

Author Responses to Anonymous Referees:

All author responses appear in grey italics below specific comments from Anonymous Referees. The authors thank all referees for the time and energy they have spent providing constructive feedback on this body of work. We have summarized our responses below, have responded to each comment individually on the following pages, and we believe we have adequately addressed the feedback provided.

Previous reviewer comments have helped us greatly improve the readability of this manuscript through the reorganization of the main text and supplement, which focuses the main text on variability trends observed and moves the additional supporting work to the Supplement to ensure readers have access to all work associated with this study if interested.

The co-occurrence of precipitation events on days where enhanced nitrate availability occurred, must be recognized, even without quantitative precipitation data and it would be irresponsible for the authors to ignore the precipitation events observed in the field.

While we appreciate that Anonymous Referee #1 has stylistic preferences and respect their opinion, Anonymous Referees #2-3 do not take issue with these same areas of concern. Anonymous Referee #1's lack of specific details made it challenging to understand/address their comments. For example, what in the abstract is not discussed within the text? This is not specified, nor why the reviewer considers the abstract information not new. Is the reviewer aware of other studies that have compared pore water nitrate concentrations in Alaskan Alder permafrost systems? If so, we would be glad to cite them. However, there do not appear to be any that exist. So, how can this work not be new? It is unfounded to claim a lack of new contributions because Alder nitrate studies exist within other landscapes when Alaskan Arctic systems have had so little investigation and the data and findings presented here are original to this study and provide insights to these understudied systems.

Furthermore, Anonymous Referee #1 did not believe this study should be published partially based on design, but reconnaissance studies are valuable to the scientific community, and this study contributes original data from a region that does not have alder influenced soil pore water nitrate variability published previously. Anonymous Referees #2-3 were able to see the merit in our reconnaissance study outcomes and have provided constructive feedback that has allowed this body of work to improve. The authors firmly believe that comments from Anonymous Referee #1 serve as unnecessary gatekeeping and that our study provides original data and important insights to the scientific community that are worthy of publication.

Anonymous Referee #1:

I see that the authors tried to restructure the text, but the MS in the presented state cannot be published, sorry.

The structure is still not satisfying: several repetitions (on flushing by rainfall), part of the discussion is presented like result section, etc.

Because the whole MS needs to be restructured again, I will not give here a specific line- per-line comment. And please (a detail), do not reply to reviewers by using the same sentence/expressions all the time, this is more than boring.

The authors thank Anonymous Referee #1 for their time and input but respectfully and strongly disagree on final recommendation and stylistic notes of Anonymous Referee #1. We can appreciate the frustration

of Anonymous Referee #1 and understand that they would have designed this study differently. However, just because this is a reconnaissance study (which helps form the basis of more detailed experimental designs at our site and elsewhere) does not mean this work is unworthy of publication. The reviewer has lost sight of the fact that these data are a unique new data set that benefits scientific understanding in Arctic ecosystems that are changing rapidly. What benefit is served by denying the scientific community access to the data and findings presented in this study, because the reviewer simply doesn't like the design? The data are valid and insightful, and this study certainly serves as a useful baseline for future Arctic studies on Alder impacts and these results are important to communicate to the broader scientific community.

Here, my main reason why the MS cannot be accepted for publication:

1) There is a general and deep misunderstanding, an article needs to be written and condensed to a unit, important pieces of information have to be incorporated in the article and not presented in the Supplementary Material or in a co-located study or somewhere (by the way, this was stated already in the first review). You cannot formulate statements in the abstract and the data are fully absent in the article. The reader of your article MUST find your "main story" in the MS, not in the Supplementary Material.

This comment cannot be sufficient as the main reason that Anonymous Referee # 1 recommends the article be rejected as the comment indicates. It is simply based on a stylistic difference of opinion. Rejection of a paper should not be based mainly on what the reviewer "prefers" when all data and cited information are available for anyone who is interested, and the main points of the paper are easily understood within the main text. We find this recommendation is inappropriate given that it is based on one reviewer's rather narrow idea of how to formulate a manuscript, and because the other reviewers appreciate the edits made that make the text compact and to the point. The authors have significantly condensed the text to follow a common theme surrounding variability and all data central to the main text is included within the main text. Data outside the scope of the findings in our main body of text have been shifted to the Supplement so that if a reader is interested in additional parameters measured, they have access to that data for additional context, but it does not distract from our main story. We believe that the transition of the nitrate source (isotopic) content to the Supplement has greatly benefitted this body of work to highlight the degree of nitrate variability observed more than any other study outcome. The authors also argue that the abstract does not present anything that is not included within the main body of text and are unsure as to what this comment refers to.

