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

Jun Murasea, Atsuko Sugimotoa, c, Ryo Shingubarad, Maochang Liang, c, c, Tomoki Morozumib, c, Shinya Takano, c and Trofim C. Maximovd,e

*Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan; Arctic Research Center, Hokkaido University, Sapporo, Japan; Graduate School of Environmental Studies, Hokkaido University, Sapporo, Japan; Institute for Biological Problems of Cryolithozone Siberian Branch, Russian Academy of Science, Yakutsk, Russia; Biogeochemistry Educational and Scientific Training Center, North-Eastern Federal University, Yakutsk, Russia

ABSTRACT
Arctic wetlands are significant sources of atmospheric methane and the observed accelerated climate changes in the arctic could cause a change in methane dynamics. 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. An aerobic 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, while the mound soils showed no methane oxidation under the given conditions. The potential methane oxidation rates of the soils at 15°C ranged from 94 to 496 nmol h−1 g−1 dw. The immediate and active 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 methane oxidation rate was temperature-dependent, but substantial methane oxidation was observed even at 0°C particularly for the moss soil samples. Soil samples collected from the frozen layer of Sphagnum peat also showed immediate methane consumption when incubated at 15°C. The present results suggest that the methane oxidizing bacteria in the wetland soils could survive under anoxic and frozen conditions keeping their potential activities and immediately utilize methane when the conditions become favorable. On the other hand, the inhibitor of methane oxidation (difluoromethane) did not affect the methane flux from the sedge and moss zones in situ, which suggested the minor role of plant-associated methane oxidation.

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 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 result of the balance between methane production and consumption. It is estimated that 50 Tg of methane is annually produced in boreal wetlands and 15 Tg of produced methane is consumed before emitted to the atmosphere (Reeburg 2007). 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 studies, the potential methane oxidation was determined using an incubation experiment in which the collected samples were aerobically incubated with high concentrations of methane, and its controlling factors have been studied (Wagner, Horn, and Daims 2003; Wagner et al. 2005; Knoblauch et al. 2008; Christiansen et al. 2015). The potential methane oxidation rate differs spatially and temporally under the influence of different environmental conditions. For example, Wagner, Horn, and Daims (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, Horn, and Daims 2003; Wagner et al. 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 arctic 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 CO₂ produced by methanotrophs for photosynthesis (Raghoobarsing et al. 2005; Kip et al. 2010, 2011; Larmola et al. 2010; Liebner et al. 2011). The specific inhibitors such as methyl fluoride (CH₃F) and difluoromethane (CH₂F₂) are used to estimate the contribution of plant-associated methane oxidation to methane flux in wetland soils (Frenzel and Bosse 1996; Frenzel and Rudolph 1998; Kruger, Frenzel, and Conrad 2001). In this technique, it is assumed that the inhibitor injected in a flux chamber diffuses to the oxic part of the system, where inhibits methane oxidation, thus allowing estimation of methane oxidation in situ by comparing methane fluxes under the conditions with and without the inhibitor. The estimated contribution of methane oxidation to the total methane flux from the wetland rice determined by specific inhibition with difluoromethane ranged from 0% to 40% (Kruger, Frenzel, and Conrad 2001), while no contribution of methane oxidation associated with *Eriophorum* was observed in the bog of Estonia (Frenzel and Rudolph 1998). The contribution of plant-associated methane oxidation to methane flux 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 (Burns et al. 2013). Also, it is not clear if the measured methane oxidation represents the actual potential of the collected samples or if the methanotrophic activity was induced by incubation because 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. As microbial growth in Arctic soil could be limited by the availability of nutrients like nitrogen (Sistla, Asao, and Schimel 2012), the effect of minerals on the potential methane oxidation was also studied by adding nutrients mimicking the transfer from the sea and forest fire to the arctic region (de Caritat et al. 2005). Besides the potential methane oxidation of soils, we estimated plant-associated in situ methane oxidation for the first time in the arctic wetlands by measuring the methane fluxes using the specific inhibitor of methane oxidation (difluoromethane).

