Author responses to:

Interactive comment on “Impacts of temperature and soil characteristics on methane production and oxidation in Arctic polygonal tundra” by Jianqiu Zheng et al.

Anonymous Referee #2

The manuscript “Impacts of temperature and soil characteristics on methane production and oxidation in Arctic polygonal tundra” of Zheng and co-authors presents results from incubation experiments of samples from two polygon centres of the arctic tundra in Alaska. The authors sectioned two cores in three layers (active layer, transition zone, permafrost) and incubated samples of these layers under either aerobic or anaerobic conditions. They measured methane (CH4) production in the anaerobic layers and CO2 production and CH4 oxidation in all of the layers at three different temperatures (-2_C, 4_C, 8_C). Furthermore they measured low molecular weight fatty acids and ferrous iron concentrations at three time points of the incubation experiment and gradients of dissolved CO2 and CH4 concentrations at the field sites. From the data of the temperature incubation experiments they calculated Q10 values for CH4 production and oxidation at each depth layer at the two sampling sites.

The manuscript presents potentially interesting data but the study seems not clearly focussed. The main part of the study deals with CH4 production and oxidation but one of the main novel conclusions is that iron reduction is more important for the anaerobic degradation of organic matter than methanogenesis. This would be an interesting result but the methodology and data used to support this this conclusion remain unclear.

It is unclear how the authors assessed the importance of methanogenesis and iron reduction. The authors present acetate concentrations and then calculate how much of this acetate was consumed by methanogenesis and iron reduction (Fig. 8). However, it remains unclear how this was done. Acetate concentrations in the soil are a function of acetate production rates e.g. by fermentation and acetate consumption rates e.g. by methanogenesis and iron reduction. Hence concentrations give no information about production rates. Furthermore, the description of the experiments and analysis is in many parts unclear (see also specific comments). It is difficult to follow the incubation experiment and in particular the CH4 oxidation experiment. Samples were incubated at different temperatures to measure the temperature response of CH4 oxidation, but they seem to have been also pre-incubated, but at different temperatures at the different sampling sites. This is confusing and should be clarified. One of the two hypothesis rather states current knowledge than a novel research idea. Furthermore, the aim of some of the presented approaches in the manuscript remain obscure, e.g. the “calculation of net CH4 emissions” (2.5).

We will add more explanation on how the relative importance of iron reduction and methanogenesis is calculated based on reaction stoichiometry in the revised manuscript. We will also add a new figure to explain the workflow for anoxic incubations and methane oxidation assays (see attached figure and responses to Reviewer 1). We will also expand section 2.5 to clarify how the calculations were done in the revised

manuscript.

specific comments
P1, L23: To my knowledge, high latitude terrestrial ecosystems are a clear CH4 source, even if atmospheric CH4 may be oxidized in dry soils. Please rephrase.

We will rephrase in the revised manuscript to clarify the uncertainty over which soils will function as a net source or sink in high latitude ecosystems.

P2, L14: See comment above.

P3, L12: This might be right for the oxidation of atmospheric CH4, but for wetlands, showing substantial CH4 production, this is not the case. Generally, highest CH4 oxidation is found in wetlands at the aerobic/anaerobic interface, which is close to the water table.

We will rephrase in the revised manuscript with a better distinction between submerged (wetland) soils and unsaturated soils.

P3, L32: This sentence is unclear. Why is additional research on CH4 oxidation needed to improve estimates on CH4 production? Please rephrase.

We will rephrase in the revised manuscript.

P4, L1: Please specify the carbon decomposition pathways investigated.

We will specify the pathways we measured, including fermentation, iron reduction and methanogenesis in the revised manuscript. This will include the added detail on reaction stoichiometry, discussed above.

P4, L5: This is not a hypothesis but well established textbook knowledge.

We will specify the hypothesis in the context of flat centered polygons and high centered polygons, which have relatively dry organic layers and wet permafrost layers, in the revised manuscript.

P5 L24ff: Please clearly explain, which samples were incubated aerobically and which anaerobically. I assume the samples treated in the anaerobic chamber were also incubated anaerobically but this is not stated.

Organic soils were incubated under oxic conditions, while soils from transition zone and permafrost were incubated under anoxic conditions. The new figure should clarify this important point, and we will elaborate in the revised manuscript.

