Manuscript Number: egusphere-2025-3080
Title: Effects of intensified freeze-thaw frequency on dynamics of winter nitrogen resources in temperate grasslands
Biogeosciences

Responses to reviewers #2:

General Comments

This manuscript addresses an important topic in ecosystem nitrogen (N) cycling under changing winter conditions, using a ^{15}N tracer approach to examine the fate of added nitrogen in plant, microbial, and soil pools following intensified freeze-thaw cycles (FTCs). The experimental design and tracer methodology are robust, and the study offers valuable insights into seasonal N allocation across ecosystem components.

However, several broader ecological interpretations—particularly those concerning ecosystem-level N retention, plant-microbial interactions, and species trait-based responses—go beyond what is directly supported by the data. Key processes such as N leaching, gaseous emissions, and root damage are not measured, limiting the conclusions that can be drawn about system-level outcomes and mechanisms.

Additionally, there are some inconsistencies between the hypotheses, measurements, and interpretations that should be addressed. Overall, the manuscript would benefit from a clearer distinction between the results directly observed in the data and the mechanisms proposed to explain them. The interpretation of the findings should be more cautious, and the methodological limitations of the study should be more explicitly acknowledged. Major revisions are needed to ensure that the hypotheses and conclusions accurately reflect the data obtained from the experiment, as some interpretations currently extend beyond what the results support. With these changes, the study could make a valuable contribution to understanding nitrogen cycling in seasonally frozen grassland systems.

Dear reviewers:

We sincerely thank you for your constructive comments on our manuscript. We have carefully addressed this point and made comprehensive revisions throughout the manuscript. Your suggestions have strengthened the overall quality of our work. Thank you once again for your time and expertise in helping us refine this research.

The key improvements are summarized below:

(1) Refined the hypotheses and Conclusions: We have rewritten our hypotheses to focus specifically on processes measurable with our ^{15}N tracer data (i.e., contrasting plant community-level N retention, species-specific ^{15}N acquisition), removing speculative mechanisms that were not directly measured (e.g., root damage, aggregate disruption). Correspondingly, all conclusions have been carefully revised to ensure they are fully supported by our data.

(2) Incorporation of new data and analysis: In direct response to specific comments, we have incorporated new data on ^{15}N leaching losses, which provides independent support for our conclusion of limited hydrological N loss. The interpretation of statistical analyses (correlation and random forest) has been refined to focus on the ecological meaning of key drivers rather than simply listing correlations.

(3) Explicit methodological limitations: A new dedicated section ("Limitations and future work") has been added to the Discussion. This section explicitly acknowledges the scope of the ^{15}N tracer method and the constraints of our temporal sampling resolution, while also incorporating the reviewer's valuable suggestions for future research avenues.

(4) Strengthened narrative and integration: Following the reviewer's suggesting, we have reorganized the Results and Discussion sections to improve logical flow, presenting findings from ecosystem-level ^{15}N retention down to the underlying soil, microbial, and plant mechanisms. We have also strengthened the integration between sections, particularly in linking microbial processes to plant community outcomes.

We believe these comprehensive revisions have significantly enhanced the clarity, precision, and overall impact of our manuscript. We are grateful for the opportunity to improve our work and believe it now makes a stronger contribution to understanding N cycling in seasonally frozen grasslands.

Specific Comments

1. Elongation of FTC:

The manuscript assumes that climate change will lead to an elongation of FTCs. However, it is not clear why elongation rather than an earlier onset or other changes in FTC dynamics is expected, especially in temperate grasslands in China. We suggest the authors clarify this assumption and provide relevant references supporting the prediction of FTC elongation in their study region. This will strengthen the rationale for the study design and its climate relevance.

We thank the reviewer for this important comment. We agree that clarifying why elongation of the freeze-thaw cycles (FTCs) period is a key projected outcome in our study region strengthens the climate relevance of our experimental design.

