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Methane oxidation potential of the arctic wetland soils of a taiga-tundra ecotone in northeastern Siberia

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Abstract.

Arctic wetlands are significant sources of atmospheric methane and the observed accelerated climate changes in the arctic could cause the change in methane dynamics, where methane oxidation would be the key process to control methane emission from wetlands. In this study we determined the potential methane oxidation rate of the wetland soils of a taiga-tundra transition zone in northeastern Siberia. Peat soil samples were collected in summer from depressions covered with tussocks of sedges and *Sphagnum* spp. and from mounds vegetated with moss and larch trees. A bottle incubation experiment demonstrated that the soil samples collected from depressions in the moss- and sedge-dominated zones exhibited active methane oxidation with no time lag. The potential methane oxidation rates at 15 ºC ranged from 94 to 496 nmol h^{-1} g^{-1} dw. Methane oxidation was observed over the depths studied (0-40 cm) including the water-saturated anoxic layers. The maximum methane oxidation rate was recorded in the layer above the water-saturated layer: the surface (0-2cm) layer in the sedge-dominated zone and in the middle (4-6 cm) layer in the moss-dominated zone. The methane oxidation rate was temperature-dependent, and the threshold temperature of methane oxidation was estimated to be −4 to −11 ºC, which suggested methane oxidation at subzero temperatures. Soil samples collected from the frozen layer of *Sphagnum* peat also showed immediate methane consumption when incubated at 15 ºC. The present results suggest that the methane oxidizing bacteria in the wetland soils keep their potential activities even under anoxic and frozen conditions and immediately utilize methane when the conditions become favorable. On the other hand, the inhibitor of methane oxidation did not affect the methane flux from the sedge and moss zones *in situ*, which indicated the minor role of plant-associated methane oxidation.

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1 Introduction

Methane is a greenhouse gas produced in natural and anthropogenic anaerobic environments as the terminal product of organic decomposition. Arctic wetlands, where large amounts of organic carbon are stored (Tarnocai et al., 2009; Hugelius et al., 2014), are one of the largest sources of atmospheric methane (Kirschke et al., 2013; Intergovernmental_Panel_on_Climate_Change, 2014). Methane emission from the Arctic wetlands could be increased by the climate changes that include increasing temperatures, changing precipitation patterns, and permafrost thaw (Olefeldt et al., 2013; Schuur et al., 2015; Treat et al., 2015).

Methane emission from the wetlands to the atmosphere is the results of the balance between methane production and consumption. The potential methane oxidation rate is typically one order of magnitude higher than that of methane production (Segers, 1998). The oxic-anoxic interfaces such as the surface of the wetland soils and the rhizosphere of aerenchymatous plants are often characterized by the active methane oxidation thus playing a key role in controlling methane flux from the wetlands (Zhuang et al., 2004; Preuss et al., 2013).

The methane oxidation in arctic wetland soils has been reported repeatedly. In many cases the potential methane oxidation was determined by the incubation experiment in which the collected samples incubated under high concentrations of methane, and its controlling factors have been studied (Wagner et al., 2003, 2005; Liebner and Wagner, 2007; Knoblauch et al., 2008; Christiansen et al., 2015). The potential methane oxidation rate differs spatially and temporally under the influence of different environmental conditions. Wagner *et al.* (2003) reported that methane oxidation in polygon depression

in the Lena Delta, Siberia, was higher than in polygon rim with the increasing rate and expanding active depth with time in summer. The methane oxidation rate at the *in situ* temperature (0.4-7.5°C) ranged 1.9−7.0 nmol h^{-1} g^{-1} except for the boundary to the frozen ground where no methane oxidation was recorded (Wagner et al., 2003, 2005). Much higher potential methane oxidation rates were recorded in permafrost-affected soils of Northeast Siberia with 45-87 nmol h^{-1} g^{-1} for mineral soils and 835 nmol h^{-1} g^{-1} for organic soil at the *in situ* temperature (5 °C) (Knoblauch et al., 2008); 8–32% of the maximum oxidation rate was observed at 0 °C. Thus, water level, soil depth, and temperature are the major factors that affect the methanotrophic activity in the artic wetlands.

Aerenchymatous plants provide a niche for methane oxidizing bacteria in the rhizosphere where oxygen and methane are both available in wetlands (Frenzel, 2000). Moss has a symbiotic association with methanotrophs: methanotrophs use oxygen supplied from moss to oxidize methane and moss utilizes CO_2 produced by methanotrophs for photosynthesis (Raghoebarsing et al., 2005; Kip et al., 2010, 2011; Larmola et al., 2010; Liebner et al., 2011). Plant-associated methane oxidation in wetland soils has been studied by comparing the methane flux under the conditions with and without the specific inhibitor of methane oxidation (Frenzel and Bosse, 1996; Frenzel and Rudolph, 1998; Kruger et al., 2001). However, the role of plant-associated methane oxidation in methane dynamics has been poorly studied in the arctic wetlands (Liebner et al., 2011; Nielsen et al., 2017).