P6 L4ff: Which samples? Are this the same "microcosms" than presented in 2.3.1? and how much is ample?

A subset of microcosms setup in section 2.3.1 were opened to set up methane oxidation assays. We will clarify in section 2.3.2 and the additional figure to demonstrate how the anaerobic incubations and methane oxidation assays were constructed.

P6 L9: Why are there two different incubation temperatures for FCP and HCP? I understood from the preceding sentence that the samples were incubated at the tree different temperatures -2_C, 4_C and 8_C. Please clarify.

The samples were incubated at three different temperatures. We put a subset of samples on the shaker to remove gas-liquid phase transfer limitation. We will clarify in the revised manuscript.

P6, L20: Please cite the method for Fe2+ measurements.

We will add the citation to this commercial assay in the revised manuscript.

P6, L25: Table S3.

Will revise.

P7, L3ff: The concept presented here is unclear. What is the aim of these calculations? Do the authors aim to calculate CH4 emissions as stated in the header? Please clarify. Furthermore, some of the assumptions are probably not met. It is unclear why the rate of CH4 oxidation should equal the rate of CH4 production? This would mean zero emission of CH4. Is this likely? And finally the authors assume a certain Km-value for CH4 and O2 and also give a very wide range of reported Km values. It should be explained why these particular Km-values were chosen. And how would a change in the Km-values affect the calculated biomass of methanogens and methanotrophs.

We will rewrite section 2.5 to clarify. (Please see also comments in response to Reviewer 1). The aim of the simulation is to demonstrate the wide range of uncertainties in net methane production and impact of methanotroph to methanogen biomass ratios in response to temperature increase. We assumed zero net CH_4 production to help us understanding whether the soil is predicted to be a CH_4 source or sink. We will change Figure 7 in the revised manuscript with clear marks of CH_4 source and sink: CH_4 sink above the plotted lines, and CH_4 source below the plotted lines.
We intentionally included a wide range of K_m values used in models for this sensitivity analysis as we do not have enough information to preferably select certain K_m values. We will clarify in the revised manuscript and add additional lines of discussion.

P7, L19ff: It would be interesting to see the water content related to soil volume. The different depth layers show substantial differences in organic carbon concentrations, which likely are also related to substantial differences in bulk densities.

We will add a plot of soil bulk density as an additional panel in Figure 1.

P8, L1ff: Dissolved gas concentrations should be calculated based on volume soil pore water (e.g. as _M). Relating it to dry weight is misleading considering that gas cannot be dissolved in a solid.

We will also include molar calculations of dissolved gas relative to volume soil pore water. Normalizing gas concentrations to soil mass facilitates stoichiometric comparisons with organic acids, iron, and gases produced in microscosms, although it is

not physically relevant.

P8, L5: If no CH4 was detected, does this indicate the oxidation of atmospheric methane in the soil? The detection limit was given as 1 ppm, which is below atmospheric concentrations.

No. This data can only be interpreted as no CH_4 produced was measured. We infer this observation is due to the low level of total microbial activity measured as CO_2 production.

P8, L7: Which statistical test was used to test for significance?

We used a paired t-test. Will clarify in the revised manuscript.

P8 L9ff: Better give the carbon concentrations together with the other profile data in Fig. 1. What about the carbon concentrations above 10 cm soil depth? If these are missing, a general comparison between active layer and the other samples is problematic, since generally active layer carbon concentrations are highest at the surface.

We will add an SOC subplot in Figure 1.

P8, L30: What means 0 and 5 days? Were they pre-incubated for 5 days with CH4? Please clearly explain in M&M.

Pre-incubated without CH_4. The added figure illustrating the experimental workflow should clarify this point.

P9, L12ff: The data on the temperature response of CH4 production and oxidation should not be presented only in the text of the manuscript but also as a graph or table as well. According to the title of the manuscript these data are the most important ones.

We will add a figure on the temperature response of CH_4 production and oxidation in the revised manuscript.

P9, L18ff: Please explain the meaning of the error for the Q10 values and how this was calculated.

We will clarify in the revised manuscript.

P10, L15ff: Calculating Q10 values from rates derived from different fitting methods (linear and hyperbolic) at the respective temperatures is problematic. I suggest using only one fitting method for all of the incubation temperatures and then use these data to calculate Q10 values.
P10, L18f: Please explain how the Q10 value was estimated.