In the revised manuscript, we have amended the introduction (specifically in the climate context paragraph) to explicitly state the evidence for FTCs elongation and provide supporting references. The primary mechanism is asymmetric winter warming, which is particularly pronounced in northern temperate regions. This warming pattern does not simply shift the timing of a stable frozen period but extends the transitional seasons (autumn and spring) when soil temperatures fluctuate around

zero degree. This results in a later and less stable soil freeze-up in autumn and an earlier thaw in spring, thereby elongating the duration of the period susceptible to FTCs.

We believe this clarification, backed by the provided references, now solidly grounds our experimental treatment (intensifying FTCs within a potentially lengthened window) in a specific and credible climate change scenario for temperate grasslands.

2. Hypothesis (1):
The first hypothesis posits that intensified FTC would reduce retention of winter N resources due to physical disruption of soil aggregates, root damage impairing plant uptake, microbial cell lysis, and subsequent N leaching and denitrification losses. However, the study does not include direct measurements or assessments of the mentioned parameters, so it is not possible to robustly test this hypothesis. We recommend revising the hypotheses to focus on mechanisms and processes that are directly measured or can be reasonably inferred from the data.
We have rewritten our hypotheses to focus specifically on processes measurable with our ^{15}N tracer data (i.e., contrasting plant community-level N retention, species-specific ^{15}N acquisition), removing speculative mechanisms that were not directly measured (e.g., root damage, aggregate disruption). We have also ensured that the corresponding conclusions in the Results and Discussion sections are carefully aligned with this revised, measurable hypothesis. We thank the reviewer for this suggestion, which has significantly improved the clarity and scientific rigor of our work.

3. Losses of winter N sources:
The use of ^{15}N tracers allows tracking of the fate of added labeled nitrogen, but it does not account for the dynamics of native, unlabeled N pools that may be mobilized during freeze-thaw cycles (e.g., through microbial lysis or mineralization of soil organic matter). This is particularly important given the observed increase in soil NH_4^+ concentrations, which likely reflects the release of native N rather than enhanced retention of applied ^{15}N. The conclusion that intensified FTC did not lead to significant losses of winter N resources (e.g., abstract line 36; discussion lines 435, 452, 460–465) is therefore not fully supported by the data, as the study lacks direct measurements of N loss pathways such as leaching or gaseous emissions (e.g., NO_3^- leaching, denitrification, or volatilization). It would be valuable for the authors to clarify the scope and limitations of the ^{15}N tracer method in assessing winter N retention, explicitly acknowledging that it only tracks added N and does not capture mobilization and potential loss of native soil N.
We sincerely thank the reviewer for this critical comment. In this revised MS, we have supplemented our data on leaching losses of ^{15}N, which indeed show no significant increase (< 0.6%; Fig. 6) under intensified FTC treatments in the two grasslands. This new data provides direct experimental partly support for our

conclusion that significant losses of winter N sources via leaching did not occur.

The key improvements are follows:
(1) **Incorporated new leaching data** (^{15}N tracing data in deep soil, 30-50 cm): The leaching data has been added as a new figure and is described in the Results section. It provides direct evidence against significant hydrological N losses.

(2) **Refined key conclusions**: The conclusion is now more precisely phrased as "did not lead to significant losses of the added winter ^{15}N tracer", and we now explicitly cite the lack of elevated leaching losses as supporting evidence.

(3) **Added a methodological limitation section**: We have added a dedicated paragraph in the Discussion 4.5 to explicitly acknowledge the scope and limitations of the ^{15}N tracer method, as suggested by the reviewer.

We believe that the addition of the leaching data, coupled with the more nuanced interpretation of the ^{15}N results, has substantially strengthened our manuscript and addresses the reviewer's concerns. We are deeply grateful for the insightful comment that led to this improvement.

