Author response to Anonymous Referee #1 comments: High temporal and spatial nitrate variability on an Alaskan hillslope dominated be alder shrubs

All author responses appear in grey italics below specific comments from Anonymous Referee #1.

The topic of the study to investigate the N cycle, in particular shifts in the nitrate concentration in the soil pore water in permafrost soils, affected by the N_2 fixation of alder (*Alnus viridis* ssp. *fruticosa*) is generally interesting. However, the study and the structure of the article has some major flaws and is a listing of observations instead of presenting a study with a clear focus. The structure of the article may be improved, but -unfortunately- several limitations of the design of the study cannot be corrected anymore (in retrospective).

The authors thank Anonymous Referee #1 for their feedback and agree that there are some limitations of the data presented due to the disparate nature of field access and sampling campaigns. While a continuous time-series would have been preferable, we were limited due to scheduling permissions and availability but firmly believe there is inherit value in the data that we were able to collect and the following text details our motivation for the various campaigns. Since there had been no actual measurements of nitrate concentrations in soil pore water of Arctic alder stands at the time this investigation began, we conducted an initial reconnaissance study in year 1 to gain insights to the distributions and concentrations of pore water nitrate. We augmented these data with additional targeted sampling campaigns in the following year but were limited on timeframes of the follow-up investigations.

Aspects to can be improved:

Many aspects that emerge in the result section, are not adequately described in the M&M section. Some descriptions given in the Supplementary Material

- "Seep" has not been explained in M&M

  The authors thank Anonymous Referee #1 for this suggestion. The seep was initially defined in the Figure 3 caption but we will add the following definition in the main text in the next version. "The seep sampling location is defined by a direct seep from the ground located on the A1 transect at the transition between upland and lowland zones. The volume of water coming from this seep was too small to measure directly but is estimated to be < 2 cm^3 s^{-1}. However, water was actively trickling from this location during all sampling campaigns and is likely representative of active layer melt that surfaces at the upland-lowland transition."

- Patch of Alnus shrubland: dimension/ size of the patch not defined. Valuable pieces of information would be height of bushes, density of alder branches per m2. Please add.

  The authors thank Anonymous Referee #1 for this suggestion. Figure 1c visually shows the scale of the alder stands we investigate but we will incorporate respective sizes of A1 (~2500 m^2) and A4 (~11250 m^2) into the main text for reference. Additionally, Salmon et al. (2019) identifies the alder stand height (cm), basal area per shrub (cm^2 stem), basal area (cm^2 m^{-2} ground), nodule biomass (g m^{-2} ground), and nodule biomass (g m^{-2} stem) in their study that include the 'alder shrubland' and 'alder savanna' regions that we investigate here. If accepted, we will also add these details to our manuscript.

  Reference:

Salmon VG, Breen AL, Kumar J, Lara MJ, Thornton PE, Wullschleger SD, Iversen CM. 2019. Alder Distribution and Expansion Across a Tundra Hillslope: Implications for Local N Cycling. Frontiers in Plant Science 10: 1–15.

- Authors present the total numbers of measuring points or samples (including soil pits), but it remains unclear what is really a replicate; a transect contains how many measuring points? The structure of the measuring points is also key for the statistical analysis. Both, temporal and spatial variability has to been taken into account in the statistical analysis. Here, one gains the impression, that one and then the other has lumped together. To emphasize that non parametric versus t-tests have been used is not adequate here.

  The authors thank Anonymous Referee #1 for this suggestion and believe some clarifying details will provide the needed context for interpretation of the results.

  At present, Figure 4 visually represents the relative locations and number of distinct rhizon clusters along the A1 and A4 transects, this information can be found in more detail in Table S5 in the Supplement, which identifies each specific sampling location that is clustered within the different sampling regions (i.e. A1_WI_Up, A1_WI_Mid, and A1_WI_Down, represent three distinct sampling locations that are located within Alder Stand 1 (A1)).

