Interactive comment on "Methane oxidation potential of the arctic wetland soils of a taiga-tundra ecotone in northeastern Siberia" *by* Jun Murase et al.

Jun Murase et al.

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Thank you for your valuable comments. Please see our response below and also the supplement file for our revised ms.

I am concerned there is very little novelty and gain in knowledge through this study. In addition to the lack of novelty, I have some severe concerns with regard to the experimental design.

–>We added more information about the background information of the present study and experimental conditions. We believe that the novelty of the current study is 1)

methane oxidation potential of soils in the arctic wetland was determined immediately after sampling, 2) rather high potential methane oxidation was sustained either under anoxic or frozen conditions, and 3) unlike the previous reports, the samples from the moss wetland showed the less sensitivity to the increase in temperature from 0 to 5 degree-C, and 4) the effect of the inhibitor on methane flux from arctic wetlands was first studied.

Specific comments: Abstract: Lines 1-5: Make two sentences out of it.

–>Done.

Lines 19-21: Under frozen conditions, oxygen diffusion should be too low to sustain aerobic methane oxidation. This statement is too vague here because it is simply based on extrapolations but not on real measurements.

–>We do not mean that methane is oxidized under anaerobic and frozen conditions, but that methanotrophs could sustain their potential activities under the conditions. We rephrase the sentence to try to make it clear: "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." (P2L19)

Line 22: Specify which inhibitor was used.

–>Done.

Introduction: Line 13: The phrase "typically" requires more than one reference.

–>We changed the sentence citing another reference to make our message clearer.

Line 23: Be more specific here: "..differs spatially and temporally under the influence of different conditions" contains very little information.

–>The results of the previous studies are followed by this sentence. To make a clear link, we inserted the phrase "For example" in front of the next sentence.

Page 5, line 11-12: The hypothesis "methane oxidation of the organic soil may be constrained by limited amounts of mineral nutrients including nitrogen" come out of the blue, there is no introduction to it before. Please include some background guiding to this hypothesis.

–>We revised the sentence adding background information: "As microbial growth in Arctic soil could be limited by the availability of nutrients like nitrogen (Sistla et al., 2012), the effect of minerals on the potential methane oxidation was also studied by adding salts and black carbon that are supposed to be transferred from the sea and forest fire to the arctic region (de Caritat et al., 2005)." (P5L18)

Materials and Methods: The chapter on sample collection needs more details on replication.

–>We collected triplicate samples and add this information in the section on sample collection.

Chapter 2.2: Was the soil water saturated? If so please state. If not, a DO meter makes no sense I think. At which depths was oxygen measured? Was oxygen continuously monitored?

–>Yes, the soil was water saturated and we measured DO in the saturated water. We add the statement: "The soil was water saturated and we measured the DO of the saturated water."(P7L3)

Chapter 2.3: Was the injected concentration of methane according to in-situ concentrations? It appears a bit arbitrary.

–>In this study, we followed the previous studies in terms of methane concentration for the incubation experiment as we would like to compare the potential methane oxidation rate with other regions.

Page 7, line 1: There is no description on the temperature dependence measurement at all. Please include.

–>We added the temperatures for the measurement. (P7L25)

Chapter 2.4: The inhibitor concentration seems arbitrary? Were there initial tests conducted to determine inhibitor concentration? How can the authors be sure that soil methanotrophs were fully exposed to the inhibitor?

–>The previous study demonstrated that the inhibitor concentration effectively suppresses methane oxidation. The idea of this technique is that methane oxidation would be inhibited at the site where the inhibitor reaches by diffusion, e.g., the rhizosphere of aerenchymous plants. Thus, we do not assume that the inhibitor would fully stop methane oxidation in the system but selectively methane oxidation at the site where the inhibitor reaches. We add the background of the technique in introduction.

Results: Page 10, lines 2-4: Extrapolating methane oxidation measured at 5 C to subzero temperatures by simple linear regression is an invalid approach. First of all oxygen diffusion is hampered at subzero temperatures. Second, enzyme kinetics are not linear.

–>We deleted the part and instead added the result of Q10 calculation according to Reviewer 1.

Same page, line 8: This is not a rate, it is a flux.