2) The data presented as main result in the abstract, are simply not new:

"Soil pore water collected within alder shrubland had an average NO₃--N (nitrogen from nitrate) concentration of 4.27 ± 8.02 mg L⁻¹ and differed significantly from locations outside alder shrubland (0.23 ± 0.83 mg L⁻¹; p < 0.05)."

The authors do not understand the above comment as not being new because these data presented here are new and unique. The reviewer has never referred to any specific citation of any peer reviewed study that quantifies pore water nitrate concentrations in alder hillslopes from permafrost landscapes. We agree that the fact that nitrate variability exists is not new but having quantitatively documented pore water nitrate variability (especially at fine spatial resolution) in these cryosystems is new. Yes, N has been measured in leaves and stream waters in Arctic Alder landscapes, but not directly in pore waters associated

with these plants. This work increases the referenceable literature in the community and allows for a better understanding of the degree of variability that can occur on short temporal and spatial scales in permafrost systems. The data presented in this study are new records of nitrate variability in a landscape/region (Seward Peninsula, AK) where Alder expansion is occurring, yet has been understudied. Our study will greatly aid in informing future studies in these systems because it demonstrates how variable nitrate concentrations are at the individual Alder patch scale as well as within and outside of Alder patches.

I suggested that you express the nitrate as Nitrate-N = NO₃-N (without a minus sign) to enhance the comparability to other N concentrations (e.g., NH₄-N), but then you have to recalculate it:

For example: 4 mg L⁻¹ NO₃⁻ = 0.90 mg L⁻¹ NO₃-N!

[The concentrations in the text and in the figures are NOW all wrong]

Thank you for this constructive level of feedback. This comment is the result of a miscommunication on the last round of revisions. The authors originally included the data as NO₃-N but mis-referenced the concentrations within the text as NO₃⁻. The previous round of comments sparked the authors to realize this typo/mistake and so the terminology used within the text was updated to reflect the appropriate format, which is what the data was already presented in. We apologize for the confusion resulting from our original mistake and the need for additional details in communicating our previous round of revisions. Anonymous Referee #1 is completely correct that had we presented the numerical nitrate data as NO₃⁻ originally, we would have needed to convert all our data. However, we originally included the numerical data in the correct form (NO₃-N) and mis-referenced it within our figures, tables, and text. This was an oversight that we are grateful that Anonymous Referee #1 identified that we display our data as 'nitrate-N' in the first round of revisions. The authors have verified the numerical data is indeed represented in the correct NO₃-N format by returning to and verifying our values within the raw and converted data. The authors apologize for the inclusion of the negative sign in the nitrate-N notation (formatting issue) and have gone through and removed the negative signs that were mistakenly included in our NO₃-N notation.

Even correctly calculated, this difference can be expected as alder are N₂-fixing plants (write N₂-fixing not N-fixing).

The authors have changed the notation to N₂-fixing plants as correctly identified by Anonymous Referee #1. While this finding could be 'expected', having additional values published that numerically document these spatial variabilities is still beneficial to the broader scientific community. A concept doesn't need to be new to be published and can still add value to the community through increasing data that exists on the topic.

3) The design remains weak, when precipitation events seem to be the main driver for flushing events, but the precipitation has not been measured on site, there is no valid proof.

While we do not have quantitative constraints on the precipitation event, the co-occurrence of precipitation on days where trends appeared could not be ignored. While the authors may have overstated the 'proof' of this in previous versions of the text, we are unable to separate the observed trends from our knowledge that precipitation events occurred on days where notable variability occurred. The authors have lessened the level of confidence in which we directly state the influence of precipitation on trends. We now state observed trends and indicate precipitation occurred on known days and that these variations are likely linked. See responses to line items left by Anonymous Referees #2-3 for more details of the changes to our text.

4) Several references are still not cited correctly (*Alnus viridis* encroachment in the Alps occurs in the montane vegetation belt, not in the alpine, see Bühlmann et al. 2014, line 54) and more important: please make sure that you are not employing any plagiarism when using whole and/or "very similar" sentences from other articles (e.g., Salmon et al. 2019).