Most studies to determine the potential methane oxidation rates of the arctic wetlands have been done by *in vitro* incubations; the target samples were transferred from the study sites to the laboratory and the methane oxidation activity was measured after some time of storage, which may affect the enzymatic activities of soils depending on the type

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(Burns et al., 2013). Also, it is not clear if the measured methane oxidation represents the actual potential of the collected samples or the methanotrophic activity was induced by incubation since the temporal change in methane concentration in the system is poorly documented in the incubation experiments. In this study, we measured the potential methane oxidation of the wetland soils in the northeastern Siberia immediately (< 24 h) after sample collection to avoid possible bias caused by sample storage as much as possible. The incubation experiments were conducted to study the depth profile of the potential methane oxidation of wetland soils under different conditions and the temperature dependence of potential methane oxidation. The effect of mineral salts and black carbon—that are supposed to be transferred from the sea and forest fire (de Caritat et al., 2005)—on methane oxidation was studied to test our hypothesis that methane oxidation of the organic soils may be constrained by limited amounts of mineral nutrients including nitrogen. Also, we conducted the *in situ* measurement of plant-associated methane oxidation for the first time using the specific inhibitor of methane oxidation (difluoromethane) in the arctic wetlands.

2 Methods

2.1 Sample collection

Active layer samples were collected from the wetland soils of a taiga-tundra transition zone along the triburary of Indigirka River (N 70°33.8', E 148°15.9') in northeastern Siberia, Russia (Fig. 1A & B), during the summer (July) of 2012–2015. The study site was described before with the name of Kryvaya or site K (Iwahana et al., 2014; Liang et

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al., 2014; Morozumi et al., 2019). Three representative vegetation types were selected: two depression zones that were dominantly covered with tussocks of sedges (*Carex* spp.) or moss-wet (*Sphagnum* spp.) and a dry mound vegetated with moss and larch trees (~~Fig. 1C–E~~). Blocks of the surface soil (0–10 cm) was collected using a serrated knife. Different layers (0–2, 4–6, and 8–10 cm) of soil were subsampled in 2013. Soil samples in the deeper layer including the surface part of the frozen layer was also collected from the depression zones in 2015 using a metallic core sampler (i.d., 4 cm; length, 80 cm). Collected samples were stored at 4 °C and subjected to measurement of potential methane oxidation within a day.

2.2 Vertical profiling of dissolved oxygen in soil

The vertical profile of dissolved oxygen in peat soil of the sedge and moss wetlands was measured by inserting a D.O. meter (HI 2040-01, Hanna Instruments, RI, U.S.A.) into small wells (diameter: ca. 1.5 cm) that were made by drilling the peat with a ~~wodden~~ stick a few days prior to measurement.

2.3 Methane oxidation potential of soil samples

Samples were homogenized by cutting into pieces (< 5 mm) with scissors and mixing, and 10 g of wet subsamples were put into 50-ml or 100-ml GC vials (Nichiden-Rika Grass, Kobe, Japan). The vials were capped with butyl rubber stoppers and open top screw caps, and injected with 0.5 or 1.0 ml of 99% methane to give an initial concentration of 5,000–10,000 ppmv in the headspace. The samples were incubated in the dark at 15 °C;

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temperature dependence of methane oxidation was studied in a range of 0–15 °C. Methane concentration in the headspace was monitored using a photoacoustic field gas monitor (Innova 1412, LumaSense Technologies, Ballerup, Demmark). The methane oxidation rate was calculated from the linear regression of the methane concentration decreasing with time. The methane oxidation rate for the initial two days of incubation (ca. 50 hrs) was calculated and the threshhold temperature for methane oxidation was estimated from the linear regression between methane oxidation rates and incubation temperatures. The methane oxidation rate was expressed per dry weight of samples that was obtained by drying at 80 °C for 48 hrs. To examine the effect of nutrients and black carbon on methane oxidation, the potential atomospheric depositions, the samples (10 g) were applied with 1 ml of inorganic solution (10 μM NH_4NO_3, 250 μM NaCl, 40 μM $CaCl_2$, 20 μM $MgSO_4$, 10 μM KCl) and/or 1 ml of 100 μg l^{-1} charcoal powder of oak (*Quercus* L., <47 μm).

2.4 Estimation of plant-associated methane oxidation

Methane oxidation associated with the wetland plants was estimated using CH_2F_2, the specific inhibitor of methane oxidation, in 2014 and 2015 according to Kruger et al. (2001). Methane flux from the wetlands with different vegetation types was measured by a closed chamber method in which the surface of the wetlands was covered with a plexiglass chamber (height: 25 cm, innder diameter: 24.5 cm). Gas samples in the headspace were collected into 20-ml pre-vacuumed vials for 3 times with an interval of 15 min. After the first measurement, the gas phase in the chamber was refreshed with atmospheric air by using a pump and then injected with CH_2F_2 at the concentration of

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1 %(v/v). Then, the second measurement of methane flux was done 10–15 min (in 2014) or 18–19 hrs (in 2015) after injection of the inhibitor. Methane concentration in the collected gas samples were detemined by a FID-GC (GC-14B, Shimadzu, Kyoto, Japan) and the methane flux before and after the injection of the inhibitor was calculated from the linear regression of methane concentration with time. Methane flux mesurement without the inhibitor was conducted in parallel to monitor the temportal shift of methane flux which may affect our interpretation of the effect of the inhibitor on methane flux.