## 2. Materials and methods

### 2.1. Sample collection

Soil samples were collected from the thawed (active) layer of the wetland in a taiga–tundra transition zone along the tributary of Indigirka River (N 70°33.8′, E 148°15.9′) in northeastern Siberia, Russia (Supplementary Fig. S1a & b), during the summer (July) of 2012–2015. Samples collected and experiments conducted for each year are summarized in Table 1. The study site was described before with the name of Kryvaya or site K (Iwahana et al. 2014; Liang et al. 2014; Morozumi et al. 2019; Takano et al. 2019). Three representative vegetation types were selected: two depression zones that were dominantly covered with moss-wet (*Sphagnum* spp., Supplementary Fig. S1c) or tussocks of sedges (*Carex* spp., Supplementary Fig. S1d) and a dry mound vegetated with moss and larch trees (Supplementary Fig. S1e). The height difference between the depression zones and mound was approximately 40 cm and the maximum depth of active layer ranged from 20 to 40 cm (Morozumi et al. 2019; Takano et al. 2019). The ground water level in the depression zones ranged from 0 to 10 cm below the soil surface during the period of the study. The vertical profiles of soil total carbon and bulk density determined in 2011 are shown in Supplementary Figs. S2 and S3.

Blocks of the surface soil (0–10 cm) were collected in tripod using a serrated knife in 2012 and 2014; dry mound soil was collected only in 2012. 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 (below 30 and 37 cm in wet-moss and sedge depressions, respectively) were 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 DO 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 wooden stick a few days prior to measurement. The soil was water saturated and we measured the DO of the saturated

---

**Table 1. Summary of samples used and experimental setup.**

| Year | Soil layer (cm) | Effect of inhibitor | Methane flux with inhibitor |
|------|-----------------|---------------------|-----------------------------|
| 2012 | 0–10            | Vegetation type     | -                           |
| 2013 | 0–2, 4–6, 8–10  | Nutrients           | -                           |
| 2014 | 0–10            | Depth (1)           | Short exposure time         |
| 2015 | 0–10, 10–20, 30–40 (moss), 37–46 (sedge) | Depth (2) | Long exposure time |
water. The ground water table depth was about 1.5–2 cm below the soil surface when measured.

### 2.3. Methane oxidation potential of soil samples

Samples collected were gently pressed to remove the surplus water and homogenized by cutting into pieces (<5 mm) with scissors and mixing. The water content of homogenized samples was gravimetrically determined by heating subsamples at 80°C for 48 h. Ten grams of wet subsamples put into 50-ml or 100-ml GC vials (Nishiden-Rika Grass, Kobe, Japan). Frozen samples were thawed at 4°C before homogenized. The vials were capped with butyl rubber stoppers and open top screw caps and injected with 0.5 (in 2012) or 1.0 (in the other years) ml of 99% methane to give an initial concentration of ca. 5,000 or 10,000 ppmv in the headspace containing atmospheric air. The samples were incubated in the dark at 15°C. Methane concentration in the headspace was monitored using a photoacoustic field gas monitor (Innova 1412, LumaSense Technologies, Ballerup, Denmark). The small volume of the headspace (1 ml) was subsampled and injected into an empty and closed GC vial, and the methane concentration of the GC vial injected with the headspace gas was determined by circulating with the gas monitor. The methane oxidation rate was calculated from the linear regression of the methane concentration decreasing with time in 2 days (49–55 h). The methane oxidation rate was expressed per dry weight of samples that was obtained by drying at 80°C for 48 h.

To examine the effect of nutrients and black carbon – potential atmospheric depositions in the Arctic – on methane oxidation, the samples (10 g) collected in 2012 were applied with 1 ml of inorganic solution (10 μM NH₄NO₃, 250 μM NaCl, 40 μM CaCl₂, 20 μM MgSO₄, 10 μM KCl) and/or 1 ml of 100 μg l⁻¹ charcoal powder of oak (Quercus L., <47 μm). The soil incubation was done as described above.

Temperature dependence of methane oxidation was studied as described above under different incubation temperatures (0°C, 5°C, 10°C and 15°C) using the surface layer soils of the moss- and sedge-dominated wetlands collected in 2013. The temperature coefficient (Q₁₀) of methane oxidation was calculated for three temperature ranges (0°C to 5°C, 5°C to 10°C, and 10°C to 15°C):

\[
Q₁₀ = \left( \frac{R₂}{R₁} \right)^{\frac{10}{T₂ - T₁}}
\]

where R1 and R2 are the methane oxidation rates at the temperatures of T2 and T1 (T1 < T2), respectively.