The authors respectfully disagree with this point as the text in the instances Anonymous Reviewer #1 identifies have already been modified to more generalized landscapes that make these references appropriate within this context. For example, the Bühlmann et al 2014 reference in question follows text that states, '... have been investigated in alpine and upland systems...', which goes beyond strictly alpine settings. However, we have further modified this text to state, "...have been investigated in alpine, subalpine, and upland systems..." and hope that it appeases Anonymous Reviewer #1 but we could also remove the Bühlmann et al 2014 reference completely if that would be preferred but we felt that it was a useful reference to direct readers to if they would like to explore studies that have already established relationships between alder and nitrogen chemistry.

Additionally, the Salmon et al 2019 study was co-located with this study and Salmon is a co-author on this manuscript. While we appreciate the level of concern for plagiarism, we firmly believe that the brief inclusion of modified text that best captures the vegetation descriptions within our study location, with the permission of the original author and a citation, is appropriate.

Anonymous Referee #2:

Specific comments with line numbering

L 124: Is there a word missing between 'down' and 'gradient' ?

No word is missing. 'Down gradient' refers to water movement along the most likely flow pathway. In the instance of our sampling location, down gradient is usually synonymous with downslope.

L 126: Nice with the description of why you measured Mn and Fe :)

Thank you kindly.

L 145: 'hereafter' in stead of 'hereby'?

Suggestion has been adopted.

L 266: the (21-22) refers to the date, I assume – maybe change to (21-22nd) or (21-22.)

We have adopted the suggested (21-22nd) reference style here and in other text where we reference dates (L 312).

L 283: Why is the labile N inaccessible according to Darrouzet-Nardi and Weintraub (2014)?

The Darrouzet-Nardi and Weintraub 2014 article referenced here outlined that labile N could remain inaccessible if spatially isolated due to low water potential of an environment. We have added text (L 328-329) to communicate these details: "...Darrouzet-Nardi and Weintraub (2014) found evidence for spatial inaccessibility of labile N in Arctic ecosystems due to isolation in environments with low water potential, but...."

L 291: If you want a more recent reference, Rasmussen et al. 2020 also saw a flush of organic C and N during and after a rainfall event.

The authors thank Anonymous Referee #2 for this relevant reference suggestion and have incorporated it into the text.

L 335-337: Agreed. And assessing the time around snowmelt and soil thaw and the transport potential related to redox environments present there is also necessary in the future.

This is absolutely true. Perhaps there could be future collaborative potential in this area. We added text to section 4.5 Future Research (L 445-446) to denote that starting sampling/monitoring around snowmelt would be a beneficial future approach.

Supplementary: A few of the references are underlined

All references in the Supplement are now formatted appropriately.

Anonymous Referee #3:

The aim of this study is to the N cycle and soil NO₃⁻ concentrations along a topographic gradient in a permafrost area with a vegetation cover of nitrogen fixing *Alnus viridis* spp fruticose. The main conclusion from the work emphasize the temporal variation in soil NO₃⁻ within and downslope from the alder shrub-land, where soil NO₃⁻ is supposedly being flushed downslope during precipitation events. This is an interesting and relevant topic of research to investigate N-cycle and transport-processes at landscape scales in permafrost regions, and the manuscript holds important data that I believe should be made available to the science community.

This manuscript has previously been revised in response to two extensive peer-reviews and I find that the authors have responded comprehensive and careful to the criticism raised. Meanwhile, I also find that the manuscript in it's current form can be further improved and streamlined to clarify and emphasize even stronger the outcome of this study. My main concern is specifically the speculation about precipitation driven downslope nitrate transport, combined with the isotopic observations that I recommend to moderate and soften. See comments below.

The authors sincerely thank Anonymous Referee #3 for their thoughtful and constructive feedback on this manuscript. We appreciate the time and energy spent on these relevant, appropriate, and professional suggestions for improving the communication of our work and are grateful for the willingness of this referee to serve in this capacity. The type of feedback Anonymous Referee #3 provided exemplifies the benefits of a peer review process.

Introduction

Line 31: A recent paper studies lateral N-transport in a permafrost landscape and demonstrates the function of lateral N-transport for plant uptake and growth. Rasmussen, L. H., et al. (2022). "Nitrogen transport in a tundra landscape: the effects of early and late growing season lateral N inputs on arctic soil and plant N pools and N₂O fluxes." *Biogeochemistry* 157(1): 69-84.

