RC1: 'Comment on egusphere-2024-1047', Anonymous Referee #1, 19 Jul 2024

The authors investigate the effects of nutrient enrichment that comes with wildfires and permafrost thaw in subarctic peat lands on soil CO2 and CH4 production. While they name "subarctic peat lands" in the title, they investigate basically samples from two sites in the same region of Canada. That means the title promises more than is presented in the manuscript. The choice and combination of methods is valid, however, the core concern regarding this manuscript is the fact that the authors used only four samples. I doubt that the informative value of this set-up is sufficient. While the experiment itself is interesting and promising, I strongly recommend to add more samples to the experiment and do/repeat the experiment at least in duplicates, better in triplicates (of every single sample) to check if the obtained data is valid. In the given form I can not recommend the manuscript for publication as I am not convinced that the extent of the experiment is sufficient to support the authors' conclusions. However, it might be an option to address this clearly and adjust the discussion accordingly.

We thank the Reviewer for thoroughly reviewing the preprint and providing constructive criticisms. We would like to confirm that for each of the two peat soils, the incubations were carried out on duplicates [preprint l. 119]. All the raw data have been deposited in the public repository (DOI is given in the Data Availability Statement).

Completed. We agree with the reviewer's specific remark on the title of our manuscript. Another reviewer also noted that the title was too broad. We have therefore revised the title to more precisely reflect the scope of our study. It now reads:

"*Effects of nitrogen and phosphorus amendments on CO_2 and CH_4 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*"

We definitely intend to conduct future experiments to assess the generality of the results presented here. Nonetheless, we believe our results, even if preliminary, are timely to communicate an emerging topic with the (peat) soil science community and discuss the need for future research [preprint l. 26-27]. Sharing these preliminary findings through the interactive peer review process of SOIL journal offers one way to achieve such a goal. Thus, we revised the results and discussion section in a concise manner and turned the manuscript in a *Short Communication*. The latter specifically aims to "report new methods or small data sets that have significant implications." (https://www.soil-journal.net/about/manuscript_types.html). In revising our preprint manuscript, we have addressed the reviewer's comments and present the changes here, along with line numbers.

The quality of the language is good and causes at no point problems regarding the comprehensibility. However, the manuscript needs some polishing it should be taken care that a more scientific language is used. The introduction needs to be restructured and rephrased in some parts. Very often, single sentences or parts of the introduction are not connected to each other and therefore the authors' reasoning is not clear. The authors also use very general terms frequently without explaining their meaning in the given context. With the Methods section, it is the same - some parts are more evocative of a staccato than of a coherent text.

Completed. Thank you for pointing out the need to improve the writing style and clarity of the manuscript. In the revised version of the manuscript, we made consistent efforts to improve the structure of the Introduction and the explanations in the Methods sections. Also, we have carefully followed the referee's suggestions in the below point-by-point responses.

Please find my detailed comments below:

Title

Having read your manuscript, it is clear what you want to express using this title. However, I suggest to change it slightly to arouse interest in more readers, for instance, "complexity" is a very broad term. In addition, just mentioning "subarctic peatland" suggest that you investigated samples from several subarctic areas - you should clarify this.

Completed. We agree and have revised the title accordingly to: "*Effects of nitrogen and phosphorus amendments on CO_2 and CH_4 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*"

Abstract

l. 15-17 Why is an increasing N and P concentration a growing concern in this context? This should be made clear.

Completed. The adverse impacts of excessive soil nutrients have been studied in other regions [preprint l.14-15]. We propose that subarctic peatlands may also be experiencing rising exposure to nutrient inputs generated by wildfires and permafrost thawing [preprint l. 15-16]. Due to climate change, wildfires are causing the release of more of the macronutrients nitrogen and phosphorus while permafrost thaw increases the hydrological connectivity within peatland regions [preprint l. 16-17]. However, as the reviewer's comment suggests, the message was not clearly coming through in the preprint article. We have completely revised the abstract, which now reads [revised 31-49]:

