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.,

https://doi.org/10.5194/bg-2021-228-RC1, 2021

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 15N 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 N2O 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 15N-lable in the mineral N pools in organic soils is quite challenging (Mortland and Wolcott, 1965; Nõmmik and Vahtras,1982; Nieder et al., 2011). Additionally, high rate of microbial immobilization commonly causes problems for 15N tracing studies in soils with low mineral N availability, typical for arctic and sub-arctic soils: the 15N 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 15N 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 15N 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 15N 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 N2O producing processes is likely not impacted, which was the main focus of the MS. We discussion this in the MS in page 20 lines 22-23 and page 21 lines 1 to 11:

"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 (~10 mg  $N_2O$  m<sup>-2</sup> d<sup>-1</sup>) were still within the range of  $N_2O$  fluxes observed in previous years from BP surfaces (1.9–31 mg  $N_2O$  m<sup>-2</sup> d<sup>-1</sup>) (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 (< 1m, personal observation. data not shown). The  $N_2O$  emissions from labelled and non-labelled plots had similar responses to changes in temperature (R<sup>2</sup> = 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, this likely did not affect the relative contribution of different microbial pathways to the total  $N_2O$  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 15, line 1 to 3:

"At day 3, total recovery of <sup>15</sup>N 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 <sup>15</sup>N 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 cirlces) we can observe that curvilinearity starts after ~ day 9, this means that the transformation process rates are constant between 1 h and 9 days (R2 = 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 (R2 = 0.4195).

Results are more variable for 15N at% excess of NO3-, but there was a linear relationship over 24 to 72 hours (R2 =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 remineralization 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  $\mu$ g N cm-3 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  $\mu$ g N cm-3 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 mention already that 15N 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 15N label and some re-mineralization of the 15N 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  $\mu$ g N per g dry weight and  $\mu$ g cm-3 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 in methods section 2.3.3 P 11 L 20- 28:

"The gross N transformation rates were calculated from data from T1 (<sup>15</sup>N-NO<sub>3</sub><sup>-</sup>; nitrification) and T2 (<sup>15</sup>N-NH<sub>4</sub><sup>+</sup>; ammonification) between time points 24 and 72 hours (3 d) after labeling. This time-period was selected 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 15N at % excess of NO3- from day 5 (120 hours) in BP surfaces, suggest quick cycles of abiotic fixation and release of NO3- (Figure 3 and S4), therefore shorter time period for the calculations is recommended to minimize errors due recycling of the label by assimilation to microbial biomass and remineralization (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 that resulted often in negative gross N transformation, most likely because the label was not yet evenly distributed in the soil"

(4) Further, the temporal dynamics of 15N recovery in the nitrate pool after 15N-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%15N 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 15N 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 15N 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 N2O under wetter, more anaerobic conditions..." should be further specified as it is otherwise misleading. Under very anaerobic conditions N2O emissions by denitrification are expected to decline as denitrification until the terminal product N2 is favored.

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

"Compared to nitrification, denitrification releases usually more N<sub>2</sub>O under wetter, more anaerobic conditions and has been suggested as the key process for N<sub>2</sub>O 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? Preevacuated 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 N2O 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 N2O 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 that 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.

	1 01	•	1 01		
			linear interplolation	end-time point	% diference
Chamber 1	N2O Flux	(µg N2O-N m-2 h-1)	19,0	19,9	5
Chaber 2	N2O Flux	(µg N2O-N m-2 h-1)	10,6	10,7	0
Chaber 3	N2O Flux	(µg N2O-N m-2 h-1)	58,9	58,6	1

**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.

Usually pushing the chamber into the soil disturbs mainly the root system, but there were not plants and roots in BP surfaces roots. 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).