2.5 Statistical analysis

Differences in methane oxidation rate between treatments were tested with a one-way ANOVA and the effect of CH_2F_2 on methane emission was assessed by Wilcoxon's test using SPSS for Windows Ver. 22.0.

3 Results

3.1 The relationship between the vegetation types and CH_4 oxidation

The methane concentration in the headspace of the bottle with peat samples from wetlands with moss and sedge vegetations rapidly decreased with time from the intial concentration (ca. 5,000 ppmv) to an atmospheric level in 7 days (Figs. 2A & B). On the other hand, the moss sample from the mound did not show any activity of methane oxidation under the given conditions (Fig. 2C). Addition of nutrients and charcoal powder did not affect

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methane oxidation of any samples. We also tested 10 times higher concentrations of nutrients and charcoal powder but found no influence on methane oxidation of the all samples (data not shown).

3.2 Vertical profile of CH_4 oxidation

The time course incubation experiment using the surface (0-10 cm) wetland peat samples showed that methane was oxidized with ~~no induction period~~ (Figs. 3A & B). The highest activity of methane oxidation was recorded at the middle (4-6 cm) layer and the top (0-2 cm) layer of moss and sedge peat samples, respectively. Immediate methane oxidation of the moss peat sample was even observed in the frozen layer (30-40 cm) with the lower rate than the upper layers (Fig. 3C). Peat samples from the sedge up to 20 cm in depth also showed active methane oxidation, while a mineral soil collected from the top of the frozen layer did not exhibit any methane oxidation. The calculated methane oxidation rate ranged from 54 to 496 nmol h^{-1} g^{-1} (Table 1). In contrast to the high potential of methane oxidation through the active layer, the *in situ* concentration of dissolved oxygen in the pore water of the wetlands was very low and undetectable below 10 cm with one exception in the sedge wetland (Fig. 4).

3.3. Temperature dependence of methane oxidation

Methane oxidation of surface (0–10 cm) peat samples from moss and sedge wetlands showed a clear temperature dependence. A linear decrease in methane concentration was observed even at 0 °C (Figs. 4A & B) and the methane oxidation rate of the moss sample

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at 0 °C did not differ from that at 5 °C (Fig. 4C). The threshold temperature for methane oxidation was estimated to be −13 and −9 °C for the moss and sedge peat samples, respectively, when the simple linear regression model was applied.

3.4 Effect of the inhibitor on methane emission from the wetland with different vegetations

The methane emission rate for the first measurement ranged from 2.4 to 1800 μmol h^{-1} m^{-2}; the mound showed the lowest rate (Fig. 5). The methane emission rate in the second measurement ranged from 0.19 to 1840 μmol h^{-1} m^{-2} and did not differ from that of the initial measurement even when the second mesurement was conducted after the treatiment with the inhibitor.

4 Discussion

The wetland soils in the depression area of the taiga-tundra transition zone in the northeast Siberia exhibited the active methane oxidation in the incubation experiment. The potential rates estimated in this study were at the higher end of those previously reported for other Arctic regions including Siberia (Wagner et al., 2003, 2005; Liebner and Wagner, 2007; Knoblauch et al., 2008; Christiansen et al., 2015). The highest rate was recorded at the subsuface (4-6 cm) and surface (0-2 cm) of the moss and sedge dominated wetlands, respectively; these depths corresponded to the water level of the study site, where the maximum methane oxidation rate has been often reported in other wetlands (e.g., (Vecherskaya et al., 1993; Sundh et al., 1995; Whalen and Reeburgh, 2000). On the other

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hand, the mound soil in the same area showed no methane oxidation. The results show the spatial heterogeneity of the potential methane oxidation of the soils in the arctic at inter- and intra-regional scales. We tested our hypothesis that methane oxidation of organic soils may be constrained by limited amounts of mineral nutrients including nitrogen, but application of the mineral salts and charcoal that are supposed to be transferred from the sea or forest fire (de Caritat et al., 2005) did not affect the methane oxidation.

Plottinng the data in the time-course measurement showed that the wetland soils exhibited methane oxidation without time lag when incubated. The immediate methane oxidation was observed throughout the different soil depths in this study except for the mineral soil in the frozen layer of the sedge-dominated wetlands that exihibited no detectable methane oxidation. The active methane oxidation throught the active layer is in contrast with our previous study of the stable isotope signals of dissolved methane that indicated that methane oxidation is limited to the surface layer (up to 10 cm) of the soils in the same study site (Shingubara et al., 2019). This suggests that methanotrophs can survive in the deeper layer for a prolonged time under the unfavorable conditions keeping the potential activity and would be able to immediately oxidize methane when the conditions become favorable according to the change of the water level (Parmentier et al., 2011; Shingubara et al., 2019). Roslev and King (1996) reported that peat samples from the freshwater marsh maintain 30% of the initial methane oxidation capacity after 32 days of anoxic incubation and methanotrophs from anoxic peat initiated aerobic methane oxidation within 1-7 hours after oxygen addition. The subzero temperatures would not also be ~~motal~~ for methanotrophs to keep their potential activity as estimated by the temperature dependence of methanotrophic activity (Fig. 4C), which is same as the

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microbial respiration on added carbon at temperatures as low as –15 ºC in the Canadian high Arctic soil (Steven et al., 2007). The immediate methane oxidaiton upon thaw is also reported for the frozen permafrost soil from a black-spruce forest in Alaska (Mackelprang et al., 2011). In this study, the methane oxidation potential over the soil depth was estimated at 15 ºC, but the deeper sample could have the higher activity at the lower optimum temperature (Liebner and Wagner, 2007).