4. Plant-microbe interactions:
The manuscript interprets the temporal pattern of stable microbial ^{15}N retention and increasing plant ^{15}N uptake as evidence of a decoupling mechanism that stabilizes winter N resources and fosters mutually beneficial plant-microbe interactions under intensified FTC. However, there is no observed decline in microbial ^{15}N over time (Figure 6), which would be expected if immobilized N were later released to support plant uptake. Furthermore, the dynamic appears consistent across all treatments, suggesting it is a general feature of seasonal nitrogen cycling rather than a specific effect of FTC. Therefore, the conclusion that FTC induces a stabilizing mechanism through temporal decoupling is not directly supported by the data and should be removed.
We sincerely thank you for your critical and correct observation. Upon re-examination of our data, we fully agree with the opinion that the stable microbial ^{15}N pool over time (Fig. 6) does not support the mechanism of a direct temporal transfer of N from microbes to plants, and that the original interpretation of a "mutually beneficial plant-microbe interaction" and specific "temporal decoupling" induced by FTC was an overstatement beyond what the data can support.

In this revised MS, we have rewritten the relevant sections in the Discussion (section 4.2) to refocus the narrative on the data-driven conclusions.

Our revised interpretation now emphasizes the following points, which are directly supported by our data:
(1) The soil pool acted as a major sink for the added ^{15}N, with significantly elevated

recovery under high-frequency FTC (HFTC), suggesting efficient physical protection and chemical stabilization of the released N.

(2) Microbes acted as a crucial biological buffer by rapidly immobilizing ^{15}N in early spring, thereby securing the N pulse against potential loss during a period of low plant uptake.

(3) The subsequent rise in plant ^{15}N uptake is now discussed as likely originating from other soil N pools (e.g., the stabilized soil N or from ongoing mineralization), rather than from a direct release of the immobilized microbial ^{15}N. We explicitly acknowledge that the stable microbial ^{15}N pool argues against a direct transfer.

We have therefore reframed the ecosystem N retention mechanism as a combination of abiotic stabilization in the soil and initial biological immobilization by microbes, both of which are directly supported by our ^{15}N data. We believe the revised text is now more accurate and robust, and we are grateful to the reviewer for their insight which has significantly improved the quality of our manuscript.

5. Clarify scope of conclusion regarding FTC effects:
The discussion (line 454) mentions that intensified FTC increased total ^{15}N recovery, and the conclusion (line 538) states that intensified FTC reveals enhanced retention of winter N resources. However, the data show that this effect is limited to the high-frequency FTC treatment, with no comparable increase under low-frequency FTC. Therefore, the current phrasing could be misinterpreted as evidence of a general ecosystem response or resilience to FTC. Please clarify that the observed effect pertains specifically to high-frequency FTC.
We agree that our original phrasing was overly broad and could be misinterpreted as indicating a general response to any intensification of FTC. It is correct that the significant increase in total ^{15}N recovery was indeed a specific effect of the HFTC treatment, as the low-frequency FTC (LFTC) did not elicit a comparable response.

We have carefully revised the manuscript to clarify the scope of this finding. Specifically:
(1) **In the Discussion**, we have rephrased the statement to explicitly specify that the increase in total ^{15}N recovery was driven by the high-frequency FTC treatment.
(2) **In the Conclusions**, we have similarly amended the language to state that enhanced retention of winter N resources in spring was revealed specifically under intensified high-frequency FTC.
These changes ensure that our conclusions accurately reflect the specific conditions under which the observed effect occurred, preventing any potential misunderstanding about a general ecosystem resilience to FTC.

6. Temporal resolution of sampling and N cycling processes:

While the seasonal soil sampling intervals are appropriate for tracking broad patterns, the temporal resolution immediately before, during, and after the freeze-thaw treatments appears too coarse to capture short-term N cycling processes. Processes like nitrification and denitrification usually occur within days after FTCs and can contribute to substantial N losses in form of N_2O fluxes. Please consider acknowledging this limitation, especially when interpreting mechanistic effects of FTC on nitrogen transformations.