**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, P22 L 10–30, P23 L 1–6) 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 in results section 3.5 (P19 L10-21):

"As described above in the methods section 2.3.3, we chose to report gross mineralization and nitrification rates for the period between 24 and 72 hours. During this time period, the gross mineralization and nitrification rates were constant and positive, and we could assume negligible recycling of the <sup>15</sup>N label via microbial biomass back to the mineral N pool (Braun et al., 2018). For method comparison purposes, we have shown the gross mineralization and nitrification rates calculated for different periods in table S1 and S2 in supplementary material. We note that variability in the results depending on the time period chosen for the calculations was higher for gross nitrification rates than for gross mineralization rendering higher uncertainties in the nitrification data. However, the comparison between VP and BP, which is the focus here, is independent of the chosen calculation method and is valid. Further, high variability of gross N turnover rates is quite common in field labeling studies (e.g., Cookson et al., 2002; Harty et al., 2017). The high

variability in our data could also simply reflect the spatial variation at the site between the subplots destructively sampled at different time-points.

Gross mineralization and nitrification rates in BP were higher than in VP (p < 0.01) (Table 2). In BP, gross mineralization rates were four times higher than gross nitrification rates. Gross nitrification rates in VP surfaces were negligible. NH<sub>4</sub><sup>+</sup> consumption rates were similar to gross mineralization rates for both surface types and higher in BP, while NO<sub>3</sub><sup>-</sup> 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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#### Comment on bg-2021-228

#### Anonymous Referee #1 – Figures and tables



**Figure S3**. Change in natural logarithmic of 15N at% of (a - b) NH4+ an (c - d) NO3- in BP and VP soil over total study period.



**Figure S4.** 15N at% excess (APE) of (a) NH4+ (b) NO3- and (c) N2O during the sampling period for all three treatments applied in BP surfaces. (Values are mean  $\pm$  S.E, n=3). Only data from treatment 1 (T1 = 15N-NO3-) and treatment 2 (T2 = 15- NH4+) was used in the calculations of the gross N transformation rates.

		µg N cm-3 d-1	µg N cm-3 d-1	µg N cm-3 d-1	µg N cm-3 d-1
(a)		Mineralisation	NH4 consumption	Nitrification	NO3 conssumption
24 hours	average	8,76	8,10	0,30	0,23
	std	2,36	1,93	0,42	0,84
	se	1,36	1,11	0,24	0,48
72 hours	average	3,31	3,50	0,87	0,92
	std	1,97	2,70	0,88	0,53
	se	1,14	1,56	0,51	0,31
120 hours	average	5,19	5,63	0,15	0,02
	std	0,49	0,88	0,08	0,24
	se	0,28	0,51	0,05	0,14
216 hours	average	1,87	1,97	0,02	-0,17
	std	0,62	0,79	0,17	0,36
	se	0,36	0,45	0,10	0,21
360 hours	average	1,45	1,13	0,00	-0,09
	std	0,63	0,80	0,13	0,23
	se	0,37	0,46	0,07	0,13
24 days	average	0,88	0,79	0,10	0,11
	std	0,29	0,27	0,05	0,07
	se	0,17	0,16	0,03	0,04

#### Table S1. Gross N process rates from bare peat surfaces (BP)

		µg N g-1 d-1	µg N g-1 d-1	µg N g-1 d-1	µg N g-1 d-1
(b)		Mineralisation	NH4 consumption	Nitrification	NO3 consumption
24 hours	average	32,45	29,98	1,11	0,84
	std	8,72	7,13	1,57	3,11
	se	5,04	4,12	0,91	1,79
72 hours	average	12,27	12,97	3,21	3,41
	std	7,31	9,98	3,27	1,98
	se	4,22	5,76	1,89	1,14
120 hours	average	19,23	20,84	0,55	0,07
	std	1,80	3,27	0,31	0,89
	se	1,04	1,89	0,18	0,51
216 hours	average	6,94	7,31	0,08	0,00
	std	2,28	2,92	0,63	1,33
	se	1,32	1,68	0,36	0,77
360 hours	average	5,36	4,18	0,00	0,00
	std	2,35	2,96	0,47	0,85
	se	1,36	1,71	0,27	0,49
24 days	average	3,26	2,91	0,38	0,42
	std	1,06	1,00	0,19	0,26
	se	0,61	0,58	0,11	0,15

Values are mean of three plots (n = 3).