### 2.4. Estimation of the effect of plant-associated methane oxidation to in situ methane flux

The contribution of methane oxidation associated with the wetland plants to methane flux was estimated using difluoromethane (CH₃F₂) in 2014 and 2015 according to Krüger, Frenzel, and Conrad (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, inner diameter: 24.5 cm). Gas samples in the headspace were taken every 15 min into 20-ml pre-vacuumed vials for 3 times (0, 15, and 30 min). After the first flux measurement, the gas phase in the chamber was refreshed with atmospheric air by using a pump and then injected with CH₃F₂ at the concentration of 1% (v/v). Then, the second measurement of methane flux was done 10–15 min after injection of the inhibitor in 2014; as no influence of the inhibitor on the methane flux was observed, we extended the exposure time of the inhibitor to 18–19 h in 2015 in order to verify the result in 2014. Methane concentration in the collected gas samples were determined by an 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. At least two chambers were set per site and methane flux measurement without the inhibitor was conducted in parallel to monitor the temporal shift of methane flux which could 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 followed by Tukey’s multiple comparison and the effect of CH₃F₂ on methane emission was assessed by Wilcoxon’s test using SPSS for Windows Ver. 22.0.

![Figure 1. Temporal change of methane concentration in the headspace of the microcosms with soil samples collected from the different vegetation types in 2012 (a, moss; b, sedge; c, moss in the mound) treated with inorganic nutrient and black carbon. Bars indicate the standard error (n = 3).](image-url)
3. Results

3.1. The relationship between the vegetation types and CH₄ oxidation

The methane concentration in the headspace of the bottle with peat samples from wetlands with moss and sedge vegetation rapidly decreased with time from the initial concentration (ca. 5,000 ppmv) to an atmospheric level in 7 days (Figure 1a & b). On the other hand, the sample from the moss-dominated mound did not show any activity of methane oxidation under the given conditions (Figure 1c).

Addition of nutrients and charcoal powder did not affect 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 all the samples (data not shown).

3.2. Vertical profile of CH₄ oxidation and DO in pore water

The time course incubation experiment using the different layers of surface (0–10 cm) wetland peat samples in depressions in 2013 showed that methane was oxidized with no lag period (Figure 2a & b). Immediate methane oxidation of the moss peat sample was also observed in the deeper layers in the 2015 measurement even in the frozen layer (30–40 cm) (Figure 2c). Peat samples from the sedge-dominated wetland up to 20 cm in depth also showed active methane oxidation, but the mineral frozen soil collected from below the organic layer of the sedge-dominated wetland in 2015 exhibited no detectable methane oxidation during the incubation period (Figure 2d). The calculated methane oxidation rate of the organic layer soils ranged from 54 to 496 nmol h⁻¹ g⁻¹ (Table 2). The moss peat samples had higher rates per dry weight basis than the sedge peat samples; this was contrasting with the higher decreasing rate of methane concentration in the headspace in the sedge peat sample, which was attributed to the higher water content of moss peat samples (93–94%[w/w]) than the sedge peat samples (75–85%[w/w]). The highest activity of methane oxidation was recorded at the middle (4–6 cm, 496 nmol h⁻¹ g⁻¹) layer and the top (0–2 cm, 181 nmol h⁻¹ g⁻¹) layer of moss and sedge peat samples, respectively.

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 (Figure 3).

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 during incubation was observed even at 0°C (Figure 4a & b). The methane oxidation rate of the sample from the moss-dominated wetland at 0°C did not differ from that at 5°C (Figure 4c). Q₁₀ ranged from 1.13

Table 2. 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 gdw⁻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                      |
| 2015 | Moss       | 0–10       | 256 ± 1                       |
|      |            | 10–20      | 321 ± 21                      |
|      |            | 30–40      | 54 ± 1                        |
|      | Sedge      | 0–10       | 138 ± 1                       |
|      |            | 10–20      | 94 ± 1                        |
|      |            | 36–47      | 0                             |

Figure 2. Methane oxidation by the different depth layers of moss- and sedge-dominated soils in 2013 (a and b) and 2015 (c and d). Bars indicate the standard error (n = 3).

