

RC2: 'Comment on egosphere-2024-1047', Anonymous Referee #2, 24 Sep 2024

The aim of the study is to demonstrate using laboratory incubations how additions of nutrients (N and P salts) affect CO₂ and CH₄ production in subarctic bog and fen peat. This would indicate the effects of wildfire induced increase in nutrient content in peat. To get more information on the effects of fire on soil processes is relevant because wildfires will increase in northern latitudes with global warming and the control of wildfires in remote areas is difficult.

There are aspects in the text, methods and information given which should be considered when evaluating the output of the study. Enclosed comments.

We would like to thank the Reviewer for evaluating our preprint manuscript with consideration of the context of our research. We agree with the Reviewer's general and specific comments and suggestions and believe we have addressed all the comments in the revised manuscript. In the following paragraphs, each comment is addressed individually. We considered all the points raised by the Reviewer and have detailed the ways in which we have addressed them in the revised version of the manuscript.

Title: Title directly suggests that the effects of increasing wildfire and permafrost thaw were studied. The study was conducted with non-fire affected peat samples (?) and merely shows effects and fate of added nutrients in peat. Of course you can discuss the possible links to the wildfire. The title could thus be modified to avoid misleading.

Completed. We agree with this suggestion and have now revised the title to:

“Effects of nitrogen and phosphorus amendments on CO₂ and CH₄ production rates in peat soils of Scotty Creek, Northwest Territories, Canada: exploratory incubation results highlight a potential impact of wildfires and permafrost thaw on peatland carbon exchanges”

Methods:

Some more information about the sampling sites and peat cores taken should be given. Now there is no data if the cores taken had vegetation cover, or were just bare surfaces sampled?

Completed. Thank you for this comment and we have made sure to give complete descriptions of the sampling sites and the soil coring. Both sites were covered by vegetation at the time of sampling as shown in Fig. 1. The corresponding text reads: “In October 2020, two duplicate peat soil cores (0-25 cm) were taken from each of the following two sites: a thermokarst bog covered by Sphagnum mosses (hereafter referred to as ‘bog peat’ or ‘bog soil’) and a channel fen covered by herbaceous plants (hereafter referred to as ‘fen peat’ or ‘fen soil’) as shown in Fig. 1.” [revised l. 131-133]

Line 91: The text indicate that the initially frozen peat cores were placed after thawing in plastic containers and mixed with hands. There are two aspects to be considered, points which can have impact on the results obtained.

The reviewer is correct: the peat samples from each site were manually mixed prior to the incubation experiment. They were thawed and mixed inside the original sampling containers to avoid loss of any in-situ materials, including pore water.

The layers of the 0-25 cm peat profile were mixed, and possibly with vegetation? The plant material are serving easily decomposable material for microbes and could enhance their activities and differently in bog peat (moss dominated?) and fen peat (sedge vegetation?). Short-term effects on peat microbiology were studied here without impact of vegetation. If the response of the overall ecosystem is the topic then the measurements should be conducted with intact peat cores including vegetation and primary production. Response of vegetation to nutrients surely has effects also on the microbial activities. The lack of plant activity (carbon release and nutrient uptake) is causing inaccuracies when the aim of the incubations is to mimic non-growing and growing seasons. Or can we assume that in this transition phase plant activity is minor and does not have a great importance? Then it has to be stressed in the aims, even in the title, that the transition phase with minor plant growth is studied here.

To effectively address the points raised by the reviewer, we have broken our responses into three parts:

Point 1) “The layers of the 0-25 cm peat profile were mixed, and possibly with vegetation?”

The soil profiles on peat plateaus at Scotty Creek have broadly two distinct layers, an upper layer containing living and lightly decomposed organic matter, and a lower layer of peat in a more advanced stage of decomposition. Although the distinction of peat layers is not always clear, we removed the topmost surface layer with fresh, large plant debris from each core. Although it may have left some plant debris, it was not our aim to test the activity of live vegetation, which was also not possible by freezing the cores after the field sampling to transport them to our lab in Onatrio. We now state this ~~more~~ explicitly in the revised manuscript. “The top layer containing fresh, large plant debris was removed from each peat core.” [revised l. 145-146]

Point 2) “The plant material are serving easily decomposable material for microbes and could enhance their activities and differently in bog peat (moss dominated?) and fen peat (sedge vegetation?).”