  Table S6 in the Supplement displays the setup and results from the Mann Whitney rank sum tests performed for each constituent. The intention of the Mann Whitney statistical analyses is to look at the difference between 'upland' and 'lowland' sampling locations (spatial differences) and not between seasons (not temporal differences). Thus, here 'n' is the total number of samples collected in each location (upland and lowland) from both July and September 2018 combined. Based on advisement from a statistician consult, this approach appeared to be the best way to directly compare differences in 'upland' and 'lowland' sampling sites overall. The individual rhizon nests that make up 'upland' and 'lowland' are defined in Table S5.

  After reviewing these comments and discussing with coauthors who performed the statistics, we have decided that if this manuscript is accepted for publication, we would modify Supplement Tables S5 and S6 to improve clarity and add text to the main manuscript to detail that the Mann Whitney statistical tests performed on nutrient data were not intended to highlight temporal variation, but to identify significant differences between the upland and lowland site geochemistry regardless of season.

  Because of the discontinuous nature of our sampling campaigns, our data is not suited for temporal comparisons of nitrate but we were interested in the influence of seasonality and precipitation events on water isotopic signatures. Thus, the only temporal statistical analysis performed was on the water isotopes (Table S7), which looked at differences between each season and the overall mean of $\delta^{18}O$ of water (not nitrate). The total number of samples collected within each campaign define 'n' for each campaign (samples not grouped spatially). These samples are not divided into 'upland' and 'lowland' samples but are lumped holistically to show seasonal trends as opposed to spatial trends. The 'n' varies widely between months because the number of days per campaign varied.

- The term "Alnus savanna" simply does not exist and is misguiding, please remove this term throughout the whole text!

  The authors thank Anonymous Referee #1 for this comment but respectfully disagree. The authors would like to note that we use the term 'alder savanna', not 'alnus savanna'. The term 'alder savanna' is defined by Frost et al., (2013), who write, "Such shrubland communities, colloquially referred to as 'alder savannas', have been described at several locations in Low Arctic and interior montane Alaska (Racine 1976, Racine and Anderson 1979, Chapin et al., 1989). Regular spacing of alders in 'alder savannas' has been attributed to intra-specific competition for limiting nutrients (Chapin et al., 1989)."

A coauthor on this paper (Salmon) also uses this 'alder savanna' for this community in Salmon et al. (2019) and Ben Sulman's recent modeling paper (Sulman et al., 2021).

References:

Chapin FS, McGraw JB, Shaver GR. 1989. Competition causes regular spacing of alder in Alaskan shrub tundra. Oecologia 79: 412–416.

Frost GV, Epstein HE, Walker DA, Matyshak G, Ermokhina K. 2013. Patterned-ground facilitates shrub expansion in Low Arctic tundra. Environmental Research Letters 8: 015035.

Racine C H 1976 Flora and vegetation Biological Survey of the Proposed Kobuk Valley National Monument. Final Report ed H R Melchior (Fairbanks, AK: Alaska Cooperative Park Studies Unit, Biology and Resource Management Program, University of Alaska) pp 39–139

Racine C H and Anderson J H 1979 Flora and vegetation of the Chukchi-Imuruk area Biological Survey of the Bering Land Bridge National Monument: Revised Final Report ed H R Melchior (Fairbanks, AK: Alaska Cooperative Park Studies Unit, Biology and Resources Management Program, University of Alaska) pp 38–113

Salmon VG, Breen AL, Kumar J, Lara MJ, Thornton PE, Wullschleger SD, Iversen CM. 2019. Alder Distribution and Expansion Across a Tundra Hillslope: Implications for Local N Cycling. Frontiers in Plant Science 10: 1–15.

Sulman BN, Salmon VG, Iversen CM, Breen AL, Yuan F, Thornton PE. 2021. Integrating Arctic Plant Functional Types in a Land Surface Model Using Above- and Belowground Field Observations. Journal of Advances in Modeling Earth Systems 13: e2020MS002396.

The authors will add in these references with additional clarifying text to avoid any misguiding caused by the use of 'alder savannas'.