We appreciate you for raising this important point regarding the temporal resolution of our sampling. We acknowledge that our seasonal sampling intervals, while appropriate for tracking the broader seasonal patterns of plant N uptake, were likely too coarse to capture the rapid, short-term dynamics of microbial N cycling processes (such as immediate microbial ^{15}N immobilization/remobilization or N_2O fluxes) that occur within days following freeze-thaw cycles.

Our experimental design was primarily focused on investigating the fate of winter N sources in the context of plant N acquisition, which is a cumulative and ecologically decisive process over the growing season. From this perspective, our sampling strategy was sufficient to accurately quantify the ultimate utilization of the ^{15}N tracer by plants.

We have followed your suggestion and explicitly acknowledge this limitation in the revised manuscript. A statement has been added to the Discussion (Section 4.5) to clarify that our interpretations of short-term mechanistic effects, particularly regarding microbial N transformations immediately following FTCs, are constrained by the temporal resolution of our sampling. We agree that future studies targeting these rapid microbial processes would benefit from higher-frequency sampling. We thank the reviewer for this valuable comment, which helps to better define the scope and interpretation of our findings.

7. Soil sampling depth and deep roots:

The study sampled only the top 20 cm of soil, which likely captures much of the microbial and plant root activity. However, nitrate is highly mobile and may leach below 20 cm, especially following FTC-induced mineralization, potentially leading to underestimation of N losses and overestimation of retention. Moreover, Hypothesis 2 suggests that deep-rooted species may increase winter N uptake under intensified FTC. Yet, root ^{15}N retention was assessed only within the top 20 cm, which may not reflect uptake from deeper soil layers where such species may access nitrate. This could reduce the apparent contrast between shallow- and deep-rooted species. We recommend acknowledging these limitations when interpreting both N retention dynamics and species-level uptake patterns.

We thank the reviewer for raising this important point regarding soil sampling depth. We designed our soil sampling to a depth of 20 cm because approximately 80% of the root biomass is distributed within this upper soil layer in the studied grasslands. However, we agree that sampling only the top 20 cm could lead to an underestimation

of N leaching and provide an incomplete picture of N uptake by deep-rooted plant species.

Actually, our experimental design included deep soil sampling (30-50 cm). The data from this depth, now explicitly presented in Figure 6, show that the amount of ^{15}N recovered below 20 cm was negligible (consistently < 0.6% of total recovery). This provides direct evidence that significant leaching of the added winter ^{15}N tracer below the root zone did not occur, and that the top 20 cm effectively captured the vast majority of the retained tracer.

We have taken the following steps to address this limitation:
(1) **We have explicitly acknowledged this limitation in the revised Discussion 4.5**. We state that our sampling to 20 cm, while capturing the majority of microbial activity and fine root biomass (> 80%) in the grasslands, may lead to an underestimation of N leaching below the root zone and may not fully reflect the N uptake by deep roots.

(2) **We have refined our interpretation of the species-specific results for Hypothesis 2.** We now cautiously state that the measured ^{15}N acquisition in the top 20 cm reflects the competitive outcome in the surface soil horizon. We clarify that while deep-rooted species might access resources from deeper layers, their enhanced ^{15}N uptake in the surface soil still indicates a successful competitive strategy under intensified FTC. However, we concede that the absolute amount of N acquired by deep-rooted species might be higher than we recorded, and the contrast with shallow-rooted species might be even more pronounced in reality.

We believe that by acknowledging this limitation and refining our interpretations accordingly, we have provided a more accurate and nuanced discussion of our findings. We thank the reviewer for highlighting this important point.

8. Species specifics (Hypothesis 2):
You hypothesize that "intensified FTC would lead to differential utilization of winter N sources among plant species," and in the discussion, you refer to several plant traits to explain species-specific responses. However, it is currently difficult for the reader to follow these arguments without a clear overview of the relevant species and their functional traits. To strengthen the link between your hypothesis and interpretation, we recommend including a brief summary of the observed species and their key traits that you mentioned in your hypothesis 2 (competitive abilities, root system architecture (particularly rooting depth and winter root activity), temporal niche partitioning in growth phenology (early spring green-up), and susceptibility for root damage) in the Methods section. Further, we suggest to formulate the hypothesis more concretely. This would provide helpful context for understanding the mechanisms underlying your findings.