We agree with the reviewer. In our study, we focussed on the effects of nutrient enrichment on the decomposition of the soil organic matter. Increased decomposition means less carbon preservation and enhanced soil CO₂ and CH₄ emissions, and vice versa for decreased decomposition. The reviewer is correct that the different vegetation types deliver organic materials of different reactivities to the soil microbial communities and, hence, play a crucial role in soil respiration and fermentation processes.

Point 3) Short-term effects on peat microbiology were studied here without impact of vegetation. If the response of the overall ecosystem is the topic then the measurements should be conducted with intact peat cores including vegetation and primary production. Response of vegetation to

nutrients surely has effects also on the microbial activities. The lack of plant activity (carbon release and nutrient uptake) is causing inaccuracies when the aim of the incubations is to mimic non-growing and growing seasons. Or can we assume that in this transition phase plant activity is minor and does not have a great importance? Then it has to be stressed in the aims, even in the title, that the transition phase with minor plant growth is studied here."

As stated above, we would like to emphasize that the aim and scope of this study was not to examine the overall ecosystem response to nutrient amendments. Rather, we want to bring attention to the potential changes increased inputs of N and P may cause on the ability of the resident microbial populations to degrade the soil organic carbon store. Whole ecosystem carbon gas exchanges are better captured by measurements with flux towers or closed chamber systems as reported, for example, by Chasmer et al. (2012) who compared net chamber CO₂ fluxes among the fens, bogs and peat plateaus at Scotty Creek (note; we have added this reference to the revised manuscript [revised l. 267-269]). Soil organic matter incubation experiments, such as those presented in our study, provide complementary data that help interpret the ecosystem fluxes.

Chasmer, L., Kenward, A., Quinton, W., & Petrone, R. (2012). CO₂ Exchanges within Zones of Rapid Conversion from Permafrost Plateau to Bog and Fen Land Cover Types. *Arctic, Antarctic, and Alpine Research*, 44(4), 399–411. <https://doi.org/10.1657/1938-4246-44.4.399>

The second comments on the mixing of 0-25 cm peat cores is the possibility that layers of different oxygen status in situ were mixed. Then the anaerobes in deeper profile had not their optimum growth conditions in the incubations. Also the populations of aerobic microbes, including methane oxidizers, could have been "diluted" and their real activity was not included to the net release of methane. The water table of the sites should be given. Water status of the peat incubated (lines 110-111) is not clear. Does 80 and 100 % mean water content related to the water holding capacity of the peat?

We thank the reviewer for these insightful comments. These and similar ones by the other reviewers have made us thoroughly revise our methods section to remove any ambiguities about the experimental procedures and conditions and their relationships with in-field conditions. Specifically, we now include the following statement:

"The moisture conditions for the incubation experiment were selected using field-based growing-season averages measured in adjacent meteorological stations equipped with soil sensors. The selected moisture conditions were a volumetric water content of 80% for the bog peat and 100% for the fen peat. These values were then used to calculate the volumes of artificial porewater to add to the experimental containers." [revised l. 174-178]

Note that the water contents of the peat samples were measured gravimetrically (i.e., drying of the sample to measure the water mass loss), and the resulting values converted to volumetric ratios (the conversion equation and related data values are provided in the linked public dataset – DOI given in the Data Availability Section). The water contents for the incubation samples were those

corresponding to saturation to reflect the field conditions. This aligns with the goal of the experiment to test nutrient enrichment scenarios relevant to possible conditions created by the combined occurrence of wildfires and increased hydrological flows due to thermokarst intensification. Admittedly, our experimental scenarios are not exhaustive and cannot simply be translated to whole-ecosystem impacts in northern peatlands. Nonetheless, we feel that the real value of our findings is to highlight the need for further studies on nutrient enrichment in these peatlands. This is also the main reason for choosing publication as a *Short Communication* type article in the SOIL journal to reach researchers interested in global soil and environmental changes.