We used linear fitting to estimate the initial production rate of CO_2 for Q10 calculation. We will clarify in the revised manuscript.

P10, L19f: This sentence should go to the discussion.

Will move this sentence.

P10, L23ff: Please explain in the M&M how these fatty acids were analysed.

We will add more explanation of organic acid analysis in the revised manuscript.

P10, L30: Please explain how significance tests were conducted. There seem to be no replicate analysis before day 90.

We used paired t-test with additional technical replicates.

P11, L5: please explain this approach in M&M.

We will add the explanation in the revised manuscript.

P11, L14: Please explain how the rates were calculated. Over the whole incubation period or only for certain incubation intervals?

Iron reduction rates were estimated by the changes in Fe(II) concentration. We will clarify in the revised manuscript.

P11, L15: How were Q10 values "estimated"?

The Q10 values of iron reduction were estimated using the ratio of iron reduction rate measured at 8 degree C and -2 degree C. We will clarify in the revised manuscript.

P11, L28: This sentence is unclear. Why does lower active layer than permafrost CH4 concentrations indicate CH4 oxidation in the active layer? Permafrost CH4 is not released from the permafrost since it is frozen. Please clarify.

We clarify in the revised manuscript.

P11, L29ff: This statement is incorrect. There are numerous studies on CH4 production and CH4 oxidation in the Arctic also showing that CH4 is produced in the anoxic soil layers and oxidized in oxic soil layers. This is an obvious fact, which likely needs no further testing if there is no evidence against it. Furthermore, differences in the temperature response of CH4 production and oxidation has been shown also for Arctic environments and respective studies were also cited by the authors.

We will rephrase the questions to be more specific to polygonal tundra with fine scale microtopographic features.

P12, L4f: This statement is not completely correct. It is current knowledge and obvious, that CH4 production depends on both CH4 and O2 supply. Therefore, indeed CH4 oxidation depends on oxygen supply but if CH4 is present. Hence, many studies on CH4 oxidation in wetlands (including those in the Arctic) demonstrate that the oxic/anoxic interface is the zone of most intense CH4 oxidation, which are not necessarily the aerobic surface soil layers, since there, as the authors correctly stated, low CH4 concentrations limit CH4 oxidation. Hence the soil water table is often more informative than the gravimetric water content for identifying the zone of maximum CH4 oxidation.

We will clarify in revision that both CH_4 and O_2 diffusion can limit aerobic methane oxidation. We appreciate the reviewer's perspective on the importance of the oxic/anoxic interface as the hotspot for aerobic CH_4 oxidation in wetlands. A cited review by Segers (1998) provides a valuable overview of potential methane oxidation rates as a function of distance to oxic/anoxic interface (p. 39). Average rates are highest near the water table as expected, but maximum values are on the anoxic side of the interface. However, this distance factor explains only a small part of the variance in observed in the distribution of methane oxidation potential. Therefore, other factors must influence methane oxidation potential as well.

We are still surprised that the maximum methane oxidation potential in flat-centered polygon soils was observed in the transition (40-50 cm) and permafrost (50-70 cm) layers –far below the near-surface water table and overlapping with areas of anaerobic methanogenesis and iron reduction. One could interpret this as a result of a fluctuating water table (as the reviewer suggests, below). However, there is no evidence for recent fluctuations in the near-surface water table at this flat center polygon, as discussed on page 12. Alternatively, we could hypothesize that the oxic/anoxic interface comprises a large part of this soil column rather than the narrow horizontal line usually drawn near the water table in conceptual diagrams. Such a broad suboxic zone would be consistent with the dissolved Fe(II) and CH_4 profiles show in in Figure 1. Proximity to CH_4 sources would be more important than proximity to the water table in this model. Future studies will be required to understand the complex O_2 transport mechanisms in this cold, saturated FCP soil. We will clarify this discussion in the revised manuscript.

P12, L30f: The meaning of this sentence is unclear. Do the authors assume, that the main oxygen source in the saturated zone is from dissolved oxygen in rain water percolating through the soil and not from molecular transport through the gas phase through unsaturated pores? Please clarify?

Based on the high water table of flat-centered polygons and the substantial precipitation preceding our sampling campaign, we do not expect much gas transport through unsaturated pores in this soil. We will clarify this point in revision.

