

Biogeosciences Discuss., author comment AC2
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Reply on RC1

Jenie Gil et al.

Author comment on "Sources of nitrous oxide and the fate of mineral nitrogen in subarctic permafrost peat soils" by Jenie Gil et al., Biogeosciences Discuss.,
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We thank the reviewers for their interest in our work and for their insightful comments that have greatly contributed to improve our manuscript. We have addressed the general and specific comments provided by the reviewers and have made necessary changes accordingly to their indications as follows:

Comment on bg-2021-228

Anonymous Referee #1

Referee comment on "Sources of nitrous oxide and fate of mineral nitrogen in sub-Arctic permafrost peat soils" by Jenie A. Gil et al., Biogeosciences Discuss.,

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The manuscript by Gil et al. addresses a pronounced research gap by investigating gross N turnover and inorganic N fates as well as N₂O emissions in permafrost peatlands, including N₂O source partitioning. They report much higher gross ammonification and nitrification for vegetation free compared to vegetated peatlands, which is explained e.g., by absent plant competition for N. Such detailed N cycle process knowledge is very scarce for permafrost ecosystems, which still makes prediction of permafrost nitrogen climate feedbacks highly uncertain. So this clearly is a timely study even if the experiments were already conducted more than 10 years ago. The overall manuscript quality is fine.

The quality of such field ¹⁵N studies to assess gross N turnover is strongly depending on a thorough experimental setup and this difficult task mostly appears to have been done very competent and thoroughly. On the other hand,

1. The chosen experimental setup with mirrored ¹⁵N labelling and all of its advantages and disadvantages was obviously designed to run the Ntrace model to estimate N turnover rates which then was not done. So the reader wonders, why not?

A: The experimental design was chosen with the aim to quantify the relative contribution of nitrification and denitrification to the overall N₂O fluxes from the bare peat surfaces (BP) using single and double ¹⁵N labeled ammonium nitrate method, previously

introduced by Baggs et al., (2003). Further, this experimental set-up allowed us to apply the traditional pool dilution technique to assess gross N transformation rates (mineralization, nitrification) in the field (Kirkham and Bartholomew, 1954). We considered that even though ^{15}N tracing studies in combination with analyses via process-based models are the current "state-of-the-art" technique to quantify gross nitrogen (N) transformation rates and N_2O emission pathways in soils, it would be very ambitious to apply the Ntrace model in an arctic peat soil in a field experiment as our first attempt to study N cycling in this soil. We wanted to first use a simpler approach and learn how the system behaves, before using the Ntrace model.

Although the Ntrace model has been used successfully for determination of gross nitrogen transformations in field studies and from organic soils (e.g., Holz et al., 2015), it has never been successfully applied to study nitrogen cycling in situ in natural peatlands so far. This could be related to the fact that the accurate quantification of ^{15}N -label in the mineral N pools in organic soils is quite challenging (Mortland and Wolcott, 1965; Nömmik and Vahtras, 1982; Nieder et al., 2011). Additionally, high rate of microbial immobilization commonly causes problems for ^{15}N tracing studies in soils with low mineral N availability, typical for arctic and sub-arctic soils: the ^{15}N labeled mineral N can be very quickly immobilized and, thus, cannot be seen in the soil extracts (Nordin, Schmidt & Shaver, 2004; Sørensen et al., 2008, Marushchak et al., 2021). In our study, the ^{15}N label was recovered mostly in the bulk soil (up to 79%) during the entire experiment period, supporting the suggestion that biotic and/or abiotic process contribute to remove the ^{15}N added from the extractable N pool. The uncertainties associated with these immobilization processes cause problems for the Ntrace model, and we need more knowledge about the fate of ^{15}N after application before the model can be applied for peatlands in general, and Arctic peatlands in particular.

2. Due to addition of ammoniumnitrate in all treatments, gross nitrification rates are likely stimulated by substrate addition, which needs to be considered and discussed.