"Impacts of nutrient enrichment on soil carbon cycling have been extensively studied in temperate and tropical regions where intensive agriculture and land development have led to large increases in anthropogenic emissions of nitrogen (N) and phosphorus (P). However, how soil carbon sequestration and soil-atmosphere gas exchanges in cold regions respond to greater inputs of N and P remains poorly known, despite recent observations showing significant increases in porewater N and P in burned subarctic peatlands and downstream waters. Wildfires plus enhanced hydrological connectivity due to permafrost thaw have therefore the potential to change carbon turnover and gas emissions in soils of northern peatlands. To start exploring the sensitivity of peatland soil biogeochemistry to variations in N and P availability, we measured the carbon dioxide (CO_2) and methane (CH_4) production rates during a month-long incubation experiment with soils from a bog and fen collected at the long-term Scotty Creek research station in the Northwest Territories, Canada. Subsamples of the peatland soils were divided into a series of containers to which artificial porewater solutions were added. These solutions were amended with

either dissolved inorganic N, dissolved inorganic P, or both N and P together. Unamended controls were run in parallel. The containers cycled through pre-set temperature steps of 1, 5, 15, and 25°C. Overall, the fen soil yielded higher CO_2 and CH_4 production rates than the bog soil. Amendment of N to the bog soil produced more CO_2 compared to its control, while amendment of P increased CO_2 production in the fen soil. Amendment of N and P together reduced CO_2 production but increased that of CH_4 in both the fen and bog soil incubations. Porewater chemistry at the end of the 30-day experiment showed aqueous C, N, and P stoichiometric ratios that trended toward those of the soil microbial biomasses, hence, implying that the initial microbial nutrient status played a crucial role in determining the responses to the different nutrient amendments. Our preliminary results show that the effects of nutrient enrichment on peatland soil biogeochemistry requires further investigation."

l. 18 I suggest to add "which" here: "...from subarctic bogs and fens, which aimed at..."

Completed. We agree with this suggestion and now explicitly mention "subarctic peatlands" [revised. 35] and clarify the provenance of the soils ("Scotty Creek research station in the Northwest Territories, Canada") in the revised abstract [revised l. 39-40].

l. 23-25 Please rephrase this sentence to make it clearer ("It was unexpected..."). Do you mean N and P were added together or both treatments?

Completed. Thank you for this comment. We meant the addition of N and P together. We have change the phrasing in the revised manuscript accordingly as follows: "Amendment of N and P together reduced …" [revised l. 44-45].

l. 20-26 It is not really clear how you treated the samples. You had three treatments + control (N, P, N+P, none), but what about the different temperatures you name in line 21? And how long did your experiment last? In line 25 you mention "after a month" - was this the end of the experiment or just the point where you observed that C, N, and P ratios approached initial soil microbial biomass ratios? This needs to be more precise.

Completed. We agree that a more precise formulation would be helpful and have revised the abstract accordingly. We invite the reviewer to read the revised abstract above. The full duration of the experiment is now explicitly given ("a month-long incubation experiment"; "at the end of the 30-day experiment"). The temperature cycling through pre-set temperatures is also explicitly stated ("pre-set temperature steps of 1, 5, 15, and 25°C"), as well as the timing of the pore water chemistry ("Porewater chemistry at the end of the 30-day experiment"). [revised l. 39; revised l. 43-44; revised l. 46]

l. 26 What do you mean by "nutrient recycling" here and how do the approaching ratios suggest this? Please explain.

Completed. In the revised abstract, we are no longer using the term "nutrient recycling" but point to the observed convergence of the pore water nutrient ratios to those of the microbial biomasses as an indicator of the role of the initial microbial nutrient status as a determinant of the observed responses to the N and P amendments. A full (and admittedly speculative at this stage) explanation

of this observation can be found in the main text of our manuscript (this would be too long to include in the abstract). The statement in the abstract now reads:

"Porewater chemistry at the end of the 30-day experiment showed aqueous C, N, and P stoichiometric ratios that trended toward those of the soil microbial biomasses, hence, implying that the initial microbial nutrient status played a crucial role in determining the responses to the different nutrient amendments." [revised l. 46-49]

Introduction

l. 30-32 In line 30 you name "subarctic regions", in line 31 you refer to "the region". In line 32 you name additionally western Canada, Siberia, and Alaska. Which region is meant here? Do western Canada, Siberia, Alaska not belong to (or contain) subarctic regions? Please check these sentences and clarify your statements.