Thank you for this valuable suggestion. We agree that providing a clearer overview of the plant functional traits could strengthen the link between our hypothesis and interpretation. Accordingly, we have added Supplementary Table 1, which provides a concise summary of the key traits for the species studied, as recommended. Revised Hypothesis 2 to make it more specific, directly linking the expected outcomes to the functional traits described.

9. Site specifics:
The manuscript provides useful site-specific information; however, the methods section does not specify how these data were obtained—whether from field measurements, previous studies, databases, or modeling (e.g. bulk density). Including details on the sources and collection methods for these site parameters is important for transparency and reproducibility. Moreover, while site differences are documented, the manuscript lacks a discussion of how these environmental and edaphic differences may have influenced the observed results. Since the study compares two distinct sites, integrating an analysis or interpretation of how site characteristics might drive differences in nitrogen cycling, microbial activity, plant uptake, or freeze-thaw responses would strengthen the ecological context.
Thank you for these insightful and constructive suggestions. We have addressed these points comprehensively in the revised MS:

(1) Enhanced methodological transparency:
In the Methods section (now Section 2.1), we have specified the source and collection method for each key site parameter. We explicitly state that soil bulk density, texture, pH, total C and N were determined from our own field measurements and laboratory analysis of soil samples collected during the study establishment. Meteorological data were obtained from the China Meteorological Administration, with specific station codes and access details now provided.

(2) Integrated site comparison in Discussion:
As suggested, we have added a dedicated analysis in the Discussion (Section 4.3) that explicitly links the observed responses between the two grasslands to their contrasting environmental and edaphic conditions.

Initially, we hypothesize that at an ecosystem level, the effects of intensified FTC on retention of winter N resources would be mediated by ecosystem type, with the sandy steppe experiencing a greater reduction than the meadow steppe due to its inferior edaphic conditions (Table 1). Contrary to our first hypothesis, plant 15N recovery showed no significant difference between the meadow and sandy steppes under intensified FTC.

In the revised MS, the text as follows:
“4.4 Ecosystem-Level Convergence in Plant N Uptake Despite Divergent Soil

Conditions

At the ecosystem level, the absence of a significant difference in the magnitude of plant ^{15}N uptake between the meadow steppe and sandy steppe is noteworthy, given their pronounced differences in soil fertility (Table 1). This apparent convergence can be explained by several compensatory mechanisms that operated across the two contrasting sites.

First, the dynamics of nitrogen availability differed. While the meadow steppe exhibited a higher net N mineralization rate in early spring, providing a larger initial pulse of inorganic N, the sandy steppe likely compensated through more efficient plant uptake of the available NH_4^+-N pool, as indicated by its significantly lower soil NH_4^+-N concentrations. This suggests that plant communities in the resource-limited sandy steppe are adapted for rapid nitrogen acquisition when it becomes available.

Second, the physical pathway of nitrogen loss was similarly constrained in both ecosystems. The lack of a significant difference in ^{15}N leaching losses indicates that intensified FTCs did not disproportionately enhance hydrological N losses in the coarser-textured sandy steppe soil. This physical retention created a similar baseline condition for nitrogen conservation in both systems.

Finally, the biotic component in the sandy steppe demonstrated inherent resilience. The microbial community, adapted to arid and nutrient-poor conditions, likely possessed metabolic traits that buffered it against FTC-induced physiological stress. This microbial stability, coupled with a plant community dominated by deep-rooted and drought-tolerant species, contributed to an ecosystem-level N retention capacity that was functionally comparable to that of the more nutrient-rich meadow steppe. Therefore, the similar levels of plant ^{15}N uptake emerged not from identical processes, but from different yet effective strategies in each ecosystem."