The authors thank Anonymous Referee #3 for this highly relevant reference and have incorporated it into the text accordingly.

2.6 Statistical analyses

Line 194: The method for determining normal distribution needs to be mentioned.

The authors have added text to provide these relevant details on L 213: “Data collected had normal distribution (identified through comparison of p-values to significance levels)...”

Since data were all normally distributed, why was non-parametric test applied rather than more powerful parametric statistical tests?

The authors chose to apply a non-parametric test after speaking with a statistical consultant who advised us that parametric approaches were unnecessary for our purposes. The non-parametric tests applied are valid for both normally and non-normally distributed data. Anonymous Referee #3 correctly identified that parametric tests on normally distributed data could provide additional insights to statistical interpretations. However, the statistical insights gleaned from the Mann-Whitney rank sum tests performed are appropriate for assessing our data for the purposes of this study.

Line 205: Calcium, Sodium and Chloride statistical analysis is explained. Confusing as these ions have not been described in previous section on chemical analysis of soil water (only that cations and anions in general were analyzed)?

The authors thank Anonymous Referee #3 for identifying this point of confusion. These statistical analyses were performed to compare common conservative chemical species (Cl^- and Na^+) to a common non-conservative species (Ca) to gain insights to processes likely influencing the system. However, as noted, this text is distracting from the content of the main manuscript and these details were intended for inclusion in the Supplement. We have thus removed this text (and associated Brown 1998 reference) from the main article and included these details solely in the Supplement to support interpretations of processes influencing source of NO_3^- .

Results

3.1 Soil depth and moisture, line 216: the equation percent dry/wet weight is not gravimetric soil moisture content but rather dry matter content. Please, specify or give correct equation.

The authors thank Anonymous Referee #3 for bringing this oversimplification to our attention and have corrected the equation to: “((weight of wet soil – weight of dry soil) / weight of dry soil x 100)”

3.2 Phase 1:... line 228: It's rather unfortunate that the local rain gauge malfunctioned at the time of the ongoing field work. Perhaps it could be relevant though to somehow provide indications for the amount of precipitation deposited during this event? The term ...brief from the manuscript. Along this line, I also find the data on isotopic observations somewhat over-interpreted; e.g. no “real-time” data on nitrate in precipitation or isotopic values of nitrate and water in precipitation is presented to support the statement about precipitation isotopic imprints on soil water NO_3 . Also, with reference to Fig S2, is the predicted denitrification driven shift in isotopic values significant as this is based on very few data points? If not, I suggest to remove this line. (later in line 331 it's referred to as a trend in data).

The authors agree and were quite disappointed by the timing of this malfunction that we only discovered after the fact. Unfortunately, the closest functioning quantitative rain gauge was over 60 km away from

our field location and due to geographical differences (coastal versus inland with topographic barriers in between) the data from this other location is not representative of the precipitation occurrence at our sampling location. There is no way to retroactively glean this information from our field site so we are left with our qualitative knowledge that rain occurred on certain days that we sampled. Because we do not have reliable records of the duration of rain or the volume of rain, we have removed the term 'brief' from the text since we cannot constrain the precipitation event with additional details.

The authors revisited the text with references to Fig. S2 and have removed the language that interprets isotopic results from the main text.

We also revisited text in section 4.2 and modified some of the language to lessen the confidence with which observed measurements were a direct result of precipitation events. Ex: we have modified 'associated with precipitation events' to 'that co-occurred with precipitation events' and we have removed a sentence that overinterpreted the isotopic 'trend' from two sample points.

Additionally, we have added the following line of text to the supplement to clearly state that the co-occurrences in nitrate variability with precipitation events is not well constrained due to the lack of isotopic analyses from precipitation samples: "However, without the direct isotopic analyses of precipitation samples, these interpretations are based solely on the co-occurrence of precipitation events with observed chemical variability and are not quantitatively verified."

4.3. Effects of redox...: Line 328: It's not clear to me how the lack of mobility of NO₃ beyond the first 20-30 m downslope can be seen from Fig. 4 as this shows no downslope-scale (see comment above).

The authors thank Anonymous Referee #3 for bringing the lack of spatial scale to our attention. We have added a line of text to the Figure 4 caption that adds these clarifying details: "Each sampling rhizon nest (depicted as histograms in these transects) is spaced ~10 m apart."