Figure 3. 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.
Figure 4. 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) (2014). 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).

Figure 5. Temperature coefficient (Q10) of methane oxidation estimated between different temperature ranges.

Figure 6. Effect of CH₄F₂ on methane flux from wetland estimated by the closed chamber method. Methane flux 1, 1st measurement without CH₄F₂; Methane flux 2, 2nd measurement after injection with or without CH₄F₂.

to 2.10 with an exclusively low value for the moss peat samples incubated at 0°C and 5°C (Figure 5).

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

The methane flux in the first measurement ranged from 2.4 to 1,800 μmol h⁻¹ m⁻² (Figure 6). The methane flux in the second measurement ranged from 0.19 to 1,840 μmol h⁻¹ m⁻² and the inhibitor did not affect the methane flux in the second measurement for all the vegetation; this was demonstrated by the 1:1 relationship between the first and second measurements either with or without injection of the inhibitor. The prolonged exposure time of the inhibitor from 10–15 min (in 2014) to 18–19 h (in 2015) did not cause difference.

4. Discussion

The wetland soils in the depression area of the taiga–tundra transition zone in the northeastern Siberia exhibited the active methane oxidation in the incubation experiment. The potential rates estimated in this study (54–496 nmol h⁻¹ g⁻¹) were at the higher end of the rates measured in other Arctic regions including Siberia using a similar headspace concentrations of methane (0–835 nmol h⁻¹ g⁻¹; Knoblauch et al. 2008; 50–66 nmol h⁻¹ g⁻¹; Christiansen et al. 2015). The highest rate was recorded at the subsurface (4–6 cm) and surface (0–2 cm) of the moss and sedge-dominated wetlands, respectively; these depths corresponded to the ground 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). The color of the soils collected from layers under the ground water level darkened soon after sample collection in 2013 and 2015. This is most likely due to a browning reaction exposed to oxygen, which in turn indicates the soil below the ground water was under anoxic conditions. The vertical profile of DO verified the low oxygen concentration in the soil.
under the ground water, though the measurement was done in the different year (2014). On the other hand, the mound soil in the same area showed no methane oxidation under the experimental conditions of this study that targeted low-affinity methane oxidation. The mound has a low water level giving the drained conditions. Our previous study demonstrated that the soil in the mound is characterized by the positive redox potential (Shingubara et al. 2019); thus, the methane production is very low and the low-affinity methane oxidation, which is adapted to high concentration of methane, may be also low in the mound soil. The results show the spatial heterogeneity of the potential methane oxidation of the soils in the taiga-tundra ecotone in northeastern Siberia at inter- and intra-regional scales. We tested our hypothesis that the methane oxidation of the soils may be constrained by limited amounts of mineral nutrients including nitrogen, but application of the mineral salts and charcoal that would be transferred from the sea or forest fire (de Caritat et al. 2005) did not affect methane oxidation.

Plotting 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 exhibited no detectable methane oxidation. The immediate methane oxidation upon thaw is also reported for the frozen permafrost soil from a black-spruce forest in Alaska (Mackelprang et al. 2011). The high methane oxidation potential throughout the active layer is in contrast with 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 in the deeper layers actually do not oxidize methane in situ but survive for a prolonged time under the anoxic/frozen conditions; they keep the potential activity and would be able to immediately oxidize methane when the soil become oxic by decrease in the ground 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.

A clear temperature dependence on potential methane oxidation was observed in the top 10 cm layer of the wetland soils. The result is mostly consistent with the previous reports (e.g., Knoblauch et al. 2008). One different observation was that the soil from the moss wetland incubated at 0°C showed a comparative activity of methane oxidation to the soil incubated at 5°C with the low value of Q_{10} (1.13 between 0°C and 5°C). This suggests that the methanotrophs in the soil sample from the moss wetland would be less sensitive to change in the low temperatures compared to the sedge-dominated wetland soil sample and implies that methanotrophs in the soil samples of moss and sedge-dominated wetlands could differently respond to the increasing temperature in future. The depth profile of methane oxidation potential was estimated at 15°C, 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, Gregson, and Marshall 1998; Kutzbach, Wagner, and Pfeiffer 2004; Petrescu et al. 2008). Addition of the inhibitor of methane oxidation did not affect the methane flux from all the vegetation studied. Stable-isotopic studies of dissolved methane in the Alaska Tundra indicate the minor role of methane oxidation during the transport from the deeper layer 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 microcosm study in Greenland using $^{13}$CH$_4$ labeling (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 (Sarnio et al. 1997).