–>corrected

Discussion: Page 11, line 1: Lack of methane oxidation in mounds could be caused by too high methane concentrations in your incubations. The mounds are likely colonized by atmospheric oxidizers so that methane concentrations of 5000-10000 ppm would be way too high.

–>We agree with it. Our experimental conditions target a low affinity methane oxidation and high-affinity methane oxidation was not examined. Our target was specified in the text: "the mound soil in the same area showed no methane oxidation under the experimental conditions of this study that targeted low-affinity methane oxidation."

(P12L6)

Same page, lines 22-24: Strange wording. English should be corrected throughout the entire manuscript.

–>We revised our discussion giving a separate paragraph and focusing of Q10.

Page 12, lines 10-14: This conclusion lacks proof. See comment before. The inhibitor concentration may simply be too low or the inhibitor was not sufficiently distributed.

–>We revised the expression of the conclusion and also added explanation regarding the aim of the experiment in more detail in introduction.

Page 13, lines 1-6: I do not understand what is meant here, please clarify? The methanotroph inhibitor has no effect on uptake of oxygen?!

–>In this technique, it is assumed that the inhibitor injected in a flux chamber diffuses to the oxic part of a system, where effectively inhibits methane oxidation.

Conclusion: Page 13, lines 14-17: Active methanotrophs in Arctic wetlands have already been studies. See for example Graef et al., 2011, EM Reports.

–>We are aware of it. However, we can not assume that active methanotrophs are same over arctic wetlands as the bacterial communities in the arctic soils would be different between the regions (Jansson et al., 2014). Jansson JK, Tas N. 2014. The microbial ecology of permafrost. Nat Rev Micro 12:414-425.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-98/bg-2019-98-AC2-supplement.pdf

Table 1. Summary of samples used and experimental setup

Year	Bottle incubation experiment		Methane flux with the inhibitor
	Soil layer (cm)	Effect of	
2012	0-10	Nutrients	-
2013	0-2, 4-6, 8-10	Depth (1)	-
2014	0-10	Temerature	Short exposure time
2015	0-10, 10-20, 30-40(moss), 37-46(sedge)	Depth (2)	Long exposure time

Fig. 1.

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} 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
2015	Moss	0-10	256	±	1
		10-20	321	±	21
		30-40	54	±	1
	Sedge	0-10	138	±	1
		10-20	94	±	1

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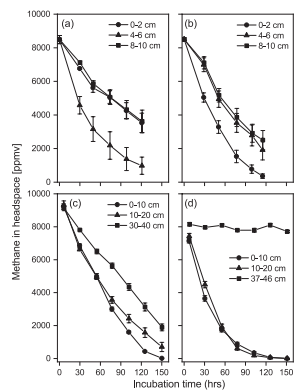
Figure 3. Methane oxidation by the different depth layers of moss (a, c) and sedge (b, d) dominated soils in 2013 (a, b) and 2015 (c, d). Bars indicate the standard error (n=3).

Fig. 3.
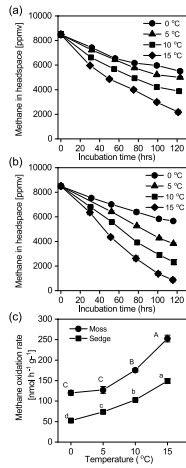
Figure 5. Effect of incubation temperature on methane oxidation by (a) moss and (b) sedge dominated peat samples and (c) the temperature dependence of the methane oxidation rate (0-10 cm) (2014). Bars indicate the standard error (n=3). Data marked with different letters are significantly different (P < 0.05, as determined by Tukey's honestly significant difference test).

Fig. 4.
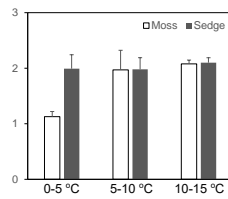
Fig. 6. Temperature coefficient (Q_{10}) of methane oxidation estimated between different temperature ranges .

Fig. 5.
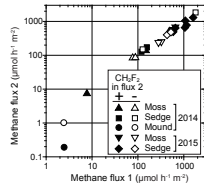
Figure 7. Effect of CH₂F₂ on methane flux from wetland estimated by the closed chamber method. Methane flux 1, 1st measurement without CH₂F₂; Methane flux 2, 2nd measurement after injection with (filled symbols) or without (open symbols) CH₂F₂.

Fig. 6.