4.4 Spatial and temporal... Line 344: As for my previous comments, I find the interpretations on soil NO₃ in relation to precipitation basically unsupported as no precipitation data are presented. Moreover, in Fig. S1 it is shown that soil NO₃ and soil moisture was not correlated, which somehow is conflicting the statement that "...the notable day-to-day changes in soil NO₃...was driven primarily by the presence of rainfall."

The authors have modified the language in this line from "correlated" to "co-occurred".

The authors have also modified the language, "the notable day-to-day changes in soil NO₃...was driven primarily by the presence of rainfall.", to "the notable day-to-day changes in soil NO₃...was likely influenced by the co-occurrence of rainfall as a mobilization mechanism"

With regard to Figure S1, we do not observe a correlation between soil moisture and nitrate concentrations in our study location. However, while the soil moisture samples are co-located with our soil pore water samples, they are not temporally correlated (collected during same sampling season but not same time as soil pore water samples). This is because sampling for gravimetric soil moisture content physically perturbs the system and could have influenced the pore soil water compositions if done at the same time. Thus, we collected soil moisture samples on our final days in the field to avoid influencing the chemistry of the water we collected and the resulting divergence between these parameters is likely a result of the temporal offset between sampling the different parameters. To clarify, the following text was added to Figure S1 caption:

“It is worth noting that soil moisture and NO₃-N samples were co-located but collected on different temporal scales to avoid perturbing the rhizon nests during our time-series sampling campaigns.”

We have also added a line of clarifying text to section 2.4, “It is worth noting that while our soil moisture and soil pore water samples were co-located, they were offset temporally with our soil moisture sampling occurring at the end of our campaigns to avoid perturbing the rhizon nests while we were actively collecting soil pore water samples.”

Thus, this lack of correlation between data collected at different times does not negate the variability in nitrate observed during or immediately following precipitation events at our study location.

4.5 Future research. Line 357, see aforementioned paper by Rasmussen et al., 2022.

The authors have incorporated this reference and amended the future work to reference the importance of snowmelt influence on NO₃ variability at the start of the grow season: “Future studies would benefit from the additional incorporation of continuous monitoring of NO₃⁻ throughout a growing season (starting around snowmelt; Rasmussen et al., 2022),”

Supplement

Isotopic insights: The inclusion of isotopic water and nitrate data is acknowledged as these can be important indicators for dominant processes in the present site. However, I do on the other hand also recommend data being interpreted and extrapolated with due attention to the fact that inputs and characteristics of e.g. NO₃ in precipitation is not established in this study – see comment above.

The authors appreciate this recommendation. While we were not able to measure the NO₃ in precipitation, we do have useful information regarding where our isotopic values plot relative to known compositions/ranges associated with likely sources of NO₃ within our environment. These interpretations are not the main focus of our body of work and this is thusly why this section has been moved to the Supplement rather than the main text. The authors have also added the following text to caution the reader of this possible issue: “These precipitation interpretations should be considered carefully as we were unable to directly measure the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of local precipitation due to logistical and sampling constraints. However, we apply logical inferences about likely influences of precipitation on observed chemical trends from our field location based on the known occurrence of rain events on specific sampling days and the corresponding shifts in site chemistry observed.”

Please, explain how 15N in TDN was measured.

The following text was added before equations S2-S3 in the Supplement for clarity: “ $\delta^{15}\text{N}_{\text{TDN}}$ was measured from the sample aliquots that underwent persulfate oxidation for TDN, which converts all ammonium, DON, and nitrite to nitrate. Thus, the $\delta^{15}\text{N}_{\text{TDN}}$ is from $\delta^{15}\text{N}$ analyses of the TDN sample aliquot.”

Coefficient of Variability: Table S6 (first line in section) should refer to Table S3.

The authors have corrected the Table reference as suggested.

Table S2. It's briefly mentioned (section 2.5) that DO, pH and conductivity didn't correlate with NO₃⁻, but did the authors assess if there's any particular spatial or temporal pattern in variation of these parameters? Maybe worth to mention.

The in situ parameters (DO, pH, and conductivity) collected from our field site were highly variable without any obvious spatial or temporal trends. The following line of text has been added to Table S2 caption and on L 204 within the main text: "no obvious spatial or temporal trends were observed within these parameters."