A symbiotic relationship between methanotrophs and wetland mosses is well known for Sphagnum species (Basiliko, Knowles, and Moore 2004; Raghoebarsing et al. 2005; Kip et al. 2010) and also for brown mosses (Liebner et al. 2011). The lack of an effect of the added inhibitor 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 unlike aerenchymatous plants but use oxygen released from moss by photosynthesis (Raghoebarsing et al. 2005). The moss peat samples sustained the methane oxidation potential over the depth studied. Moss-associated methanotrophs may keep their potential 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 northeastern Siberia keep the high methane oxidation potential even under anaerobic/frozen conditions that is expressed upon aerobic incubation with methane. The difference in temperature dependence on methane oxidation at the lower temperatures between the moss- and sedge-dominated wetland soils would give an insight for understanding and predicting methane dynamics in the arctic wetland under global warming. 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 as the methane oxidation potential is maintained over the depth. As microbial community in the arctic wetlands is geographically heterogeneous (Jansson et al., 2014), ecology of methane oxidizing bacteria actively involved in the methane cycle in the wetland of northeastern Siberia should be studied considering the vegetation types to better understand methane dynamics in this region.

Acknowledgments

This research was partly supported by Grant-in-Aids, the Global COE Program “Establishment of Center for Integrated Field Environmental Science” (IFES-GCOE) from the Ministry of Education, Culture, Sports, Science and Technology-Japan (MEXT), the Green Network of Excellence.
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.

Jansson, J., and N. Taj. 2014. “The microbiological ecology of permafrost.” Nature Reviews Microbiology 12: 414–425. doi:10.1038/nrmicro3262.

King, G. M. 1996. “Physiological Limitations of Methanotrophic Activity in Situ.” In Microbiology of Atmospheric Trace Gases: Sources, Sinks & Global Change Processes, edited by J. C. Murrell and D. P. Kelly, 17–32. Berlin: Springer.

Kip, N., J. F. van Winden, Y. Pan, L. Bodrossy, G.-J. Reichart, A. J. P. Smolders, M. S. M. Jetten, J. S. S. Damste, and H. J. M. Op den Camp. 2010. “Global Prevalence of Methane Oxidation by Symbiotic Bacteria in Peat-moss Ecosystems.” Nature Geoscience 3 (9): 617–621. doi:10.1038/ngeo939.

Kip, N., W. Ouyang, J. van Winden, A. Raghoebarsing, L. van Niftrik, A. Pol, Y. Pan, et al. 2011. “Detection, Isolation, and Characterization of Acidophytic Methanotrophs from Sphagnum Mosses.” Applied and Environmental Microbiology 77 (16): 5643–5654. doi:10.1128/aem.05177-11.

Kirschke, S., P. Bousquet, P. Ciais, M. Saunois, J. G. Canadell, E. J. Dlugokencky, P. Bergamaschi, D. Bergmann, D. R. Blake, and L. Bruhwiler. 2013. “Three Decades of Global Methane Sources and Sinks.” Nature Geoscience 6 (10): 813. doi:10.1038/ngeo1955.

Knoblauch, C., U. Zimmermann, M. Blumenberg, W. Michaelis, and E.-M. Pfeiffer. 2008. “Methane Turnover and Temperature Response of Methane-oxidizing Bacteria in Permafrost-affected Soils of Northeast Siberia.” Soil Biology & Biochemistry 40 (12): 3004–3013. doi:10.1016/j.soilbio.2008.08.020.

Kruger, M., P. Frenzel, and R. Conrad. 2001. “Microbial Processes Influencing Methane Emission from Rice Fields.” Global Change Biology 7 (1): 49–63. doi:10.1046/j.1365-2486.2001.00395.x.

Kutzbach, L. D. Wagner, and E.-M. Pfeiffer. 2004. “Effect of Microrelief and Vegetation on Methane Emission from Wet Polygonal Tundra, Lena Delta, Northern Siberia.” Biogeochernomy 69 (3): 341–362. doi:10.1023/B:BIOG.0000031053.81520.db.