The methane emission rate in the moss- and sedge-dominated wetlands observed in this study ranging from 7.36 to 1,840 μmol m^{-2} h^{-1} was mostly comparable to or more than that reported in the previous studies (Cao et al., 1998; Kutzbach et al., 2004; Petrescu et al., 2008). Addition of the inhibitor of methane oxidation did not affect the methane flux with any rates, which indicated the methane emission is not accompanied by the concurrent plant-associated methane oxidation in the *in situ* emission process at a wide range of the flux rate in the both vegetations. Stable-isotopic studies of dissolved methane in the Alaska Tundra also indicates the minor role of methane oxidation during the transport from the deeperlayer to the surface (Throckmorton et al., 2015). The minor role of plant-associated methane oxidation in methane emission from aerenchymatous plants in arctic peatlands was also demonstrated by the recent microcosms study in Greenland using $^{13}CH_4$ labelling (Nielsen et al., 2017). The potential activity of methanotrophs may be sustained by the release of oxygen from the aerenchymatous plant roots at the very low level (Nielsen et al., 2017), which may not affect the methane flux from the vegetation. The low *in situ* temperature could be another reason for the undetectable level of the rhizospheric methane oxidation (Saarnio et al., 1997). A symbiotic relationship between methanotrophs and wetland mosses is well known for *Sphagnum* species (Basiliko et al., 2004; Raghoebarsing et al., 2005; Kip et al., 2010) also for brown mosses (Liebner et al.,

2011). The no effect of the inhibitor added in the headspace on the methane flux from the moss-dominated wetlands suggests that moss-associated methanotrophs may not use oxygen diffused from the atmosphere but use oxygen released from moss by photosynthesis (Raghoebarsing et al., 2005). Moss-associated methanotrophs may keep their activity even after the moss is dead and accumulated in the deeper layer where the conditions are not favorable for methane oxidation due to the anoxia (King, 1996).

In conclusion, the wetland soils of the taiga-tundra ecotone in the northeastern Siberia keep the high methane oxidation potential even under the unfavorable conditions. Though the plant associated methane emission may be influenced by methane oxidation, the oxic-anoxic interface of the wetland soils would be an important niche for methane oxidizing bacteria. The vertical shift of the oxic-anoxic interface caused by the fluctuation of the water level may not affect the methane oxidation at the site because the methane oxidation potential is maintained over the depth. Further study should focus on ecology of methane oxidizing bacteria actively involved in the methane cycle in the wetland of the northeastern Siberia.

Author contributions. JM and AS designed the experiments and JM carried them out. RS, TM, ST, and TCM helped with sampling, in situ data collection, and preparing resources for the fieldwork. JM prepared the paper with contributions from all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

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References

Basiliko, N., Knowles, R., and Moore, T. R.: Roles of moss species and habitat in methane consumption potential in a northern peatland, Wetlands, 24, 178-185, https://doi.org/10.1672/0277-5212(2004)024[0178:ROMSAH]2.0.CO;2, 2004.

Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., Weintraub, M. N., and Zoppini, A.: Soil enzymes in a changing environment: Current knowledge and future directions, Soil Biol. Biochem., 58, 216-234, https://doi.org/10.1016/j.soilbio.2012.11.009, 2013.

Cao, M. K., Gregson, K., and Marshall, S.: Global methane emission from wetlands and its sensitivity to climate change, Atmos. Environ., 32, 3293-3299, https://doi.org/10.1016/S1352-2310(98)00105-8, 1998.

Christiansen, J. R., Romero, A. J. B., Jorgensen, N. O. G., Glaring, M. A., Jorgensen, C. J., Berg, L. K., and Elberling, B.: Methane fluxes and the functional groups of

methanotrophs and methanogens in a young Arctic landscape on Disko Island, West Greenland, Biogeochemistry, 122, 15-33, https://doi.org/10.1007/s10533-014-0026-7, 2015.

de Caritat, P., Hall, G., Gislason, S., Belsey, W., Braun, M., Goloubeva, N. I., Olsen, H. K., Scheie, J. O., and Vaive, J. E.: Chemical composition of arctic snow: concentration levels and regional distribution of major elements, Sci. Total Environ., 336, 183-199, https://doi.org/10.1016/j.scitotenv.2004.05.031, 2005.

Frenzel, P., and Bosse, U.: Methyl fluoride, an inhibitor of methane oxidation and methane production, FEMS Microbiol. Ecol., 21, 25-36, 1996.

Frenzel, P., and Rudolph, J.: Methane emission from a wetland plant: the role of CH_4 oxidation in Eriophorum, Plant Soil, 202, 27-32, https://doi.org/10.1023/a:1004348929219, 1998.

Frenzel, P.: Plant-associated methane oxidation in rice fields and wetlands, in: Advances in Microbial Eology, edited by: Schink, B., Springer, Boston, 85-114, 2000.