P12, L34ff: Which observations? I do not see that the survival of methanotrophs under changing redox conditions argue against highest CH4 oxidation at the water table. I assume the authors mean here in situ CH4 oxidation and not potential CH4 oxidation measured in the laboratory. It has been shown repeatedly that highest CH4 oxidation is found in the soil layer where elevated CH4 concentrations overlap with oxygen. This is in soils generally close to the water table. However, if the water table fluctuates, potential CH4 oxidation rates measured in the laboratory do not need to correlate with the current water table, but likely in situ CH4 oxidation rates do. There is no way to aerobically oxidize CH4 without the presence of CH4 and oxygen.

See response above.

P13, L13F: Why should this be? Please explain.

Sharp temperature gradients along soil depth.

P13, L20f: What is meant by "outcompete"? Methanogens and CH4 oxidizers are

not competitors. I understand that it is meant that CH4 production is expected to be higher than CH4 oxidation. But why is this likely. It has been shown that even at 8_C the potential CH4 oxidation with the current community size is 7 times higher than methanogenesis. I would rather say that it is highly unlikely that CH4 production will be higher than potential CH4 oxidation.

We will replace "outcompete" in the revised manuscript to better describe the kinetics of these two processes. Our point in the simulation shown in Figure 7 is to illustrate the disparate effects of temperature on methanogenesis and methane oxidation activity and address model sensitivity to assumptions of half saturation rates. We will use an example to clarify interpretation of this figure.

P13, L21-L29: This part of the discussion is unclear and in part speculative. The purpose of these calculations was not clearly stated in the description in the M&M section (see above) nor is it here. It might be interesting if the authors would have data on the microbial biomass of methanogens and CH4 oxidizers. But as it is now, it gives no substantial additional information.

See above.

P14, L1: Which incubations are referred to? The permafrost only or also the active layer?

This refers to all incubations, including permafrost and active layer.

P14, L4f: To which samples is referred to here? To the FCP samples and the HCP samples?

FCP samples. We will clarify in the revised manuscript.

P15, L5f: The described pattern was obviously not observed for the HCP in this study. What could be the differences to the cited study?

We did not see evidence of cryoturbation in the HCP core used in this study. The organic layer of HCP contained much lower level of organic acids comparing to the FCP organic layer, so overall the substrate level is low.

P14, L9f: It is obvious that organic carbon oxidation processes contribute to anaerobic CO2 production, which is the result of organic carbon oxidation. Please rephrase.

We will rephrase in the revised manuscript to distinguish decomposition from mineralization processes.

P14, L12ff: This sentence should be split into two. Furthermore, the information content is limited. It seem obvious that CH4 isotopes are consistent with either acetoclastic methanogenesis or hydrogenotrophic methanogenesis since these are the mayor pathways of methanogenesis. Does this sentence mean that acetate is mainly oxidized via methanogenesis and not via iron reduction? This seems to contradict the first sentence of this paragraph.

We will revise this sentence to more clearly justify the use of acetoclastic methanogenesis reaction stoichiometry.

P14, L15: These calculations should be described in the M&M section. The acetate concentrations are rising during the incubations. Hence, there is a net production over time. But how was gross acetate production calculated? This is not possible from the concentration data alone. The data presented in Fig. 8 are not comprehensible.

We will clarify the calculation and provide an example. The net production of acetate over time was measured. The consumption of acetate was calculated based on the stoichiometry of iron reduction and methanogenesis utilizing acetate as electron donor. Thus we estimated the overall gross production of acetate.

P14, L29ff: This last paragraph gives the current and well-established view of organic matter decomposition in wetlands. It might fit to the introduction but is not needed at the end of the discussion. The relative importance of iron reduction versus methanogenesis is an interesting issue but the data collected here does not allow a meaningful comparison of these two processes. Hence, I rather suggest omitting Fig. 9.

We believe the conceptual figure 9 will help readers to integrate the numerous processes discussed in this paper. Therefore, we prefer to keep it.

Fig 5: Please show in the panels which samples were incubated aerobically and which anaerobically.

We will clarify in the revised manuscript and the new figure.

Fig. 8: Acetate concentrations rather than acetate production are presented in this Figure. Please rephrase.

We will revise the Figure caption in the revised manuscript.

Fig S1: This figure is unclear. What do the red circles mean?

The circles show the combined soil sections used for incubations. We will clarify in the revised manuscript.