Table S2. Gross N process rates	from vegetated surfaces (VP)
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		µg N cm-3 d-1	µg N cm-3 d-1	µg N cm-3 d-1	µg N cm-3 d-1
(a)		Mineralisation	NH4 consumption	Nitrification	NO3 conssumption
24 hours	average	0,00	0,00	0,02	0,04
	std	1,94	3,32	0,13	0,13
	se	1,12	1,92	0,08	0,08
72 hours	average	0,55	0,43	0,00	0,01
	std	0,96	0,97	0,02	0,02
	se	0,55	0,56	0,01	0,01
120 hours	average	1,15	1,11	0,00	0,00
	std	0,76	0,66	0,01	0,01
	se	0,44	0,38	0,01	0,01
216 hours	average	0,63	0,63	0,00	0,00
	std	0,46	0,43	0,00	0,00
	se	0,26	0,25	0,00	0,00
360 hours	average	0,37	0,36	0,00	0,00
	std	0,25	0,25	0,01	0,00
	se	0,15	0,14	0,00	0,00
24 days	average	0,22	0,23	0,00	0,00
	std	0,18	0,15	0,00	0,00
	se	0,10	0,09	0,00	0,00

		µg N g-1 d-1	µg N g-1 d-1	µg N g-1 d-1	µg N g-1 d-1
(b)		Mineralisation	NH4 consumption	Nitrification	NO3 consumption
24 hours	average	0,00	0,00	0,43	0,87
	std	38,70	66,35	2,61	2,61
	se	22,34	38,31	1,51	1,51
72 hours	average	10,96	8,60	0,00	0,20
	std	19,21	19,42	0,45	0,45
	se	11,09	11,21	0,26	0,26
120 hours	average	22,97	22,22	0,00	0,00
	std	15,15	13,15	0,18	0,18
	se	8,75	7,60	0,10	0,10
216 hours	average	12,63	12,63	0,08	0,10
	std	9,18	8,62	0,08	0,08
	se	5,30	4,98	0,05	0,05
360 hours	average	7,33	7,21	0,06	0,06
	std	5,10	4,93	0,14	0,05
	se	2,94	2,84	0,08	0,03
24 days	average	4,46	4,62	0,07	0,08
	std	3,53	2,96	0,06	0,06
	se	2,04	1,71	0,03	0,03

Values are mean of three plots (n = 3).

#### Comment on bg-2021-228 Anonymous Referee #2

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., https://doi.org/10.5194/bg-2021-228-RC1, 2021

The manuscript 'Sources of nitrous oxide and fate of mineral nitrogen in sub-Arctic permafrost peat soils' by Gil and co-authors is an interesting study on nitrogen cycling in vegetated and bare soils of the Russian sub-arctic. The authors used an isotope pulse labelling approach to identify the microbial pathways responsible for N2O emissions at their study sites and concluded that emitted N2O mainly originated from microbial denitrification. However, also nitrification was an essential process for N2O production since it produced the nitrate required for denitrification.

The formation and release of N2O from arctic and subarctic soils has not yet gained the attention it deserves. It is crucial to understand how N2O fluxes will respond to future environmental and climatic changes in particular in the high northern latitudes, since there the changes will be most severe. The presented study adds important information on the formation and release of the important greenhouse gas N2O in a remote area of this world. The study is well designed and the conclusions are supported by the presented data. I have only few comments, which hopefully are helpful to improve the manuscript.

# (1) The title of the study indicates that these soils are affected by permafrost, but in the description of the study site and the soil characteristics I did not find any information on permafrost, except that the sites are situated in the zone of discontinuous permafrost. Are the sites still affected by permafrost? If yes, please give more information e.g. on active layer depths, if not, the title should probably be adapted.