Completed. We agree that a more precise use of geographical terminology is called for. Western Canada, Siberia and Alaska host parts of the subarctic regions. We have now replaced "the region" to "the subarctic regions" (plural) in the revised manuscript to be consistent and explicit [revised l. 53].

l. 36-40 Again, please explain why increasing nutrient inputs are a rising concern here. Above, you only mentioned the increase in POC and DOC, you do not give a reason for the enrichment in N and P.

Completed. Upon reflection, we have dropped the "rising concern". Our point is that recent observations have reported substantial enrichment of dissolved nitrogen (N) and phosphorus (P) in western Canada [preprint l. 37-40 and Table 1]. We realize that the statement "as summarized in Table 1" was out of context and not meant to refer to the case studies on DOC and POC increases cases. Thus, we have now corrected the two related sentences in the revised manuscript [the phrase was removed from revised l. 56-57 and placed at revised l. 63 where it is relevant].

Additionally, we have reformulated the motivation for our experiment as follows: "Increasing N and P inputs to peatland soils have the potential to perturb soil carbon cycling in peat soils. For instance, by relieving nutrient limitations on the soil microbial community, additional N and P may accelerate the decomposition of soil organic matter." [revised l. 59-61]

l. 40-44 I suggest to separate the two parts of this sentence and emphasize the effects of permafrost thaw you name here. And still, you do not mention why all of this is a problem. What is the effect of dissolved nutrients? What is the effect of an increased hydrological and geochemical connectivity?

Completed. Thank you for this suggestion, which is helpful in clarifying this part. We have revised the text and separated the sentences as follows:

"Thawing permafrost may release additional dissolved nutrients previously bound in frozen sediments (Treat et al., 2019; Schuur et al., 2022; Wright et al., 2022). Furthermore, at the

landscape level, permafrost thaw-induced ground subsidence increases the hydrological and geochemical connectivity among landscape components, including forested areas, fens, thermokarst bogs, and adjacent peatlands (Connon et al., 2014; Gibson et al., 2018; Haynes et al., 2018; Post et al., 2019; Carpino et al., 2021). Thus, there is growing probability for organic carbon stored in peat soils to experience enhanced microbial decomposition driven by nutrient enrichment." [revised l. 64-70]

l. 52 Where does "generally most poor in soil nutrients" refer to? To the N-fixing microbes? And what do you mean by "most poor"?

Completed. We admit that 'most poor' did not fully reflect the intended context. We meant to emphasize the nutrient poor status of isolated bogs among the various peatland types in the subarctic and boreal regions. To avoid confusion, especially for those who are not familiar with this ecosystem type (*i.e.,* peatland), we have changed the sentence in the revised manuscript to:

"For example, isolated ombrotrophic bogs rely on direct atmospheric deposition of N and P plus microbial N fixation. Comparatively, minerotrophic channel fens capture nutrients from surrounding water bodies through groundwater and surface water pathways." [revised l. 77-80]

l. 53-56 Here it could help to add one sentence that describes the impacts in temperate and tropical peatland soils.

Completed. Thank you for this comment and suggestion. We have now expanded this part in the revised manuscript to describe the impacts of increasing nutrient in temperate and tropical peatland soils with more specific cases:

"Rapid increases in nutrient inputs to peatlands have long been studied in temperate and tropical regions, where there is a higher chance of fertilizer spillover and growing agriculture or fossil fuel-driven atmospheric deposition (Amador and Jones, 1993; Hoyos-Santillan et al., 2018; Lin et al., 2014; Moore et al., 2019; Qualls and Richardson, 2000; Schillereff et al., 2021). Early research on subtropical peatlands in the Everglades, Florida, showed that experimentally P-enriched soils released significantly more CO_2 than controls, seemingly because the added nutrient P stimulated soil heterotrophic respiration (Amador & Jones, 1993; Qualls & Richardson, 2000). Recently, however, a study suggested that nutrient availability is not always a rate-determining factor as microbial communities in tropical peatland soils have developed strategies to process site-specific plant litter even without adequate nutrient supply (Hoyos-Santillan et al., 2018). Still, other studies support the important control of nutrient increases in peat decomposition rates. For example, a data synthesis across temperate regional bogs revealed that a long-term P inputs to surface peat enhanced microbial decomposition activities and reduced net organic C burial to deeper peat layers (Schillereff et al., 2021). Another study of a 12-year P fertilization field experiment of temperate mountainous peatlands showed that the extra P supply increased soil respiration and CO_2 release, reducing the overall carbon sink function of these peatland ecosystems (Lu et al., 2022)." [revised l. 83-96]

l. 60-63 The connection between the first part ("Recent observations...") and the second one ("but the effects...") is not clear.

Completed. Thank you for this comment. We have now revised the sentences to make the connection clear in the revised manuscript by changing the sentences to: “Recent observations highlighted the increase of wildfire impacts, the acceleration of permafrost thaw and the degradation of permafrost stability. As a result, peatland ecosystems and their associated hydrological processes and pathways are rapidly transforming (Gibson et al., 2018). However, the potential effects of sudden nutrient inputs to peatland soils have not been explored for subarctic regions, despite the possible increase of organic carbon decomposition and carbon greenhouse gas emissions suggested by studies in other climate zones.” [revised l. 110-115]

l. 64 To me it is still not clear what you mean by "nutrient recycling".

Completed. We appreciate this comment on the term “nutrient recycling” used in this manuscript. We meant to describe the microbial utilization of nutrients from in-soil stores (*i.e.,* soil minerals and organic materials, including their necromass), rather than from external inputs. Based on this comment, as well as other detailed comments by the Reviewer, we acknowledge that such recycling cannot be readily inferred from the experimental results presented here. Thus, we have revised the sentence to not include this terminology. It now reads: “In this study, our aim was to investigate the potential acceleration of organic matter decomposition and carbon gas production in subarctic peatland soils following the addition of readily bioavailable dissolved inorganic N and P.” [revised l. 115-118].

Methods

Figure 1 Please check the caption. It could also help to mark your sampling area in the small map in the upper left corner.

Completed. Thank you for pointing this out. We have revised the caption as well as Figure 1 accordingly. The sampling area is marked in the small inset map in the upper left corner in the revised Figure 1.

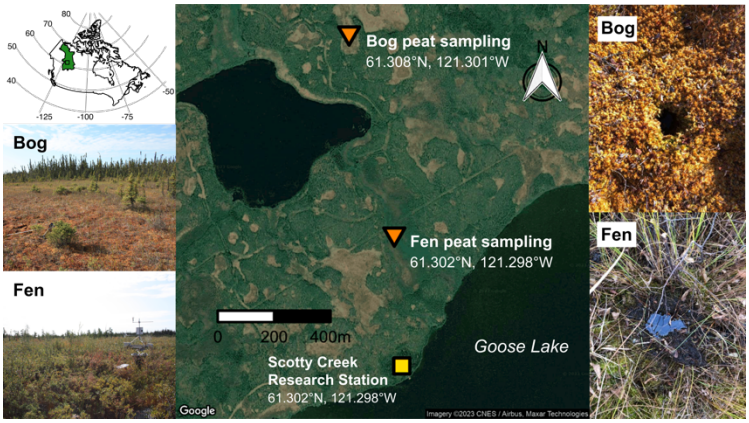

Figure 1: Scotty Creek watershed bog and fen peat sampling site locations (Map data: Google ©2023 CNES / Airbus, Maxar Technologies) and photos (credit: Mason Dominico). The location and extent of the Taiga Plains Ecozone of Canada (National Ecological Framework for Canada, 2017) is shown in the inset map with a small box indicator for the site locations.

l. 93-96 Please split this sentence in at least two sentences. And please clarify its second part: it should be made clear that the named steps belong to the MgNO3 digestion method.