Hugelius, G., Strauss, J., Zubrzycki, S., Harden, J. W., Schuur, E., Ping, C.-L., Schirrmeister, L., Grosse, G., Michaelson, G. J., and Koven, C. D.: Estimated stocks of circumpolar permafrost carbon with quantified uncertainty ranges and identified data gaps, Biogeosciences, 11, 6573-6593, https://doi.org/10.5194/bg-11-6573-2014, 2014.

Intergovernmental_Panel_on_Climate_Change: Climate Change 2013: The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, 2014.

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, 96-113, https://doi.org/10.1016/j.polar.2014.01.005, 2014.

King, G. M.: Physiological liminations of methanotrophic activity in situ, in: Microbiology of atmoshpheric trace gases: sources, sinks an global change processes, edited by: Murrell, J. C., and Kelly, D. P., I 39, Springer, Berlin, 17-32, 1996.

Kip, N., van Winden, J. F., Pan, Y., Bodrossy, L., Reichart, G. J., Smolders, A. J. P., Jetten, M. S. M., Damste, J. S. S., and Op den Camp, H. J. M.: Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems, Nat Geosci, 3, 617-621, https://doi.org/10.1038/Ngeo939, 2010.

Kip, N., Ouyang, W., van Winden, J., Raghoebarsing, A., van Niftrik, L., Pol, A., Pan, Y., Bodrossy, L., van Donselaar, E. G., Reichart, G.-J., Jetten, M. S. M., Sinninghe Damsté, J. S., and Op den Camp, H. J. M.: Detection, isolation, and characterization of acidophilic methanotrophs from *Sphagnum* mosses, Appl. Environ. Microbiol., 77, 5643-5654, https://doi.org/10.1128/aem.05017-11, 2011.

Kirschke, S., Bousquet, P., Ciais, P., Saunois, M., Canadell, J. G., Dlugokencky, E. J., Bergamaschi, P., Bergmann, D., Blake, D. R., and Bruhwiler, L.: Three decades of global methane sources and sinks, Nat Geosci, 6, 813, https://doi.org/10.1038/Ngeo1955, 2013.

Knoblauch, C., Zimmermann, U., Blumenberg, M., Michaelis, W., and Pfeiffer, E. M.: Methane turnover and temperature response of methane-oxidizing bacteria in permafrost-affected soils of northeast Siberia, Soil Biol. Biochem., 40, 3004-3013, https://doi.org/10.1016/j.soilbio.2008.08.020, 2008.

Kruger, M., Frenzel, P., and Conrad, R.: Microbial processes influencing methane

emission from rice fields, Glob. Chang. Biol., 7, 49-63, https://doi.org/10.1046/j.1365-2486.2001.00395.x, 2001.

Kutzbach, L., Wagner, D., and Pfeiffer, E. M.: Effect of microrelief and vegetation on methane emission from wet polygonal tundra, Lena Delta, Northern Siberia, Biogeochemistry, 69, 341-362, https://doi.org/10.1023/B:BIOG.0000031053.81520.db, 2004.

Larmola, T., Tuittila, E. S., Tiirola, M., Nykanen, H., Martikainen, P. J., Yrjala, K., Tuomivirta, T., and Fritze, H.: The role of Sphagnum mosses in the methane cycling of a boreal mire, Ecology, 91, 2356-2365, https://doi.org/10.1890/09-1343.1, 2010.

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, 327-341, http://dx.doi.org/10.1016/j.polar.2014.07.008, 2014.

Liebner, S., and Wagner, D.: Abundance, distribution and potential activity of methane oxidizing bacteria in permafrost soils from the Lena Delta, Siberia, Environ. Microbiol., 9, 107-117, https://doi.org/10.1111/j.1462-2920.2006.01120.x, 2007.

Liebner, S., Zeyer, J., Wagner, D., Schubert, C., Pfeiffer, E. M., and Knoblauch, C.: Methane oxidation associated with submerged brown mosses reduces methane emissions from Siberian polygonal tundra, J. Ecol., 99, 914-922, https://doi.org/10.1111/j.1365-2745.2011.01823.x, 2011.

Mackelprang, R., Waldrop, M. P., DeAngelis, K. M., David, M. M., Chavarria, K. L., Blazewicz, S. J., Rubin, E. M., and Jansson, J. K.: Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw, Nature, 480, 368-

371, https://doi.org/10.1038/nature10576, 2011.

Morozumi, T., Shingubara, R., Suzuki, R., Kobayashi, H., Tei, S., Takano, S., fan, R., Liang, M., Maximov, T. C., and Sugimoto, A.: Estimating methane emissions using vegetation mapping in the taiga-tundra boundary of a north-eastern Siberian lowland, Tellus B: Chemical and Physical Meteorology, https://doi.org/10.1080/16000889.2019.1581004, 2019 (in press).

Nielsen, C. S., Michelsen, A., Ambus, P., Deepagoda, T. K. K. C., and Elberling, B.: Linking rhizospheric CH_4 oxidation and net CH_4 emissions in an arctic wetland based on $^{13}CH_4$ labeling of mesocosms, Plant Soil, 412, 201-213, https://doi.org/10.1007/s11104-016-3061-4, 2017.

