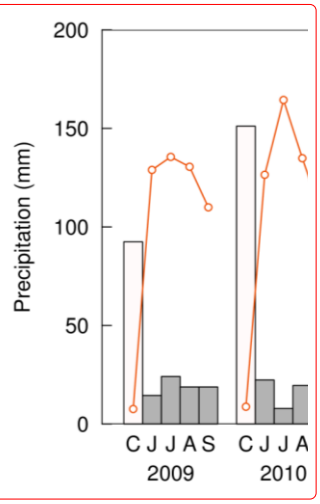


Figure 2: Interannual variations in precipitation (bars) and air temperature (solid lines) observed at a weather station at Chokurdakh for the cold season with snow cover (C: total from October in the previous year to May in the current year) and the warm season (JJAS), and water level (dotted lines) 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.

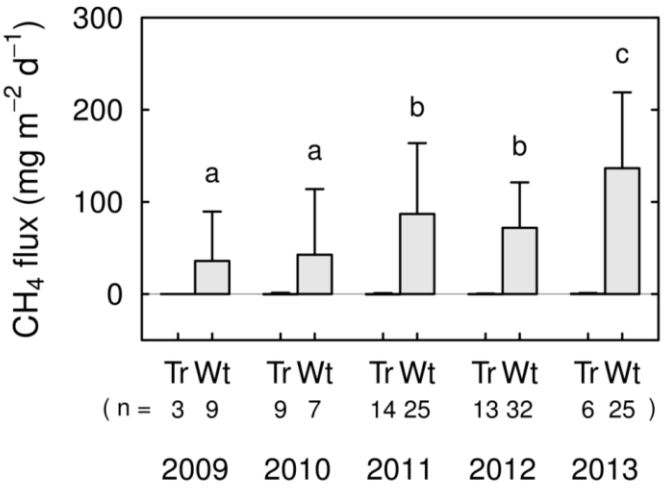
削除: those in

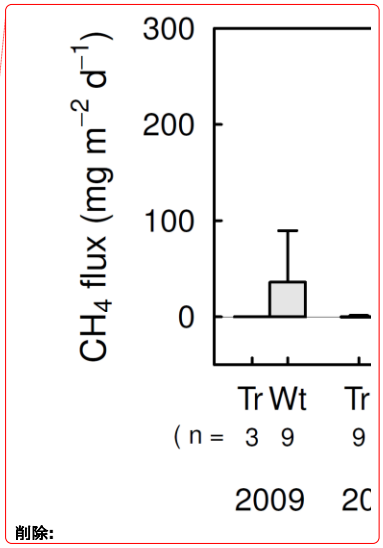
移動（挿入）[1]

書式変更: フォント：（英）+見出しのフォント（Times New Roman）

削除: Methane flux was observed from early July to early August in these years.

上へ移動 [1]: Error bars represent standard deviations.





削除:

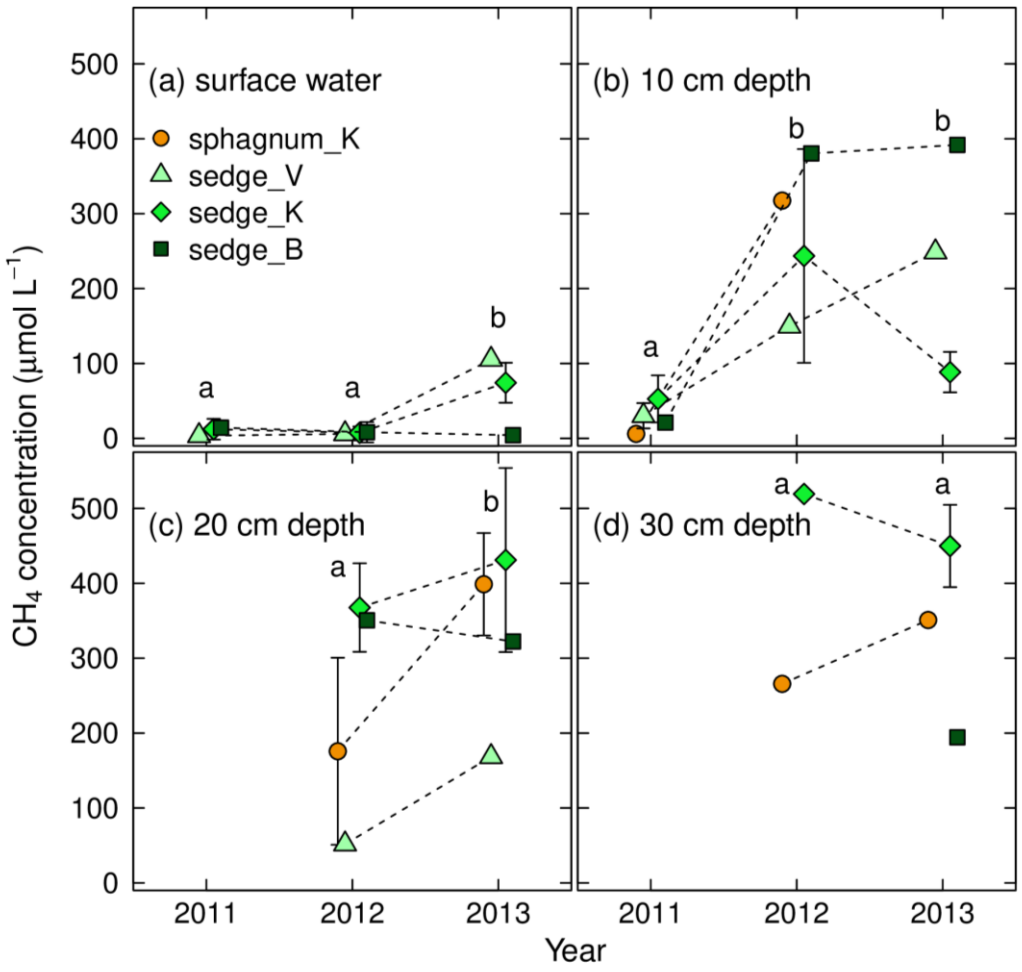
Figure 3: Interannual variations in averaged CH_4 flux in tree mounds (denoted as "Tr") and wet areas ("Wt") for main summer seasons from 2009 to 2013. Replication numbers ("n") are shown for each averaged flux value and standard deviations are represented by error bars. Different letters show statistical interannual differences in the flux values for wet areas. See Table S2 for flux values at respective observation points.

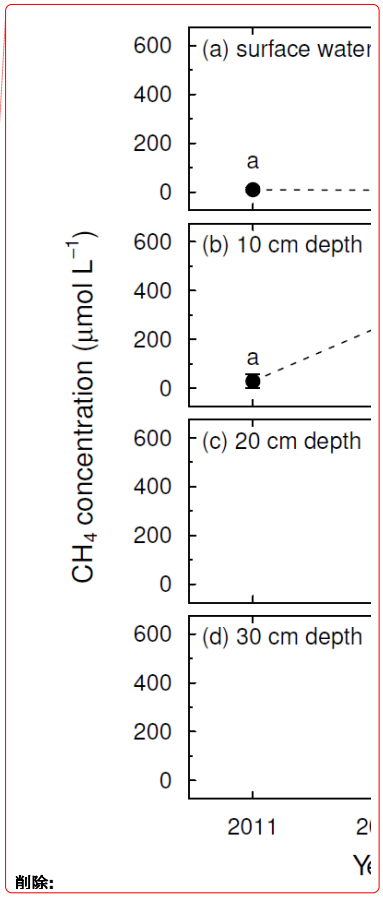
削除: s

書式変更: フォント：斜体（なし）

削除: every

削除: Table S1





削除:

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.