A: The application rate of the label solutions was based on soil inorganic N concentrations measured from soils extracts from previous years (2007-2008). We have applied approx. 50% of the soil average inorganic N concentrations measured previously to avoid or minimize the effect of substrate adding on the soil N processes. The total amount also never exceeded maximum amounts measured in the soils. All this information is mentioned already in the manuscript (see below). Importantly, the contribution of N_2O producing processes is likely not impacted, which was the main focus of the MS. We discuss this in the MS in lines 14 to 23 on page 15:

"The concentration of inorganic N was at most doubled by adding labelled NO_3^- and/or NH_4^+ , but the final nutrient content never exceeded maximum content of native NO_3^- or NH_4^+ observed in the soil (data not shown). The bulk N_2O fluxes from the labelled plots ($\sim 10 \text{ mg N}_2\text{O m}^{-2} \text{ d}^{-1}$) were still within the range of N_2O fluxes observed in previous years from BP surfaces ($1.9 - 31 \text{ mg N}_2\text{O.m}^{-2}\text{d}^{-1}$) (Repo et al., 2009; Marushchak et al., 2011). The differences in the N_2O fluxes from BP labelled and non-labelled plots could be also attributed to the natural spatial variation in the N_2O fluxes within the BP surfaces, which can be large even on small spatial scales ($< 1\text{m}$, personal observation. data not shown). The N_2O emissions from labelled and non-labelled plots had similar responses to changes in temperature ($R^2 = 0.391$, $p < 0.005$), which was likely the major factor controlling the temporal variation in the N_2O fluxes from BP surfaces during the study period. Even if some stimulation occurred, there likely was no change in the relative contribution of various processes underlying the N_2O emissions because BP surfaces were not N limited during the study period (see discussion below)."

3. Another issue could be that gross rates of N turnover were calculated based

on day 1 to day 3 data with day 3 being a clear outlier in 15N recovery for bare peat (much lower than at day 1 and day 5; Fig. S1) – did this low 15N recovery lead to a bias in gross N turnover estimates, eventually because 15N was quickly leached in some labelling plots?

A: The low recovery of 15N on day three was indeed surprising and a bit difficult to explain. We had teams which were responsible for labelling and sampling on different days, which could have potentially introduced some experimental artefacts and biased the results on total 15N recovery on day 3. However, the extractable N pools which were used to calculate gross N turnover rates and contributions of different processes to N₂O emissions accounted only for a minor proportion of the total 15N pools and followed different dynamics (as in detail explained below). Thus, our main results were not biased by the low recovery of 15N on day 3, and we have sufficient confidence in the gross turnover estimates to report them here (more details below and in the following answer). It has to be also noted that if high leaching losses would have occurred in some labelling plots, mainly the calculations of N consumption would have been affected, and less so the calculations of production; the latter being one of the key aims of the study.

We added a sentence to the manuscript on page 10, line 14 to 16:

“At day 3, total recovery of ¹⁵N was lower than expected and although we have no explanation for these findings, this low recovery did not impact on the main results which were calculated from ¹⁵N in mineral nutrient pools.”

To corroborate the reliability of the gross N turnover rates, we plot here a couple of more figures for the reviewer. In the figure S3a – b (in supplement file with answer to the referee), the natural logarithm of 15N atom percent excess of NH₄⁺ is plotted against sampling time. Given constant rates this plot provides a linear relationship, while declines or increases in isotope pool dilution rates cause curvilinearity. For BP (blue circles) we can observe that curvilinearity starts after ~ day 9, this means that the transformation process rates are constant between 1 h and 9 days ($R^2 = 0.9125$), so we can calculate and report values between this time period for BP. Gross transformation rates for VP were low (open squares) but there was also some linearity between day 1 and day 5 ($R^2 = 0.4195$).