Flaws in the design (cannot be correct anymore)

- The nitrate concentrations in soil pore water of Alnus shrubland along the hill is compared to those in the soil pore water in the lowland. However, authors stated that the soil was partly covered by standing water and the alder bushes were much smaller in the lowland area or do not even exist in the lowland (Figure 4!) So, standing water on one hand may dilute the nitrate concentrations and create denitrifying conditions, and on the other hand smaller Alnus shrubs have for sure a lower N_2 fixation. These two aspects cannot be disentangled. So, it is obvious that the nitrate concentration is lower under such conditions, described here as major result (please avoid overstatements in general). Furthermore, it has already been shown that the nitrate concentrations in the soil water under Alnus stands is much higher than under non-Alnus stands due to the N_2 fixation (not a new result!)

  The authors thank Anonymous Referee #1 for this comment and will make sure our field location descriptions are clarified to accurately represent the conditions present in the uplands versus the lowland sampling sites. The lowland sampling sites are comprised of alder savanna, where alder are present but not in dense stands as they are in the uplands. The findings observed provide insight to the transport and mobility potential of nitrate from dense alder stands in upland environments: topography/gradient and precipitation/moisture in tundra environment with alders largely control this potential. So although nitrate availability may increase directly in the soil-pore water of dense alder stands, the moisture/precipitation and topography/gradient conditions of the alder environment likely limit/control of the mobility potential of this critical nutrient. The authors will ensure they properly clarify this point and critically assess the major results discussion and remove instances of overstating outcomes.

- On site weather station is fully missing. Although authors stated precipitation events as key

for leaching processes.

A weather station has been installed for continuing studies at this NGEE field location but was unavailable at the time of the study presented here. We agree that measurements provided by a weather station would have been useful but are unable retroactively obtain this information. Weather records of towns ~50 km away from the field site are accessible and referenceable but not a direct representation of the weather conditions at our remote field site. Field observations of precipitation events are included for additional context of trends and variability observed. While we are not able to quantify the extent of precipitation events, we are able to identify trends correlating with observed precipitation events that provide insight to nutrient transportation responses to weather observed.

- A measuring campaign during 4 days (year 2017) is simply not representative for a seasonal measure (overstatement).

  The authors agree with Anonymous Referee #1 and will modify language accordingly to avoid temporal overstatements. While we do not have a full seasonal time series, the multiple short time-series we have captured provide valuable insights to snapshots of compositions and variations that may exist within short timeframes within specific seasons.

Detailed comments:

Abstract: Line 11: in the Arctic with capital letter, but arctic ecosystems with small letter. Please adapt throughout the MS.

The authors thank Anonymous Referee #1 for pointing this inconsistency out and the text will be updated accordingly.

Line 13: Simply not true, please consult the literature and adapt

While some details are known, many are not. The authors stand by this statement but will modify the text to include additional details of where knowledge gaps exist (ex: variable nature and transport potential of nitrate) and where further characterization is needed.

Line 14: Edaphic controls for the nitrate concentrations has not been shown.

'Edaphic' will be deleted from the text.

Line 18: I do not agree that all the nitrate is produced by degradation of N-rich alder shrub organic matter…or depends on your definition of organic matter..Are the corolla structure of the alder roots/nodules where the N2 fixation takes place, organic matter (I think not!)? The majority of the nitrate is already released during the N2 fixation.

The authors thank Anonymous Referee #1 for this comment and will modify the language here to include text referencing the role of root nodules in nitrate production.

Line 19, etc.: In general, better to express the nitrate concentrations as nitrate**-N** (enhanced comparability to other N compounds, atmospheric N deposition)

The authors will change references of nitrate to references of nitrate-N.

Line 23: denitrification buffers nitrate mobility. Strange description! Nitrate is transformed into N2 (complete) or N2O (incomplete) denitrification. From an ecological point of view the production of N2O is worse than nitrate mobility! Please adapt.

The authors are not claiming that the production of N2O isn't ecologically important, we are stating that nitrate-N is unable to be transported if it is converted to other N-species. We will modify the text to read 'denitrification limits the mobility of nitrate-N by transforming it to other N-species.'

Line 24. Nutrient production is a misguiding term. Through N2 fixation nitrogen as a nutrient gets available, then it is transformed or lost again through complete denitrification..please adapt.