Completed. Thank you for this comment and suggestion. We have now clarified this part in the revised manuscript as follows: "In addition, a sub-sample of each of the peat soils was freeze-dried for analysis of the total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) concentrations. TOC and TN were determined using a CHNS analyzer (Carlo Erba NA-1500 Elemental Analyzer; detection limit of 1% by mass for both TOC and TN). TP was determined following the method of Aspila et al. (1976): an aliquot of soil was ashed in a muffle furnace at 500 °C with magnesium nitrate added as an oxidant. The ashed soil was subsequently extracted by mixing with 1 M hydrochloric acid on a shaker for 16 hours. The extract was then analyzed for total dissolved P by ICP-OES (Thermo Scientific iCAP 6300 Duo)." [revised l. 148-155].

l. 96-97 I do not understand the last sentence here. Do you mean you did not attempt to compare the pools prior to the treatment with the pools after the treatment?

Completed. Thank you for this comment. The sentence meant to express technical concerns with expressing the TOC, TN and TP mass concentrations relative to the peat soil weight, given the contributions to the total soil weight from salts. However, this is considered to have had very minor effects on the results presented. In addition, all measured values are accessible in the associated public dataset. Given the potential confusion our original comment may create, we have deleted this sentence in the revised manuscript. [revised l. 155-156]

l. 101-104 Please rephrase this part.

Completed. Thanks for this suggestion. We have now revised this part in the revised manuscript as follows: "The pre-incubation caused some reduction of the peat moisture contents. We therefore re-measured the moisture contents at the end of the pre-incubation period by the oven-drying method to determine how much porewater solutions needed to be added to return the peat moisture contents to the selected levels. Note that the latter were selected to be close to average field conditions." [revised l. 160-165]

l. 105-106 Why did you measure microbial biomass C, N, and P after the pre-incubation?

We estimated the microbial biomass C, N, and P contents after the pre-incubation to directly compare the elemental contents at the start and the end of the incubation. This enabled us to see the impact of the various nutrient treatments of the microbial nutrient status (i.e., the end of the pre-incubation period yielded the baseline nutrient status). We have clarified this point in the revised manuscript: "After the pre-incubation, each of the bog and fen peat samples was subsampled for fumigation to measure microbial biomass C, N and P concentrations. These concentrations were considered the baseline values against which changes in microbial C, N, and P accompanying the various nutrient treatments were evaluated." [revised l. 167-169]

l. 102-103/108-111 You repeated the measurement of moisture content after pre-incubation to decide how much porewater solution should be added, but then you use data from adjacent meterorological stations to obtain this information? Please explain what you did clearly and in comprehensible steps.

Completed. Thank you for this comment. We have now clarified the steps in the revised manuscript as follows: “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]

l. 165-166 "The subsamples were treated either with chloroform for 24 hours to fumigate in a vacuum desiccator, or with no fumigation."? Please explain why, this way it sounds like you just randomly fumigated some samples and some not.

Completed. Thank you for this comment. We followed the standard fumigation method described in the literature (Brookes et al., 1984; Vance et al., 1987; Joergensen, 1996; Jenkinson et al., 2004 cited in the preprint). We have now clarified the experimental procedure in section 2.3.2 of the revised manuscript. [revised l. 228-244]

Results and Discussion

l. 190 It should be "resulted in" (whole section, not only this line).

Completed. Thank you for this comment. We have now made the corrections throughout the revised manuscript.

l. 192-194 "...are consistent with its higher microbial biomass than the bog initially."? Please check again thoroughly your manuscript if references within sentences are correct.

Completed. Thank you for this comment. We have now changed this statement to: “The higher CO_2 and CH_4 production rates in the fen soil are consistent with the higher initial biomass of the fen soil (MBC 4.3 mg C g^{-1}) compared to that of the bog soil (MBC 0.7 mg C g^{-1}).” [revised l. 266-267]

l. 196-203 It would massively enhance the comprehensibility if you would focus on one treatment after the other.