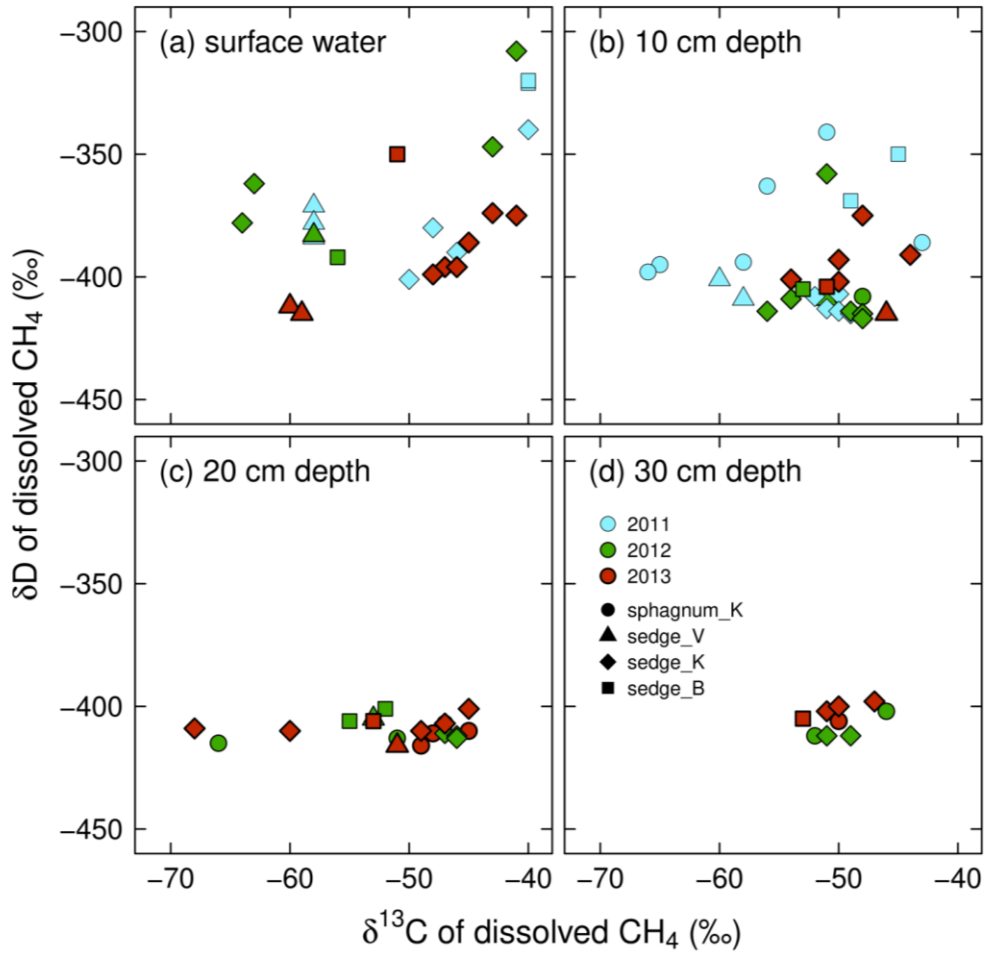
削除: averaged

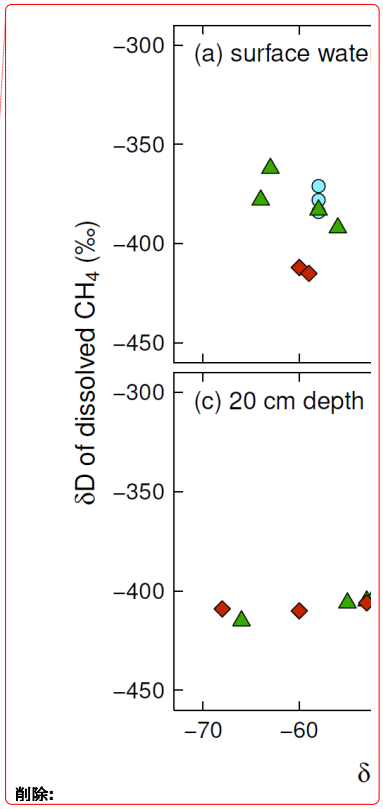
削除: the wet event in

削除:

削除: Table S2

削除: concentration values at respective observation points





削除:

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.