Results are more variable for 15N at% excess of NO₃⁻, but there was a linear relationship over 24 to 72 hours ($R^2 = 0.6957$) for BP (Figure S3c - d). For VP surfaces, gross nitrification rates are negligible. High uncertainty is quite common in field labeling studies (e.g. Cookson et al., 2002; Harty et al., 2017). The high variability in our data could simply reflect the spatial variation at the site. Since constant process rates are a prerequisite for estimating gross N transformation rates (Kirkham and Bartholomew, 1954), we chose to report gross mineralization and nitrification rates for the period between 24 and 72 hours because gross mineralization and nitrification rates for BP were constant during this period (Figure S3). In addition, the changes in 15N at% excess of NO₃⁻ from day 5 (120 hours) in BP surfaces, suggest quick cycles of abiotic fixation and release of NO₃⁻, as mention by the reviewer. To follow the recommendations from Braun et al., 2018 (and references therein) about shorter time period during pool dilution experiments to minimize errors due to re-mineralization of the label added, we decided not to include data from day 5 and beyond in the calculations of gross N process. We also observed low recovery of the 15N in the inorganic N pools at time 0 (1 hour after labeling) so we assumed that the 15N label was not well homogenized after 1h and did not use this time point for the calculations either.

Table S1 and S2 (in supplement file with answer to the referee) summarized gross mineralization and nitrification rates for all sampling points calculated from 24h for both surface types. We would like to mention that while re-calculating the data we have

detected an error in our previous calculations of the gross N transformation rates and have now updated the rates. The gross rates have changed to some extent, but not largely. Our apologies for the previous mistake.

Looking at the table S1, the range of gross mineralization rate for BP at 72 hours is between 1.34 and 5.28 $\mu\text{g N cm}^{-3} \text{ d}^{-1}$. This range included the mean rates calculated for 120 (5 days), 216 (9 days) and 360 hours (15 days). The same is observed for gross nitrification rates from BP (range from -0.01 to 1.75 $\mu\text{g N cm}^{-3} \text{ d}^{-1}$), and gross mineralization rates from VP (Table S2, -0.41 to 1.51). Gross nitrification rates from VP are negligible. Consequently, we are confident that gross N transformation rates calculated for the period from 24 to 72 hours are representative of the gross N transformation rates for most of the experiment period. Exception to this are the gross rates calculated for 24 h and 24 days. To calculate the 24 hours rates, we used the data between 1h and 24h period. We have mentioned already that ^{15}N label added was probably not well homogenized after 1h and we did not include this time point in the final calculations. For 24 days, probably too long period after adding the ^{15}N label and some re-mineralization of the ^{15}N label occurred resulting in smaller rates compared to previous days.

In the supplementary material in MS we have added two summary tables (Table S1 and Table S2) with gross N process for all sampling points for both surfaces and in both units $\mu\text{g N per g dry weight}$ and $\mu\text{g cm}^{-3} \text{ d}^{-1}$. In the main text of the MS, we report and discuss gross N transformation rates calculated for the period between 24 to 72 hours. We have added the following explanation in results section 3.5 P 14 L 5-15:

"We chose to report gross mineralization and nitrification rates for the period between 24 and 72 hours because (1) gross nitrification rates for BP were constant during this period (Figure S3) and constant process rates are a prerequisite for estimating gross N transformation rates by Kirkham and Bartholomew, 1954 (2) the changes in ^{15}N at% excess of NO_3^- from day 5 (120 hours) in BP surfaces, suggest quick cycles of abiotic fixation and release of NO_3^- (Figure 3 and S4), therefore shorter time period for the calculations is recommended to minimize errors due to re-mineralization of the label added (Braun et al., 2018) (3) the first time point of measurement (between 1 hour and 1 day after label application) could not be included in the calculations since negative rates were observed most likely because the label was not yet evenly distributed in the soil. We acknowledge that there are several assumptions in this approach but since gross mineralization and nitrification rates were constant during the first days after labelling (with exception of day 0 to 1; Figure S3), we have confidence in the data presented. We further note that variability was higher for gross nitrification rates rendering higher uncertainties in these data, however, since all data were sampled and analyzed the same way, the comparison between VP and BP which is the focus here is valid."