A: Yes, the site is affected by permafrost. We were studying permafrost peatlands (peat plateaus), which are bogs raised after permafrost aggredation. The active layer depth in 2010 was  $60 \pm 12$  cm on average in vegetated peatlands (VP) and  $70 \pm 5$  cm on average in bare peat (BP) (this is mentioned in table 1 in the MS). We have now added a sentence specifically stating that the sites are underlain by permafrost which reads (section 2.1, P5 L 2–8):

"The experiment was carried out at the Seida study site which is located in sub-Arctic northwestern Russia (67°03'N, 62°57'E) in the discontinuous permafrost zone. Some common geographical features occurring in discontinuous and sporadic permafrost zone are the so-called palsas and peat plateaus (Seppälä, 2011, Sannel and Kuhry, 2011, Borge et al., 2017). They are formed by permafrost aggradation, which lifts the peat surface, leading to drier conditions than the surrounding unfrozen peatland surface (Seppälä, 2003). As a result of wind abrasion, parts of the palsas and peat plateaus lack vegetation (Seppälä, 2003)."

#### Specific comments:

**P2, L1-4:** Which processes do you mean? Could you be here more specific and start with the explanation of the processes you mean?

A: We believe that the reviewer refers to the processes contributing to increased CO2 and CH4 emissions under a changing climate, and have specified them now (decomposition processes). The sentence reads now:(section 1, P2 L14-17):

"The Arctic and sub-Arctic regions store more than 50% of the Earth's soil carbon (C) pool (1330 - 1580 Pg) (Schuur et al., 2015). The possible increase in release of the greenhouse gases carbon dioxide (CO2) and methane (CH4) from these carbon stocks as a result of increased decomposition processes (aerobic and anaerobic) to the atmosphere under a changing climate has been intensively studied"

P2, L9-10: This sentence is hard to understand.

A: To make the sentence better understandable, we have now changed it so that it reads:

"Soils world-wide are important N<sub>2</sub>O sources responsible for 60% of the global emissions (IPCC, 2013). Traditionally it has been suggested that N<sub>2</sub>O emissions from Arctic soils are negligible because of the low concentrations of mineral N in soils underlain by permafrost (Ma *et al.*, 2007; Takakai *et al.*, 2008; Siciliano *et al.*, 2009; Goldberg *et al.*, 2010)."

**P5 L35-P6 L2:** What are the results of the comparison between the two methods? In Fig. 1 I only see the results of the current approach. I would expect that inserting the collar only 1h before measurement would disturb the system and affect the outcome of the measurement. Furthermore, a one point measurement introduces uncertainty in comparison to multi-point measurements and it would be good to comment on this uncertainty.

A: 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 (small, non-permanent chambers) were higher compared to N2O fluxes from non-labelled plots (large, permanent chambers) but never higher that 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.

			linear interplolation	end-time point	% diference
Chamber 1	N2O Flux	(µg N2O-N m-2 h-1)	19,0	19,9	5
Chaber 2	N2O Flux	(µg N2O-N m-2 h-1)	10,6	10,7	0
Chaber 3	N2O Flux	(µg N2O-N m-2 h-1)	58,9	58 <i>,</i> 6	1

**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.

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.

P6 L16: Do you mean Herrman et al., 2005?

A: Yes, we have corrected in the text. Thank you for noticing it!

**P7 L16:** If I get this right the 15N in the soil was only considered until a depth of 6 cm, since this is the sampling depth of the soil cores. It was not described how deep the 15N tracer solution was injected into the soil, but I can imagine that the label diffuses relatively quickly below this depth. Would N2O production from the 15N label below the sampled soil depth introduce a bias into their mass balance calculations?

A: yes, the reviewer was right, the label was injected up to 6 cm but soils were sample 0 -10 cm. Some losses due to lateral and vertical movement of N forms, particularly  ${}^{15}NO_{3}$ , including downward leaching, were expected. Since immediately after labelling (~1h) and by end of the experiment (24 days), most of 15N label was still retained in the bulk peat soil (71-92% across VP and BP) we can assumed that the losses by leaching were minimum. We discuss and acknowledge the possibility for downward leaching, and production of 15N2O from labelled nutrients below the sampling layers, in the current MS, see discussion section 4.3, P22 L 10–30:

"The total recovery of applied <sup>15</sup>N within 24 hours was close to 100% in both studied surface types. The recovery percentage decreased during the course of experiment in both VP and BP, which might be a consequence of lateral and vertical leaching of N forms within the soils, particularly in the case of  ${}^{15}NO_3^-$  (Clough et al., 2001). Also, part of the label could have been lost as gaseous fluxes of NO and N2, which were not measured here. Both downward leaching and gaseous N losses as NO and N2 were likely higher in BP than in VP because of effective plant N uptake and microbial immobilization in VP. Indeed, the total recovery of <sup>15</sup>N was higher in VP than in BP surfaces during the whole 24-day experiment ( $\sim$ 79% vs.  $\sim$ 62%, respectively). It is also likely that the <sup>15</sup>N might increasingly accumulated as <sup>15</sup>N-N<sub>2</sub>O and <sup>15</sup>N-N<sub>2</sub> in pore water/gas in BP. Soil gas concentrations of N<sub>2</sub>O can be very high (up to 4500 ppb) particularly in BP (Gil et al., 2017). However, since more than 60% and 80% of <sup>15</sup>N was recovered in VP and BP, respectively, we did account for all the major sinks of  $NO_3^-$  and  $NH_4^+$  in both soils throughout the 24-day experiment. In both VP and BP, the largest relative proportion of <sup>15</sup>N label after 24 days of experiment was observed in bulk peat (71–92% of total <sup>15</sup>N recovered), comprising physically adsorbed, dissolved, chemically or electro-chemically fixed and microbially immobilized <sup>15</sup>N. Peatlands are known to be able to efficiently retain nutrients to deal with low N inputs, which has given them ecological functions as nutrient buffers (Vikman et al, 200). Recovery of <sup>15</sup>N in bulk peat was higher for <sup>15</sup>NH<sub>4</sub><sup>+</sup> than for <sup>15</sup>NO<sub>3</sub><sup>-</sup> (Figure 2a and 2b). This suggests that fixation of nutrients to SOM is one of the main reasons for the high retention of <sup>15</sup>N, since soil particles are negatively charged (Schlesinger, 1997) and since fixation capacity is high under acidic conditions (Huber, Oberhauser & Kreutzer, 2002). This is supported by other studies that have found evidence for similarly high fixation of nutrients, particularly of  $^{15}NH_4^+$ , to organic peat material (e.g., Munchmeyer et al., 2000). Microbial immobilization likely is another reason for high <sup>15</sup>N recovery in bulk soil in BP and VP, since NH4<sup>+</sup> and NO3<sup>-</sup> consumption rates were as high as production rates in both soils as obtained from the pool dilution approach (Table 2; see discussion below). Rapid uptake of <sup>15</sup>N by microbes in soils with low N in from arctic and sub-arctic ecosystems has been documented during the first days after the addition of label in previous experiments (Nordin, Schmidt & Shaver, 2004; Sørensen et al., 2008)."

#### P9 L13: Please explain how WFPS was calculated.

A: We have now included this explanation in the MS, section 2.3.4, P9 L14–24:

"Soil water filled pore space (WFPS) in the topsoil was calculated using equation (11). For this, soil moisture sensor data (in mV) measured with a ML3 ThetaProbe (Delta-T Devices, Cambridge, UK) was converted to volumetric water content ( $\theta$ v), applying a sensor calibration as instructed by the manufacturer (see Gil *et al.*, 2017, supplementary material). Bulk density (BD) was measured in the field from volumetric soil samples. Particle density (PD) was estimated from soil organic matter content (SOM) as previously described (Marushchak *et al.* 2011). The total porosity (TP) was calculated using equation (10):"

TP = 1 -	$\left(\frac{BD}{PD}\right)$	(10)
WFPS =	θv TP	(11)"

#### **P9 L24:** Figure 3

A: Figure 1 is correct, but we have added Figure 3c to this sentence.

#### P10 l3: 'no'

A: thanks, we have changed accordingly in the text.

#### P14 L8-10: This was yet said in the introduction.

A: We agree with the reviewer, but still think that it is important to reinforce this fact (that N2O emission from permafrost peatlands are a novel discovery) and that it is important to put the fluxes into the context of N2O emission from other soils. We would thus like to keep that sentence.