Larmola, T., E.-S. Tuittila, M. Tiirola, P. J. Hannu Nykanen, K. Y. Martikainen, T. Tuomivirta, and H. Fritze. 2010. “The Role of Sphagnum Mosses in the Methane Cycling of a Boreal Mire.” Ecology 91 (8): 2356–2365. doi:10.1890/09-1343.1.

Liang, M., A. Sugimoto, S. Tei, I. V. Bragin, S. Takano, T. Morozumi, R. Shingubara, and et al. 2014. “Importance of Soil Moisture and N Availability to Larch Growth and Distribution in the Arctic Taiga-tundra Boundary Ecosystem, Northeastern Siberia.” Polar Biology 37 (8): 1075–1087. doi:10.1007/s00300-014-1502-8.

S. Takano, R. Frenzel, R. Shingubara, R. Suzuki, K. Kobayashi, S. Tei, S. Takano, R. Fan, M. Liang, T. C. Maximov, and A. Sugimoto. 2019. “Estimating Methane Emissions Using Vegetation Mapping in the Taiga-tundra Boundary of a North-eastern Siberian Lowland.” Tellus Series B-Chemical and Physical Meteorology 71 (1): 1–17. doi:10.1080/16000889.2019.1581004.

Nicola, H. J. Ammann, T. K. Per Ambus, C. Deepagoda, and B. Elberling. 2017. “Linking Rhizospheric CH4 Oxidation and Net CH4 Emissions in an Arctic Wetland Based on 13C/12C Labeling of Mesocoms.” Plant and Soil 412 (1): 201–213. doi:10.1007/s11104-016-3061-4.

Olefeldt, D., M. R. Turetsky, M. O. Mill, and A. David McGuire. 2013. “Environmental and Physical Controls on Northern Terrestrial Methane Emissions across Permafrost Zones.” Global Change Biology 19 (2): 599–603. doi:10.1111/gcb.12071.

Parmantier, J. F. W. J. van Huisteden, M. K. van der Molen, G. Schampaert-Strub, S. A. Karsanaev, T. C. Maximov, and A. J. Dolman. 2011. “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.
Petrescu, A. M. R., J. van Huisteden, M. Jackowicz-Korczynski, A. Yurova, T. R. Christensen, P. M. Crill, K. Backstrand, and T. C. Maximov. 2008. "Modelling CH4 Emissions from Arctic Wetlands: Effects of Hydrological Parameterization." *Biogeosciences* 5 (1): 111–121. doi:10.5194/bg-5-111-2008.

Preuss, I., C. Knoblauch, J. Gebert, and E. M. Pfeiffer. 2013. "Improved Quantification of Microbial CH4 Oxidation Efficiency in Arctic Wetland Soils Using Carbon Isotope Fractionation." *Biogeosciences* 10 (4): 2539–2552. doi:10.5194/bg-10-2539-2013.

Raghoebarsing, A. A., A. J. P. Smolders, M. C. Schmid, W. I. C. Rijpstra, M. Wolters-Arts, J. Derksen, M. S. M. Jetten, et al. 2005. "Methanotrophic Symbionts Provide Carbon for Photosynthesis in Peat Bogs." *Nature* 436 (7054): 1153–1156. doi:10.1038/nature03802. ISI:000231416600045.

Reeburgh, W. S. 2007. "Oceanic Methane Biogeochemistry." *Chemical Reviews* 107 (2): 486–513. doi:10.1021/ch050362v.

Roslev, P., and G. M. King. 1996. "Regulation of Methane Oxidation in a Freshwater Wetland by Water Table Changes and Anoxia." *FEMS Microbiology Ecology* 19 (2): 105–115. doi:10.1016/0168-6496(95)00084-4.

Saarnio, S., J. Alm, J. Silvola, A. Lohila, H. Nykänen, and P. J. Martikainen. 1997. "Seasonal Variation in CH4 Emissions and Production and Oxidation Potentials at Microsites on an Oligotrophic Pine Fen." *Oecologia* 110 (3): 414–422. doi:10.1007/s004420050176.

Schuur, E. A. G., A. D. McGuire, C. Schadel, G. Grosse, J. W. Harden, D. J. Hayes, G. Hugelius, et al. 2015. "Climate Change and the Permafrost Carbon Feedback." *Nature* 520 (7546): 171–179. doi:10.1038/nature14338.