Olefeldt, D., Turetsky, M. R., Crill, P. M., and McGuire, A. D.: Environmental and physical controls on northern terrestrial methane emissions across permafrost zones, Glob. Chang. Biol., 19, 589-603, https://doi.org/10.1111/gcb.12071, 2013.

Parmentier, F. J. W., van Huissteden, J., van der Molen, M. K., 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, https://doi.org/10.1029/2010jg001637, 2011.

Petrescu, A. M. R., van Huissteden, J., Jackowicz-Korczynski, M., Yurova, A., Christensen, T. R., Crill, P. M., Backstrand, K., and Maximov, T. C.: Modelling CH_4 emissions from arctic wetlands: effects of hydrological parameterization, Biogeosciences, 5, 111-121, https://doi.org/10.5194/bg-5-111-2008, 2008.

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, 2539-2552, https://doi.org/10.5194/bg-10-2539-2013, 2013.

Raghoebarsing, A. A., Smolders, A. J. P., Schmid, M. C., Rijpstra, W. I. C., Wolters-Arts, M., Derksen, J., Jetten, M. S. M., Schouten, S., Damste, J. S. S., Lamers, L. P. M., Roelofs, J. G. M., den Camp, H. J. M. O., and Strous, M.: Methanotrophic symbionts provide carbon for photosynthesis in peat bogs, Nature, 436, 1153-1156, https://doi.org/10.1038/nature03802, 2005.

Roslev, P., and King, G. M.: Regulation of methane oxidation in a freshwater wetland by water table changes and anoxia, FEMS Microbiol. Ecol., 19, 105-115, https://doi.org/10.1016/0168-6496(95)00084-4, 1996.

Saarnio, S., Alm, J., Silvola, J., Lohila, A., Nykanen, H., and Martikainen, P. J.: Seasonal variation in CH_4 emissions and production and oxidation potentials at microsites on an oligotrophic pine fen, Oecologia, 110, 414-422, https://doi.org/10.1007/s004420050176, 1997.

Schuur, E. A. G., McGuire, A. D., Schadel, C., Grosse, G., Harden, J. W., Hayes, D. J., Hugelius, G., Koven, C. D., Kuhry, P., Lawrence, D. M., Natali, S. M., Olefeldt, D., Romanovsky, V. E., Schaefer, K., Turetsky, M. R., Treat, C. C., and Vonk, J. E.: Climate change and the permafrost carbon feedback, Nature, 520, 171-179, https://doi.org/10.1038/nature14338, 2015.

Segers, R.: Methane production and methane consumption: a review of processes underlying wetland methane fluxes, Biogeochemistry, 41, 23-51, https://doi.org/10.1023/a:1005929032764, 1998.

Shingubara, R., Sugimoto, A., Murase, J., Iwahana, G., Tei, S., Liang, M., Takano, S., Morozumi, T., and Maximov, T. C.: Multi-year effect of wetting on CH_4 flux at taiga–

tundra boundary in northeastern Siberia deduced from stable isotope ratios of CH4, Biogeosciences, 16, 755-768, https://doi.org/10.5194/bg-16-755-2019, 2019.

Steven, B., Niederberger, T. D., Bottos, E. M., Dyen, M. R., and Whyte, L. G.: Development of a sensitive radiorespiration method for detecting microbial activity at subzero temperatures, J. Microbiol. Methods, 71, 275-280, https://doi.org/10.1016/j.mimet.2007.09.009, 2007.

Sundh, I., Mikkela, C., Nilsson, M., and Svensson, B. H.: Potential aerobic methane oxidation in a Sphagnum-dominated peatland - controlling factors and relation to methane emission, Soil Biol. Biochem., 27, 829-837, https://doi.org/10.1016/0038-0717(94)00222-M, 1995.

Tarnocai, C., Canadell, J. G., Schuur, E. A. G., Kuhry, P., Mazhitova, G., and Zimov, S.: Soil organic carbon pools in the northern circumpolar permafrost region, Global Biogeochem. Cy., 23, GB2023, https://doi.org/10.1029/2008GB003327, 2009.

Throckmorton, H. M., Heikoop, J. M., Newman, B. D., Altmann, G. L., Conrad, M. S., Muss, J. D., Perkins, G. B., Smith, L. J., Torn, M. S., Wullschleger, S. D., and Wilson, C. J.: Pathways and transformations of dissolved methane and dissolved inorganic carbon in Arctic tundra watersheds: Evidence from analysis of stable isotopes, Global Biogeochem. Cy., 29, 1893-1910, https://doi.org/10.1002/2014gb005044, 2015.

Treat, C. C., Natali, S. M., Ernakovich, J., Iversen, C. M., Lupascu, M., McGuire, A. D., Norby, R. J., Roy Chowdhury, T., 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, Glob. Chang. Biol., 21, 2787-2803, https://doi.org/10.1111/gcb.12875, 2015.

Vecherskaya, M. S., Galchenko, V. F., Sokolova, E. N., and Samarkin, V. A.: Activity and

species composition of aerobic methanotrophic communities in tundra soils, Curr. Microbiol., 27, 181-184, https://doi.org/10.1007/bf01576018, 1993.

Wagner, D., Kobabe, S., Pfeiffer, E. M., and Hubberten, H. W.: Microbial controls on methane fluxes from a polygonal tundra of the Lena Delta, Siberia, Permafrost and Periglacial Processes, 14, 173-185, https://doi.org/10.1002/ppp.443, 2003.