### P14 L 13: I would admit in this paragraph that addition of label likely increased N2O fluxes. At least that is what the data show. I am sure the heterogeneity is high but the authors do not present these data.

**A:** The reviewer is right, but we already mention and discuss this in the manuscript (related to Fig. 1). 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 that the highest N2O fluxes measured on previous years. Addition of label could have stimulated the fluxes to some extent, but as we argue this likely does not impact on the process contribution to the fluxes and on the N processes between BP and VP which were clearly different, and the main focus of the MS remain.

#### P15 L14: Sx?

A: Sorry, this was a typo and we meant table S1. Table with N transformation rates expressed in unites of µg N per g dry weight has been added to supplementary material (Table S1)

P16 L2: What do you mean by 'evolutionary advantage'? A: Maybe this term is confusing, we have deleted it.

P17 L28: When was the 'exceptionally dry year'? 2010 is the year of the current study. A: The 'exceptionally dry year' was 2011. We have corrected the text accordingly.

Figure 2: The y-axis label is misleading. It seems that not the total recovery is presented but the relative proportion of the different pools in the recovered 15N. It might be more informative to present the absolute 15N recovery in the different pools, which would than not always sum up to 100%. Please add error bars.

A: The absolute recovery is shown in figure S1 (total). We think it would be redundant to show then also absolute recovery of different pools (which is a combination of Fig. S1 and Fig. 2). The main point here was to compare the fate of 15N in plants vs. N2O in vegetated and bare peat. For that purpose, we believe that the relative recovery is more informative.

Error bars have been added to figure 2.

Figure 3: Could the authors comment on the large differences in 15NH4-N and 15NO3-N concentrations in the different plots. In particular the 15NH4-N concentrations steeply decrease during the first days, but the increase of 15NO3-N concentrations or 15-N2O-N fluxes seems much lower. Where is the label gone?

A: It is easier to follow the changes in the movement of the 15N label 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. We recovered some of the label in the mineral N pools, N2O emissions and plants (for vegetated surfaces) but most of the 15N label was recovered in the bulk soil (up to 79%) during the entire experiment period (Figure 2 and Figure S1). This could be consequence of different processes including physical and chemisorption of the 15N added to the soil organic matter, (Mortland and Wolcott, 1965; Nõmmik and Vahtras, 1982; Nieder et al., 2011) and microbial immobilization, particularly in the vegetated peat surfaces (VP), something previously observed from soils with low N availability in arctic and sub-arctic ecosystems (Nordin, Schmidt & Shaver, 2004; Sørensen et al., 2008, Marushchak et al., 2021). This biotic and/or abiotic process could contribute to remove the 15N added from the extractable N pool. It is reflected in the consumption rates we calculated. Some discussion on this issue was already in the previous version of the manuscript, but we have added a sentence to address it further (section 4.3 in discussion, page 22, L10-30.

In addition, we observed generally higher enrichment of 15N2O than any of the source pools (e.g. NO3-, NH4+). One possible explanation for this could be that the production of N2O occurred from NO2- rather that NO3- and the isotopic enrichment of N2O would be much more similar to that of NO2- than of NO3- (Mulvaney et al., 1997). However, the presence or accumulation of NO2- was never detected in the soils before or during the 15N tracer experiment (data not shown). This is expected because in soils, NO2- typically is rapidly consumed and therefore, we could not detect it in the soil extracts. Another explanation for the mismatch in 15N between sources and mixture is

that there was a gap between N2O production in soil (reflecting the source pools at that time point) and the N2O measured in the headspace due to for example time diffusion and or N2O production at deeper soil layers.

#### Figure 4: Could you add error bars to the average values presented

A: Error bars have been added to figure 4.

#### References

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#### Comment on bg-2021-228

#### Anonymous Referee #2 - Figure



**Figure S4.** 15N at% excess (APE) of (a) NH4+ (b) NO3- and (c) N2O during the sampling period for all three treatments applied in BP surfaces. (Values are mean  $\pm$  S.E, n=3). Only data from treatment 1 (T1 = 15N-NO3-) and treatment 2 (T2 = 15- NH4+) was used in the calculations of the gross N transformation rates.