Shingubara, R., A. Sugimoto, J. Murase, G. Iwahana, S. Tei, M. Liang, S. Takano, T. Morozumi, and T. C. Maximov. 2019. "Multi-year Effect of Wetting on CH4 Flux at Taiga–tundra Boundary in Northeastern Siberia Derived from Stable Isotope Ratios of CH4." *Biogeosciences* 16 (3): 755–768. doi:10.5194/bg-16-755-2019.

Sistla, S. A., S. Asao, and J. P. Schimel. 2012. "Detecting Microbial N-limitation in Tussock Tundra Soil: Implications for Arctic Soil Organic Carbon Cycling." *Soil Biology and Biochemistry* 55: 78–84. doi:10.1016/j.soilbio.2012.06.010.

Sundh, I., C. Mikkélæ, M. Nilsson, and B. H. Svensson. 1995. "Potential Aerobic Methane Oxidation in a Spahgnum-dominated Peatland—controlling Factors and Relation to Methane Emission." *Soil Biology & Biochemistry* 27 (6): 829–837. doi:10.1016/0038-0717(94)00222-M.

Takano, S., A. Sugimoto, S. Tei, M. Liang, R. Shingubara, T. Morozumi, and T. C. Maximov. 2019. "Isotopic Compositions of Ground Ice in Near-surface Permafrost in Relation to Vegetation and Microtopography at the Taiga–Tundra Boundary in the Indigirka River Lowlands, Northeastern Siberia." *Plos One* 14 (10): e0223720. doi:10.1371/journal.pone.0223720.

Tarnocai, C., J. G. Canadell, E. A. G. Schuur, P. Kuhry, G. Mazhitova, and S. Zimov. 2009. "Soil Organic Carbon Pools in the Northern Circumpolar Permafrost Region," *Global Biogeochemical Cycles* 23 (2): GB2023. doi:10.1029/2008GB003327.

Throckmorton, H. M., J. M. Heikko, B. D. Newman, G. L. Altmann, M. S. Conrad, J. D. Muss, G. B. Perkins, et al. 2015. "Pathways and Transformations of Dissolved Methane and Dissolved Inorganic Carbon in Arctic Tundra Watersheds: Evidence from Analysis of Stable Isotopes." *Global Biogeochemical Cycles* 29 (11): 1893–1910. doi:10.1002/2014gb005044.

Treat, C. C., S. M. Natali, J. Ernakovich, C. M. Iversen, M. Lupascu, A. D. McGuire, R. J. Norby, et al. 2015. "A pan-Arctic Synthesis of CH4 and CO2 Production from Anoxic Soil Incubations." *Global Change Biology* 21 (7): 2787–2803. doi:10.1111/gcb.12875.

Vecherskaya, M. S., V. F. Galchenko, E. N. Sokolova, and V. A. Samarkin. 1993. "Activity and Species Composition of Aerobic Methanotrophic Communities in Tundra Soils." *Current Microbiology* 27 (3): 181–184. doi:10.1007/bf01576018.

Wagner, M., A. Loy, M. Klein, N. Lee, N. B. Ramsing, D. A. Stahl, and M. W. Friedrich. 2005. "Functional Marker Genes for Identification of Sulfate-reducing Prokaryotes." *Methods Enzymol* 397: 469–489. doi:10.1016/S0076-6879(05)97029-8.

Wagner, M., M. Horn, and H. Daims. 2003. "Fluorescence in Situ Hybridisation for the Identification and Characterisation of Prokaryotes." *Curr Opin Microbiol* 6 (3): 302–309. https://www.ncbi.nlm.nih.gov/pubmed/12831908.

Whalen, S. C., and W. S. Reeburgh. 2000. "Methane Oxidation, Production, and Emission at Contrasting Sites in a Boreal Bog." *Geomicrobiology Journal* 17 (3): 237–251. doi:10.1080/01490450050121198.

Zhuang, Q., J. M. Mellilo, D. W. Kicklighter, R. G. Prinn, A. D. McGuire, P. A. Steudler, B. S. Felzer, and S. Hu. 2004. "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 Biogeochemical Cycles* 18: 3. doi:10.1029/2004gb002239.