The authors will alter text from 'nutrient production' to 'nutrient availability' here and in the remaining text.

Line 40: Nutrient availability instead of nutrient production
The authors will alter text from 'nutrient production' to 'nutrient availability' as suggested.

Line 46: Latin names – italic
The authors will verify that all Latin names are in italic before any resubmission occurs.

Line 48: instead of microbes, add here: *Frankia* bacteria
The authors thank Anonymous Referee #1 for this suggestion and will alter the text from 'microbes to 'Frankia bacteria' as suggested.

Line 51, line 57: Wrong!! Brühlmann et al. 2014, Hiltbrunner et al. 2014: Both studies are located in the montane (not in the alpine vegetation belt of the Alps), but clearly not on permafrost soils! Increase of alder shrubland due to changes in land use, not increasing temperatures. Please correct and add this aspect of land use changes.
The authors thank Anonymous Referee #1 for this observation and will modify this section accordingly to appropriately cite these references and identify land use change as an additional driver of shrub expansion.

Line 68-70: Necessary?
The authors will delete this text from the Study Objectives section because these details are already included in the Acknowledgements section.

Line 73-76: Hypotheses rather weak as already widely known that alder shrubs through their N2 fixation are source for nitrate. And your measuring campaigns cover some days in July and September, not seasons. And see comments on line 18 and 23 (comments are not repeated here).
The authors thank Anonymous Referee #1 for their feedback and will strengthen the hypotheses listed with additional details as well as modify the language to specify that seasonal variation is not formally investigated in this study.

Line 80: unusual format for coordinates, add elevation of the KG hillslope, please adapt
The authors will add the elevation of the KG hillslope to the text but keep the formatting of the coordinates consistent with the other studies associated with this campaign.

Line 95ff, add species names of the dominant species of graminoids and dwarf shrubs.
The authors will add species names as identified in associated studies by Salmon et al. (2009a-b).

Line 101-112: rather unclear and wordy description. Be more precise here! Avoid expression such as initial phase and comprehensive informed phase (rather empty expressions).
The authors will be more precise with their wording in this section.

Line 114-135 unclear what means additional transect here, how many sampling points per transect?? Shorten! Please be more precise.
The authors will shorten this section and clarify the number of sampling points per transect within the main text (information is currently outlined in Table S5 in the Supplement).

Line 140ff: a nest of macro-rhizon: please define in the main text (not in the Supp. Material)- I wonder how long lasted the installation, for such short sampling intervals (of 4 days) the installation duration may affect the water sampling of the first day. Please explain!
The authors will add these additional sampling details to the text. Rhizons were inserted in the nests on the first morning of each campaign and left in place for the duration of each campaign. Collection syringes were hung from the rhizons each morning and emptied each afternoon to obtain an integrated sample from each day in the field.

Line 167. Unclear description, which transect (?) and soil 0-15cm has not been sampled?
The authors will add these additional details of soil sampling to the Supplement in table format and reference appropriately within the main text. Yes, soil 0-15 cm was commonly dense with

roots from the overlying peat so we did not collect samples from 0-15 cm depth because we felt it would bias the chemistry. And all rhizon-obtained pore-water samples came from a depth of 15+ cm.

Line 180: Why five litter samples when A1 and A4 have three sampling locations each, unclear…
The authors were only able to obtain 5 uncompromised leaf samples. 6 samples were collected but one sample bag ripped and the sample was possibly compromised so we didn't analyze it, which is why only 5 samples were reported.

Line 184: instead of each water sample…*In situ* parameters were measured for each water sampling location …
The authors thank Anonymous Referee #1 and will adapt the text accordingly.

The whole M & M section needs to strongly streamlined, now it is a potpourri of very different measurements and reader often does not know why for what purpose a measurement was carried out, besides when and how many times…I suggest to present all these different locations and campaigns in a Table.
The authors thank Anonymous Referee #1 for this constructive suggestion and will present the different locations/campaigns in a Table and streamline the associated M&M text with appropriate references to the table. This suggestion will greatly improve the flow of the M&M section.