4. Further, the temporal dynamics of ^{15}N recovery in the nitrate pool after ^{15}N -nitrate labelling is problematic. Data show that there is an increase in recovery between day 0 and day1, a decrease between day 1 and day 3 which is used to calculate gross ammonification, followed by another increase. I suppose therefore that atom% ^{15}N enrichment of nitrate also shows no persistent dilution. Hence, choosing other time steps for calculating gross nitrification might reveal completely different results or even negative rates. Were there probably quick cycles of abiotic fixation and release of nitrate? Or is this originating from problems with ^{15}N labelling as described above? Based on these thoughts it appears to me that gross nitrification rates in this study might be pretty unreliable. Considering this would require major changes in the discussion section.

A: We have mostly answered to these questions of the reviewer in the previous comment.

In a nutshell: due to drawbacks associated to the study of organic rich soils, such as peat soils, under field conditions using pool dilution technique, the calculations of the gross nitrogen transformation rates were indeed not straightforward. We base our decision to report gross nitrification rates between day 1 and 3 on the fact that the label is not homogeneous distributed before day 1 and that there is re-cycling of labelling after day 3. Importantly, there is a linear relationship between \ln of ^{15}N in nutrients and time, thus the analysis gives reliable results. We have backed up these assumptions with more figures and tables in the supplementary, and explanations in the text as well as relevant literature (previous comment, main MS). We acknowledge that the uncertainty is large, particularly for gross nitrification rates. However, since very few data have been published on gross nitrification rates from arctic and sub-arctic soils, we considered that our results are still valuable to the scientific community.

We further note that the results we present on gross mineralization and nitrification rates fall within the range of published gross turnover rates for Arctic soils (though only a few exist for gross nitrification) (e.g. Wild et al., 2015). Also, all data were sampled and analyzed the same way, thus even if there was some variability within the data and the choice of time points for the gross turnover calculations is associated with some assumptions, the comparison between VP and BP which was the main focus here, is still valid.

P3 L 6: The sentence "Denitrification releases usually more N_2O under wetter, more anaerobic conditions..." should be further specified as it is otherwise misleading. Under very anaerobic conditions N_2O emissions by denitrification are expected to decline as denitrification until the terminal product N_2 is favored.

A: thanks, we modified the text accordingly, and the new sentence reads now:

"Compared to nitrification, denitrification releases usually more N_2O under wetter, more anaerobic conditions and has been suggested as the key process for N_2O production in bare peat surfaces (Repo et al., 2009)."

P 3 L 23: Do you mean microbial immobilization? Please specify.

A: Yes, modified accordingly in the MS text.

P 5 L 30 probably a few more details how gas samples were transferred (overpressure? Pre-evacuated vials?)

A: Ok, few more details were added:

"Gas samples (20 mL) were transferred into 12 ml pre-evacuated exetainers equipped with butyl rubber septa (Labco Ltd, UK) the same day of sampling."

P 5 L32 Leakage test with a standard gas can be conducted only for other vials.

A: Yes, we cannot test the exact vials with samples. However, we took around 10-15 random vials from the same batch we were going to use for sampling and used the same protocol for sample collection (e.g same lids, evacuation time, sample volume) but filled those with N_2O standard. We carried those to the field with us and brought them back for analysis together with field samples.

P5 L35: the authors write that N_2O emissions calculated based on only two concentration measurements were compared with adjacent static chambers that had higher sampling frequency. Good, but what was the outcome of this comparison?

A: Also reviewer 2 mentioned that this point was not clear, and we are sorry for that. We thus repeat the answer to reviewer 2 here for reviewer 1: The comparison between small chamber, non-permanent, one-point sampling (labeled plots) and the large chambers on permanent plots with multiple samplings points (non-labeled plots) is shown graphically in figure 1 in the MS. We are sorry that it was not clear and have changed the caption and description of figure 1. N2O fluxes from labeled plots were higher compared to N2O fluxes from non-labelled plots but never higher than the highest N2O fluxes measured on previous years.

In addition, for clarification here, we have calculated N2O fluxes from three random large chambers on permanent plots by taking only the last sampling point (60 min) and compared those to N2O fluxes calculated by linear integration over all sampling points (5, 20, 45 and 60 min) (Table A). We observed that the difference between the two approaches was between 1 to 5 %. Even with $\pm 5\%$ overestimation, N2O fluxes from labeled plots (small, non-permanent chambers) are in the range of N2O fluxes from non-labeled plots from previous years.