Completed. Thank for this comment and suggestion. We have changed the text accordingly to [revised l. 278-285]: “Temperature variably affected the gas production rates in the different nutrient treatments. The bog peat soil with added P and NP (thus, P amendment effects in general) yielded distinctively lower CO_2 production rates at 25°C but higher CO_2 production rates at 15°C, compared to the no-nutrient control (Fig. 2a). The fen peat soil with added N and NP (thus, N amendment effects in general) resulted in lower CO_2 production rates at 25°C but no apparent difference at 15°C, compared to the no-nutrient control. For the fen peat soil, the P only amendment (i.e., without N) resulted in distinctively higher CO_2 rates than the N amended treatments (N only and NP) and the control (Fig. 2b). The CH_4 production rates of the fen soil

also showed noticeable temperature effects with the greatest differences between the various nutrient treatments observed at 25°C (Fig. 2b)."

l. 216 Why do you refer to Table 2 here?

Completed. Thank you for noting this. This was a mistake and it has been now corrected. The reference to Table 2 was not meant for this particular sentence, but for subsequent ones. [revised l. 299]

Table 2 Given are the Q10 values - why don't you mention this in the caption? Why are there no values for bog CH4?

Completed. Thank you for this comment and suggestion. We have now added a sentence to the methods section to explain why no Q_{10} values were given for bog peat CH_4 effluxes: "However, the CH_4 production rates in the bog peat containers were very low and yielded too little variation to derive meaningful Q_{10} values for CH_4 production." [revised l. 295-296]

l. 222 Here, it is 3.89 - in the table it is 3.90. In gnereal: Is your method sufficiently precise to give two decimals?

Completed. We appreciate the reviewer's concern. Providing two decimals for the Q_{10} estimations may give a false impression of the representativeness of the reported flux rates considering the limited number of sampling locations and number of experimental incubations. Therefore, we now present the Q_{10} values to one significant digit in the revised manuscript [revised Table 2]. Note that the individual gas flux measurements themselves are very precise and are thus given with the appropriate decimals in the open dataset made available with the manuscript.

Figure 3 Your caption should make clear which differences are given: "Relative differences (%) between x and y in the..."

Completed. Thank you for this comment and suggestion. We have now revised the caption to: "Relative differences (%) in cumulative CO_2, CH_4, and total carbon gas (Gas C) emissions integrated over the entire incubation experiment: comparing the unamended control (Ctrl) and the nutrient amended treatments N (N only), P (P only) and NP (both N and P together)." [revised l. 337-339]

l. 263-270 These are very general statements and should be better connected to your data.

Completed. Thank you for this comment. We agree that these general background statements related to possible organic carbon changes by microbial processes are not point-by-point connected with the data of this study. Accordingly, we have decided to move this part of the text to the Introduction where it now appears before stating the specific aims of our study. Subsequently, we also rephrased some parts for the context: "Given the high accumulation of plant organic materials in peatlands, the absolute amount of carbon as substrates for microbial activities is assumed not limiting in peat soils. Microbial strategies to overcome relative nutrient imbalances of N, P or other nutrients relative to carbon may fall into three broad categories: (1) increasing the production of

extracellular enzymes to acquire necessary nutrient elements from enzyme-facilitated organic matter degradation, (2) recycling of the nutrient elements assimilated in dead microbial cells (i.e., necromass recycling), and (3) releasing some extra carbon in relation to the other major nutrients via auxiliary respiration without biomass assimilation (i.e., overflow respiration) (Giesler et al., 2011; Manzoni et al., 2012; Lin et al., 2014; Hoyos-Santillan et al., 2018; Schillereff et al., 2021; Lu et al., 2022). Laboratory soil incubation experiment to measure the changes in carbon gas production rates with net microbial biomass change can help address which strategy is activated. [revised l. 98-106]

l. 273-275 Please rephrase this sentence.

Completed. Agreed – we have now rephrased this sentence in the revised manuscript to: "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." [revised l. 363-368]

Figure 4 "The shades ara indicates where the initial porewater ratios are marked"?

Completed. Thank you for this comment. We have now revised the caption of Figure 4 to clarify this as: "The blue shade indicates where the initial (i.e., at the start of the incubation experiment) compositions are marked in comparison to the compositions at the end of the incubation." [revised l. 399-401]