Line 191-203 Statistical analysis: Weak description, no information how normal distribution, outliers, etc were handled. Weird description of processes acting on nitrate production.
Additional details of the statistical analyses approach can be found in the Supplement. Authors will seek guidance from a statistical consultant for how to best display and communicate statistical outcomes obtained for next version.

Line 205: I assume that soils (and correspondingly patches) along the slope differ from soils in the inundated lowlands. And permafrost occurrence and thickness of the layer- were they similar along the hillslope as in the lowlands? Please specify!
The details of soil depth and soil moisture in the upland (UA + WA) and lowland (DA) sampling locations are included in Table S4 in the Supplement. The authors will add a statement identifying that lowland (DA) sites had shallower depths than upland (UA + WA) sites during Sept 2017 and July 2018, and add an appropriate reference to table S4 within the main text.

Line 208: personal observation of whom? Mean gravimetric soil moisture content
The personal observation was by those who participated in the field campaigns and measured active layer: ex: McCaully, Arendt, Newman, Heikoop, Wales, Musa. The authors will add this reference to the reference list to clarify. The authors will also alter current text 'mean soil moisture content (percent dry/wet weight)' to 'mean gravimetric soil moisture content' as suggested.

Line 209: unclear: which other sampling campaigns?
The authors will update the text here to specify that we are referring to the July 2017 and July 2018 sampling campaigns.

Line 212: what are logistical and sampling challenges? Unclear.
The authors feel that detailing the logistical challenges experienced over these campaigns would detract from the findings of the study but wanted to acknowledge that original plans had been disrupted and we were unable to get some of the data we had planned to get despite best intentions due to some logistical and sampling (field and equipment access) issues. However, the authors can also remove this statement from the text if it distracts from the manuscript.

Line 213: adapt subtitle, 4 measuring days do not allow to delineate synoptic results (overstatement)
The authors will modify the subtitle to "Initial results…" instead of "Synoptic results…".

Line 214: you mean.. nitrate concentration was higher … please adapt
The authors thank Anonymous Referee #1 and will adapt the text accordingly.

Line 216: Strange description on atmospheric condition, air temperature, mean or maximum , please be precise, brief precipitation event, add where this has been measured and the exact rain amount.
The authors will modify the text accordingly.

Line 220: Seep? Not introduced in M& M.
As commented on above, the authors will introduce the Seep during the M&M.

Line 221: relative to the other three sampling days in July
The authors will modify the text accordingly.

Line 222: avoid such blue sky interpretation! Adapt
The authors thank Anonymous Referee #1 for their caution and will adapt the associated text accordingly.

Line 224: 58.61 mg L-1 Typing error, such high values are not presented in the Figures nor in Tables!
The authors thank Anonymous Referee #1 for identifying this typo and will adapt the text accordingly.

Line 230: please describe the weather conditions during your campaigns properly
The authors thank Anonymous Referee #1 for this suggested and will add in more detailed daily weather information in a supplemental table., however, we were unable to obtain several quantitative weather parameters (ex: mm precip.) due to equipment access issues and equipment malfunction.

Line 234-237: two times the same results with very different outcomes? Rather weird description, please improve.
The authors will reword these lines to improve clarity.

Line 228-258: Not fully clear what you like to present as results here, rather repetitive description on the different campaigns, not really convincing, please improve
The authors will adapt the text to highlight the geochemical observations shown in Figures 2-6 and remove any repetitive language from the study designs.

Line 259-271: Rather a potpourri of observation, in M &M section you mentioned 5 litter samples, now there are 10. What do these results tell you.
The authors performed replicate measurements of the original 5 samples collected, making the total number of analyses points for the leaf litter, n = 10. The authors will add these details and clean up the text to streamline and clarify this section.

Discussion: Obvious that the N2 fixation is the main source for the nitrate! So, please reorganise the whole discussion, now it reads like another result section! Please adapt
The authors will take this feedback and use it to restructure the discussion section accordingly.

Line 325-326: what kind of additional controls? Please avoid such empty sentences.
The authors will specifically list examples of possible additional controls including denitrification bacteria, assimilation, and hydrologic flushing.