削除: -

書式変更: フォント：（日）ＭＳ 明朝

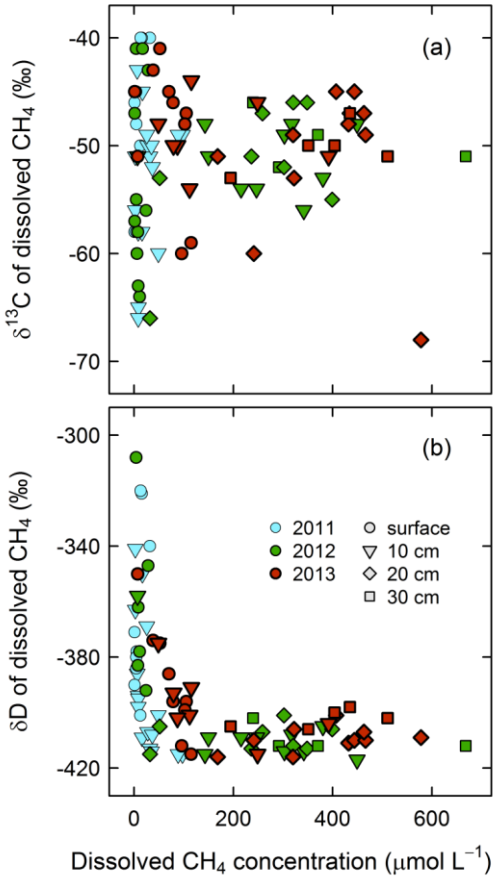
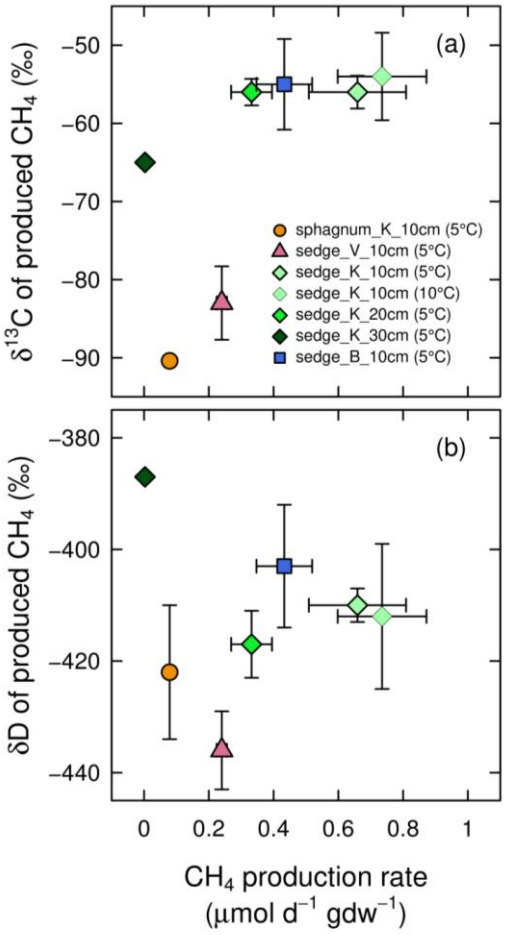
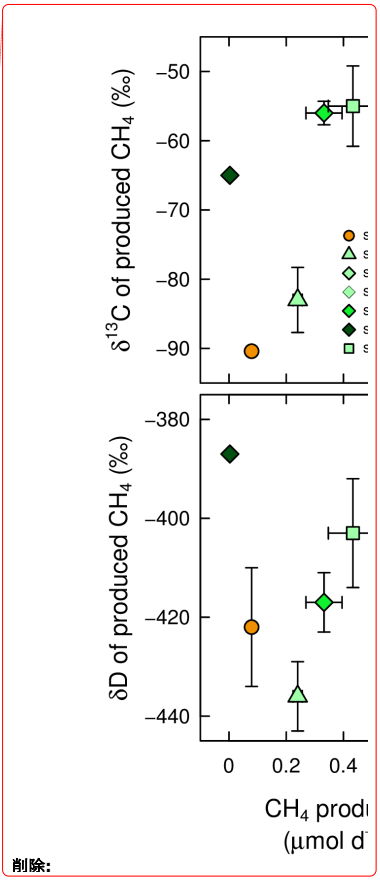