Table A. N2O fluxes from larger chambers on non-labeled plots calculated by linear integration over all sampling points and only the last sampling point.

		linear interpolation	end-time point	% difference
Chamner 1	N2O Flux ($\mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{ h}^{-1}$)	19.0	19.9	5
Chamber 2	N2O Flux ($\mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{ h}^{-1}$)	10.6	10.7	0
Chamber 3	N2O Flux ($\mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{ h}^{-1}$)	58.9	58.6	1

Usually pushing the chamber into the soil disturbs mainly the root system, but there were no plants and roots in BP surfaces roots where N2O fluxes were evident. There are several papers presenting N2O fluxes from non-permanent chambers, by pushing the chamber into the soil (e.g Weitz et al., 1999; Maljanen et al., 2007; Hyvönen et al., 2009). We thus believe that the disturbance was minimal and flux data are reliable.

P8 L18 The abbreviation "T" for treatment might be misunderstood by the reader as extraction time steps, so probably choose another abbreviation for treatment.

A: We hope that we explain the abbreviations sufficiently well in the MS. We would like to keep thus the original version right now, but if the reviewer and editor insist, we are ready to change it in another review round.

Statistics section: Only N2O mentioned, what about gross N turnover?

A: It is now added in the text.

P9 L 18: N2O measurements are reported for a little more than a month – I therefore would not speak of "seasonal patterns"

A: agreed, we deleted the word seasonal from the sentences.

Figure 3: It shows 15N excess? The caption states that it is 15N enrichment, which is not true. Please clarify. Generally, the 15NO₃⁻ seems quite problematic and cannot be explained by gross nitrification but probably by 15N nitrate fixation and release in organic matter? This would question the gross nitrification calculations – showing the atom% excess enrichment would further help to judge this. A thorough and critical discussion is needed here.

A: The reviewer is correct. The caption of the figure 3 has been corrected. It is easier to follow the changes in the movement of the 15N excess from the figure S4 (in supplement file with answer to the referee). The 15N at% excess values of NO₃⁻ and NH₄⁺ behaved in general as expected from the pool dilution experiment. The changes in 15N at% excess of NO₃⁻ from day 5 (120 hours) in BP surfaces, suggest quick cycles of abiotic fixation and release of NO₃⁻, as mention by the reviewer. Consequently, we chose to report gross mineralization and nitrification rates for the period between 24 and 72 hours as explained in previous comments.

In the discussion section of the MS (section 4.3, P15 L 24 – 36, P16 L 1 – 12) we have acknowledged that biotic and/or abiotic process could contribute to remove the 15N added from the extractable N pool and that the uncertainties associated with these immobilization processes could have an impact particularly on the gross nitrification rates calculations.

We have included in supplementary material Figure S4 and Table S1 and S2 showing the gross N transformation rates for the whole study period.

P13 L 18: "...27 and 90 times higher.." – sure, dividing by very small rates is giving such impressive numbers which are however a bit misleading as rates at VP were hardly present. And: please give the rates related to soil dry weight as well not only related to cm³.

A: The reviewer is correct, particularly gross nitrification rates from VP are negligible.

NO₃⁻ consumption is not zero (see table S1 and S2). It is small but no zero. We did set to zero all negative values. We have modified the text in MS as follow:

"Gross mineralization and nitrification rates in BP were higher than in VP ($p < 0.01$) (Table 2). For BP surfaces, gross mineralization rates were four times higher than gross nitrification rates. Gross nitrification rates in VP surfaces were negligible. NH₄⁺ consumption rates were similar to gross mineralization rates for both surface types and higher in BP, while NO₃⁻ consumption only took place in BP surface and not in VP. See Table S1 and S2 in supplementary material, for gross N transformation rates calculated on a soil weight basis."

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Please also note the supplement to this comment:

<https://bg.copernicus.org/preprints/bg-2021-228/bg-2021-228-AC2-supplement.pdf>