A basic question for the conclusions is the number of replicates. The two replicates do not allow proper statistical analyses to compare the treatments.

We agree and caution against over-generalizing the experimental results. We were limited by the amounts of peat soils available and were only able to run duplicates. Note, however, that each incubation reservoir cycled through several temperature steps. The CO₂ and CH₄ production rates measured during these individual temperature steps contribute to assessing the internal consistency of the results.

Other comments:

The effects of N and P salts on CO₂ and CH₄ evolution from two different peat were studied. The measured CO₂ evolution reflects CO₂ production from anaerobic and aerobic microbial processes. As pointed out above CH₄ evolution is the sum of CH₄ production and consumption. The peat studied here is taken from subarctic peatlands. There the effects of added nutrients on CO₂ and CH₄ evolution have not been intensively studied. However, we can well assume that the results on CO₂ and CH₄ from boreal peatlands treated with nutrients are useful when discussing the results obtained here - the basic mechanisms are the same. However, the literature from boreal peatlands has not been considered here. Especially the effects of N on CO₂ and CH₄ dynamics have been studied intensively.

We agree that the effects of N deposition in boreal peatlands have been studied in relation to plant-soil interactions. The aim of our study, however, was to compare the impacts of both N and P additions to soil organic matter degradation, within the context of intensifying wildfires and thermokarst (e.g., Gustine et al., 2022 plus the other articles cited in the preprint). The conditions addressed by our study are quite unique for the subarctic regions and more akin to the direct runoff of nutrient enriched waters seen in tropical and temperate regions impacted by direct human activities, rather than by long-range atmospheric deposition that has received most attention in northern high latitudes (e.g., Utstøl-Klein et al., 2015; Gong et al., 2019; Leroy et al., 2019; Gong et al., 2024). Thus, we believe our experimental approach may offer a more relevant starting point to address nutrient enrichment in cold regions that are remote from direct anthropogenic land (but that experience accelerating climate warming and the associated increases in permafrost thaw and wildfires). In the final analysis, we refrained from linking our study too closely with those on

boreal peatland while still including them in the referenced literature (e.g., Utstøl-Klein et al., 2015; Gong et al., 2019; Leroy et al., 2019; Gong et al., 2024).

Gustine, R.N., Hanan, E.J., Robichaud, P.R. et al. (2022), From burned slopes to streams: how wildfire affects nitrogen cycling and retention in forests and fire-prone watersheds. *Biogeochemistry* 157, 51–68. <https://doi.org/10.1007/s10533-021-00861-0>

Gong, Y., Wu, J., Vogt, J., Le, T.B. (2019), Warming reduces the increase in N₂O emission under nitrogen fertilization in a boreal peatland. *Sci Total Environ* 664, 72–78. <https://doi.org/10.1016/j.scitotenv.2019.02.012>

Leroy, F., Gogo, S., Guimbaud, C., et al. (2019), Response of C and N cycles to N fertilization in Sphagnum and Molinia-dominated peat mesocosms. *J Environ Sci* 77, 264–272. <https://doi.org/10.1016/j.jes.2018.08.003>

Gong, Y., Wu, J., Roulet, N., Le, T. B., Ye, C., & Zhang, Q. (2024), Vegetation composition regulates the interaction of warming and nitrogen deposition on net carbon dioxide uptake in a boreal peatland. *Functional Ecology*, 38, 417–428. <https://doi.org/10.1111/1365-2435.14480>

Utstøl-Klein, S., Halvorsen, R. and Ohlson, M. (2015), Increase in carbon accumulation in a boreal peatland following a period of wetter climate and long-term decrease in nitrogen deposition. *New Phytol*, 206: 1238-1246. <https://doi.org/10.1111/nph.13311>

A point which could be considered is the effects of salts as such, without any nutrient effects. There are results showing that extra salts can decrease microbial activities in acidic soils. Could this have an impact in the rather short-term incubation experiments with naturally nutrient poor soils?