Figure 6: In situ (a) $\delta^{13}C$ and (b) δD versus concentration of dissolved CH_4 at four depths (surface water, 10 cm, 20 cm, and 30 cm) in wet areas from 2011 to 2013.

削除: -

削除: for





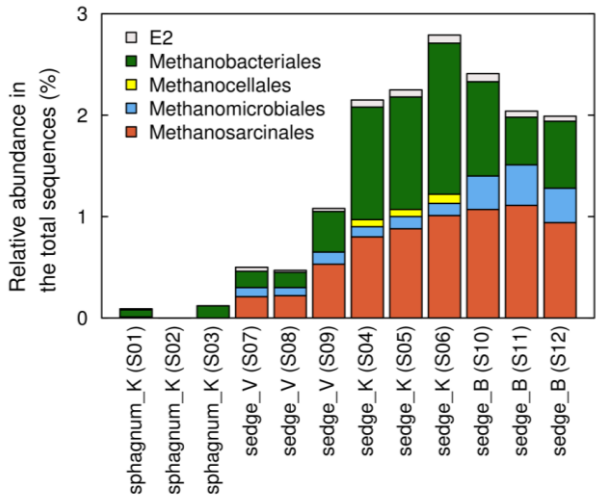
削除:

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.

削除: are

削除: that

削除: of



書式変更：中央揃え

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.

書式変更：図表番号

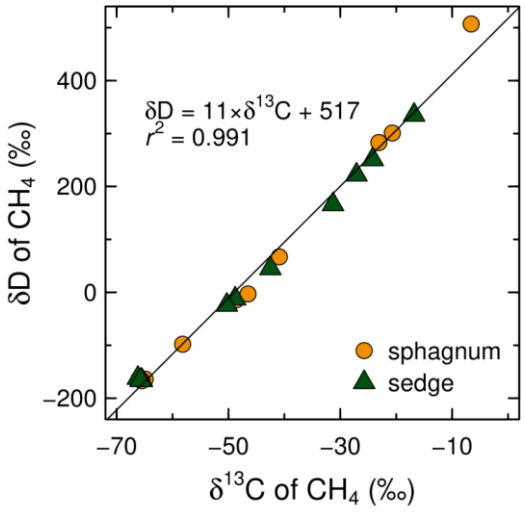
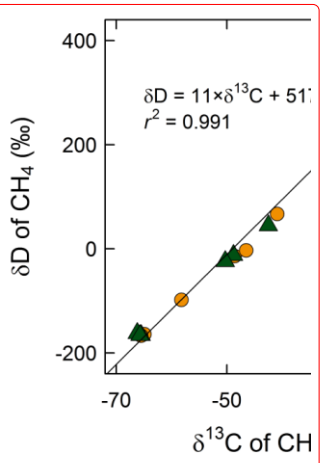


Figure 9: Enrichment of D/H (CH_4) and $^{13}C/^{12}C$ (CH_4) through CH_4 oxidation during the aerobic incubation experiment of surface organic layers from wet areas of sphagnum mosses and sedges in site K. Individual delta values of the headspace CH_4 from each incubated syringe and each day are shown. Initial isotopic compositions of the headspace CH_4 were −66‰ to −65‰ for $\delta^{13}C$, and −167‰ to −162‰ for δD.

削除:



削除: 8

削除: in

書式変更: 下付き

削除: in incubated syringes are all situated in the bottom left corner (

削除: =

削除: −66‰,

削除: = −165‰)