Wagner, D., Lipski, A., Embacher, A., and Gattinger, A.: Methane fluxes in permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality, Environ. Microbiol., 7, 1582-1592, https://doi.org/10.1111/j.1462-2920.2005.00849.x, 2005.

Whalen, S. C., and Reeburgh, W. S.: Methane oxidation, production, and emission at contrasting sites in a boreal bog, Geomicrobiol. J., 17, 237-251, 2000.

Zhuang, Q., Melillo, J. M., Kicklighter, D. W., Prinn, R. G., McGuire, A. D., Steudler, P. A., Felzer, B. S., and Hu, S.: Methane fluxes between terrestrial ecosystems and the atmosphere at northern high latitudes during the past century: A retrospective analysis with a process-based biogeochemistry model, Global Biogeochem. Cy., 18, https://doi.org/10.1029/2004gb002239, 2004.

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Table 1. Estimated potential methane oxidation rate of soil samples from the wetlands of northeastern Siberia (average ± std error, n=3)

Year	Vegetation	Depth (cm)	Oxidation rate (nmol h^{-1} g_{dw}^{-1})
2013	Moss	0-2	268 ± 34
		4-6	496 ± 87
		8-10	242 ± 27
	Sedge	0-2	181 ± 16
		4-6	126 ± 27
		8-10	117 ± 24
2014	Moss	0-10	256 ± 1
		10-20	321 ± 21
		30-40	54 ± 1
	Sedge	0-10	138 ± 1
		10-20	94 ± 1

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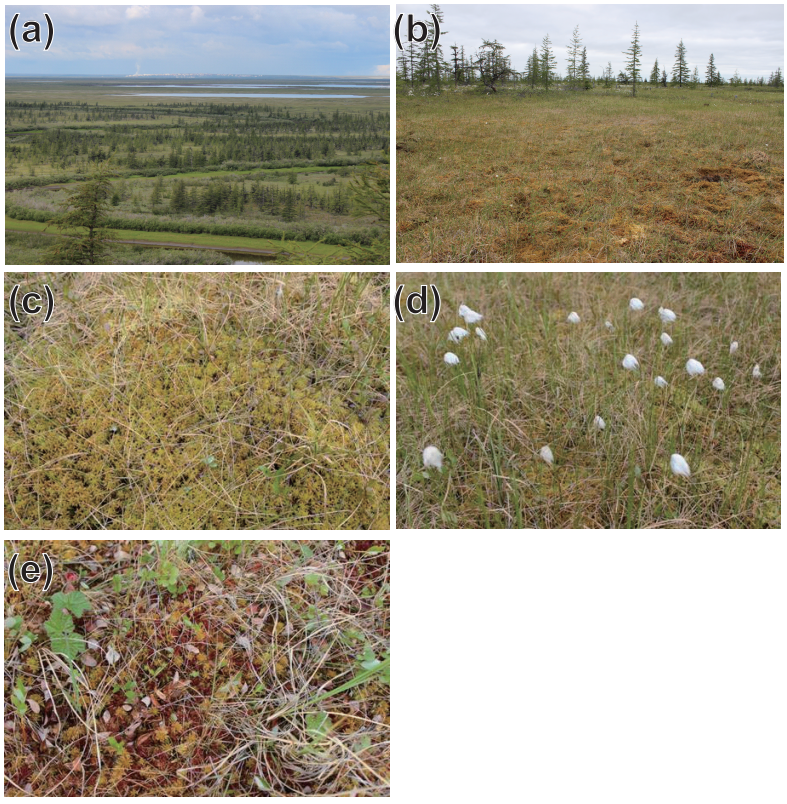

Figure 1. Distant (A) and close-range (B-E) views of the study sites. Soil samples were collected from the wetlands dominated by (C) moss and (D) sedge and from (E) moss-dominated mound.

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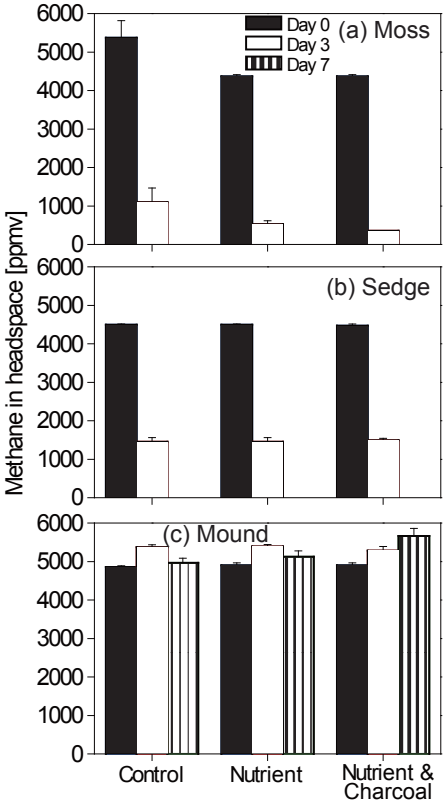

Figure 2. Temporal change of methane concentration in the headspace of the microcosms with soil samples from the different vegetation types (A, moss; B; sedge; C, moss in the mound) treated with inorganic nutrient and black carbon. Bars indicate the standard error (n=3).