Line: 329: A new aspect emerges: the comparison with the global meteoric water line! Why do you expect evaporation ins such a wet landscape? See line 244 Water flows even during periods without rain…
The authors acknowledge the evaporation was negligible and made the comparison to the GMWL as a standard practice / common reference. Text cited from Line 244 was to active layer thaw

seepage, which is a constant presence of moisture but still susceptible to evaporation. The authors can remove the reference to the GMWL if readers find it to be distracting.

Line 386: Future research? Rather bizzare that the authors list all the requirements for a more solid study. I would not declare these points as future research but as prerequisites for the current study!
The authors argue that inviting the community to participate in more research in this area with suggestions to improve research outcomes is not bizarre, it is collegial and asks for engagement from the community. The goal of this text is to call on the community to increase our collective understanding of the ways in which NO3-N availability and mobility will change with changing climate in a variety of landscapes and environments. Knowing what we now know, we would have made some modifications to this study design and want to share these insights with other studies to they can build off of what is already known and maximize the outcomes of their research. Despite the challenges and learning opportunities encountered during this study, we still obtained data that increases our understanding of the highly variable nature and limited mobility of NO3-N in permafrost landscapes with alder shrub communities despite the increasing availability of NO3-N with warming climates and expanding alder stands.

Line 401: Really unclear how such a single hillslope study should be of value for ESM
This research was completed with oversight and support of the Next-Generation Ecosystems Experiments (NGEE Arctic) project. NGEE Arctic is supported by the Office of Biological and Environmental Research in the U.S. Department of Energy – Office of Science. A major motivation for this program is the use of modeling outcomes to inform experimental design and the use of experimental observations to inform models. We obtained experimental observations during this study that were shared with the modeling team to help inform the project on the appropriateness of incorporating small-scale spatial and temporal variations into models. While a single hillslope may not be incorporated into a larger ESM, processes observed within that hillslope can inform models of the general geochemical trends expected from expanding alder shrubs on Arctic hillslopes.

Line 4902: bolster??
The authors will replace 'bolster' with 'increase'.

Conclusion: Already established that alder fix atmospheric N and therefore contribute to higher nitrate concentration in soil water. Rather redundant conclusions.
The authors will critically assess their conclusions and remove instances of redundancy for future considerations. Authors also argue that additional studies that support known relationships add to n and increase the scientific community's broader understanding on nuanced variability within this known relationship.

Figures

Figure1 (a) better to insert a map
The authors will modify Figure 1a accordingly.

Figure 2 a, b: Redundant to use different size of symbols and different colours. Please use for the same nitrate concentration the same colour! It is not really convincing to present means AND single values
The authors find that although different size symbols and different colors for different NO3-N ranges may be redundant, it is useful to visually emphasize where the higher concentrations exist. The color and range of concentrations between Figures 1a and 1b will be verified for consistency.

Figure 3: add sd (of bars) and add weather conditions (at least air, soil temperature and rain in mm)
The authors included the sd information in Table S5 in the Supplement but can also add markers to Figure 3. The authors do not have all the weather condition information suggested but can add

air and soil temperature and the presence or absence of precipitation.

Figure 4: There are no alders in the lower part of both transects, correct? I have some doubts whether the log Y scale really helps here. Table with the values (mean ± sd, number of replicates would be for sure more informative)

There are no dense alder stands in the lowland sampling locations but there are small interspersed alders present (defined by alder savanna landscape). Tables S5 and S6 in the Supplement contain this requested information. The authors will include a reference to these tables in the Figure 4 caption.

Figure 6. Boxplots largely overlap, that means no significant differences. How did you get this P<0.05? Though single t-tests? (Multiple mean test would be correct). Add n here…

Table S7 in the Supplement contains n, max, min, mean, sd, and p-values for the $\delta^{18}O$ data portrayed in Figure 6. Authors may end up moving isotopic portion of this manuscript to the Supplement because it seems to distract from transportation outcomes and is the only part of the manuscript to focus on temporal variability so it may be better to separate the isotopic work including this figure from the main body of the manuscript.