The reviewer raises a very good point. Our results do not support a major effect of salinity, however. If the extra salts would have caused a decrease in microbial activity, the N, P and NP amendments (as N and P salts) should have shown decreased carbon gas production rates compared to the no-nutrient (and thus no-salt added) controls, which is not supported by our results. Also, the microbial biomass increase was highest in the bog soil with the highest amount of salt added through both N and P amendments (Table 3).

Please note that with fen peat the fumigation extraction method gave negative microbial biomass at the end of the incubation. This means that the amount of extractable organic C was higher before the fumigation than after the fumigation (end of page 10). Would extra salts have been destroyed a substantial part of the microbes releasing their carbon in incubated salt-treated fen peat?

Completed. The reviewer is correct: the negative biomass is based on the sample weight measurements before and after the fumigation. It is hard to imagine that the organic matter decomposition inferred from the CO₂ and CH₄ production would occur without microbes. Thus, the inferred negative biomass for the fen soil is likely a methodological artefact, as we now state in the revised manuscript: In Methods, “The resulting values from these sample measurements were compiled into a calculation spreadsheet to derive MBC, MBN and MBP values for each peat

sample (Byun et al., 2024).” [revised l. 243-244] and in Results and Discussion, “It is difficult to imagine that the observed CO₂ and CH₄ production throughout the incubation period could have occurred without microbes in fen soil samples. Thus, the inferred negative biomass for the fen soil was considered a methodological artefact.” [revised l. 343-345] (including a reference to the public accessible dataset for this experiment).

Byun, E., Rezanezhad, F., Slowinski, S., Lam, C., Saraswati, S., Wright, S., Quinton, W., Webster, K., Van Cappellen, P.: Dataset for Examining the Effects of Nutrient Pulses on Biogeochemical Cycling in Subarctic Peatlands in the Context of Permafrost Thaw and Wildfires. Federated Research Data Repository. 10.20383/102.0712, 2024

Some references if exist could be given about the experiences to apply fumigation extraction method for peat. Are there reports on problems, e.g. negative biomass? If the negative biomass for fen after the incubation is not a result of biomass decomposition, then we can ask if the method has inaccuracies to determine microbial biomass in peat in general (also for bog here)?

Completed. We appreciate this point and see the need of exploring this issue further. A negative biomass result is not entirely surprising given the method’s multiple steps of sample treatment and repeated weight measurements each introducing some measure of uncertainty. We have further clarified this aspect of the fumigation method in the revised manuscript as well as in our replies to the above comment [revised l. 243-244; 343-345]. Please also note that we only had limited amounts of soil sample for the fumigation methods (several aliquots were needed for the other chemical analyses).

In the Fig. 4 microbial biomass carbon and nitrogen are used in the calculations also for fen peat although there is a comment at the end of page 10 that after the incubation the microbial biomass could not be determined for fen with the fumigation extraction method? Did the method give positive biomass for fen before the incubation (initial biomass) but negative biomass just after the incubation (see the comments above for the possible salt effect and methodological problems).

Completed. The reviewer is correct: the fumigation method gave a positive initial value for the fen soil. Full details for the method and the obtained values are given in the associated open dataset “The resulting values from these sample measurements were compiled into a calculation spreadsheet to derive MBC, MBN and MBP vales for each peat sample (Byun et al., 2024)” [revised l. 243-244]. For this reason (and those above), we used the initial biomass elemental compositions to interpret the compositional changes in the nutrient porewater ratios that occurred over the course of the experiment. We have clarified this in the revised manuscript. “We conducted the incubations in the dark without adding any organic carbon substrates. Thus, we interpret the observed changes in the porewater pools of C, N and P to be the result of the processing of existing C, N and P soil pools coupled to the net mineralization of soil organic matter by the resident microbial community. The changes in the pore water DOC concentration and the stoichiometric ratios of DOC to dissolved N and P, respectively, are shown in Fig. 4. The initial microbial biomass stoichiometry is added as a dotted line in each panel of Fig. 4.” [revised l. 363-368]