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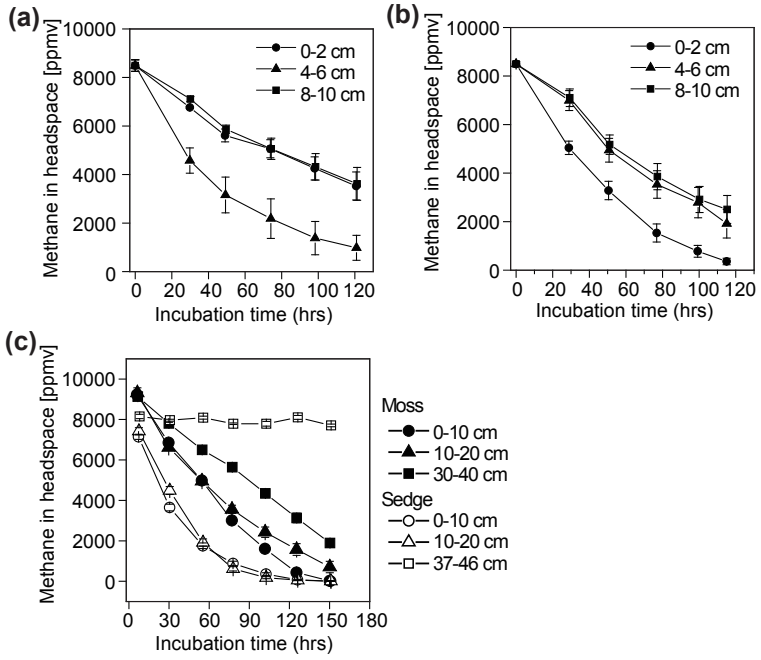

Figure 3. Methane oxidation by the different depth layers of moss- and sedge- dominated soils in 2012 (A and B) and 2015 (C). Bars indicate the standard error (n=3).

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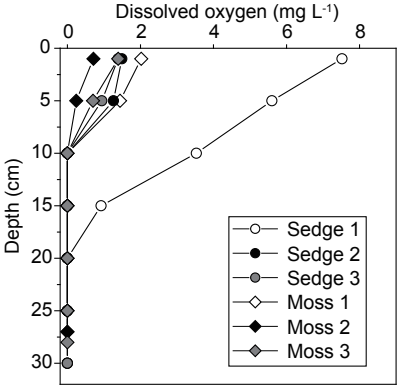

Figure 4. Vertical profile of dissolved oxygen in pore water of the wetland soils. Three independent measurements for sedge and moss dominated wetlands were done at the study site in 2014.

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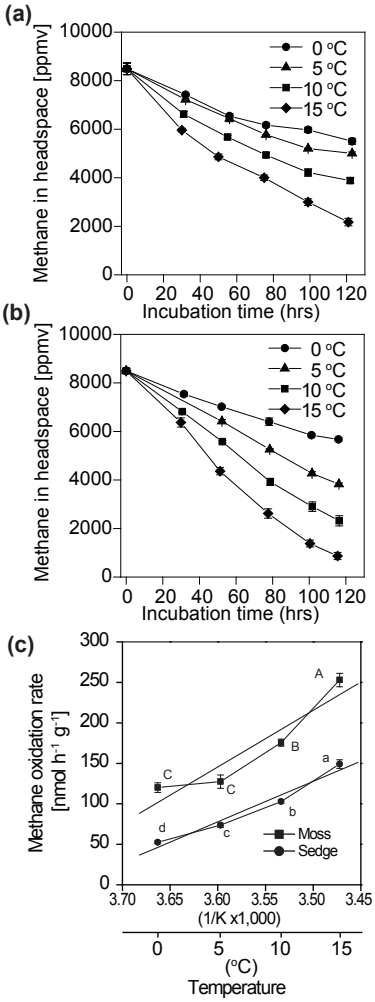

Figure 5. Effect of incubation temperature on methane oxidation by (A) moss and (B) sedge dominated peat samples and (C) the temperature dependence of the methane oxidation rate (0-10 cm) (2013). Bars indicate the standard error (n=3). Data marked with different letters are significantly different ($P < 0.05$, as determined by Tukey' s honestly significant difference test).

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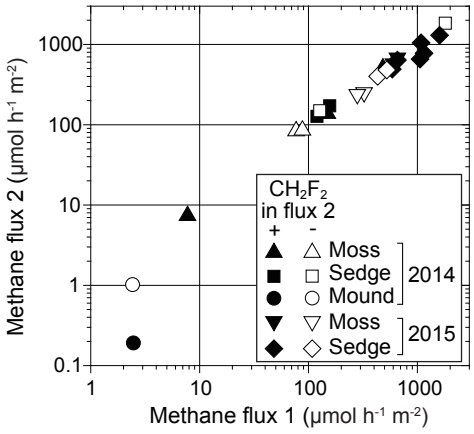

Figure 6. Effect of CH_2F_2 on methane flux from wetland estimated by the closed chamber method. Methane flux 1, 1st measurement without CH_2F_2; Methane flux 2, 2nd measurement after injection with or without CH_2F_2.