We believe these revisions significantly improve the transparency, reproducibility, and ecological depth of our study.

10. Meteorological information:

The manuscript references the China Meteorological Administration website (http://data.cma.cn/) as the source for meteorological information at the study sites. However, this website is primarily in Chinese and can be difficult to navigate for non-Chinese speaking readers. To improve accessibility and reproducibility, we recommend providing a more direct and specific link to the exact data pages used, or alternatively, suggesting an English version or database where the meteorological data can be accessed more easily by an international audience. This would help readers verify the data and facilitate broader use of the study's findings.

Thank you for this valuable suggestion to improve the accessibility and reproducibility of our meteorological data. We have added another international

website: NOAA Integrated Surface Database (ISD): (https://www.ncei.noaa.gov/maps/hourly/) in the revised MS, as an alternative or for verification. This database integrates global surface meteorological observations and includes data from numerous Chinese international exchange stations.

11. Artificial manipulation and microclimate measurements:
The study aims to address the lack of natural in situ FTC experiments (lines 71), but the use of polyester tents and air heating still introduces some artificial influences. While the manuscript notes that mesh windows were used to reduce CO_2 accumulation, it would still be helpful to clarify how other potential microclimatic changes were accounted for. For instance, air temperature was recorded at 5 cm above ground (line 174), but these data are not presented or discussed. Changes in humidity, photosynthetically active radiation (PAR), or CO_2 levels could still affect plant growth and nitrogen uptake. We recommend briefly discussing these possible side effects of the experimental setup to help contextualize the findings. Also, figure 2 shows that the soil processing period overlaps with the occurrence of freeze-thaw cycles at the sandy steppe site. Please address if this overlap may have influenced the results.
We thank the reviewer for raising these important methodological considerations. We have addressed each point as follows:

(1) **Contextualizing our in-situ approach**: While we acknowledge that the use of tents introduces some artificiality, our experimental design represents a significant improvement over previous laboratory-based FTC simulations. Unlike studies that transport soils to the lab for temperature manipulation, our in-situ approach maintains natural soil structure, root networks, and microbial communities, thereby providing a more ecologically realistic representation of FTC impacts in intact grassland ecosystems.

(2) **Temperature monitoring clarification**: We apologize for the unclear description in our original manuscript. We have now clarified that while soil temperature at 10 cm depth was continuously monitored, the 5 cm depth was periodically verified with a handheld thermometer specifically during FTC treatments to ensure target temperature thresholds were met. The continuous 10 cm depth data, which effectively captures the FTC dynamics. We have corrected this methodological description in the revised MS to avoid any confusion.

(3) **Microclimate considerations**: We acknowledge that we did not monitor humidity, PAR, or precise CO_2 levels due to equipment limitations. We recognize that measuring these parameters would have provided a more complete picture of the microclimatic changes induced by our tents. We also state that this insightful comment highlights an important aspect for future research, and we will incorporate the monitoring of these key microclimatic parameters in subsequent experiments to provide a more comprehensive understanding in the future.

However, we note that: (i) our treatments were applied before plant green-up when vegetation would be less responsive to subtle microclimatic variations; (ii) the mesh windows substantially reduced CO_2 accumulation; and (iii) the short duration of tent deployment (6-12 cycles, immediately removed after treatment) minimized potential impacts on plant growth and N uptake. We agree that monitoring these parameters in future studies would provide valuable supplementary data.

(4) **Treatment timing**: We confirm that our experimental FTC treatments were completed before the natural FTC period began, as clearly shown in Figure 2a,b. The treatments were intentionally scheduled approximately 15 days prior to the natural FTC period to simulate FTC elongation while avoiding overlap with natural cycles, thus preventing confounding effects.

We believe these clarifications and methodological improvements address the reviewer's concerns while demonstrating the ecological validity of our experimental approach.

12. Soil moisture measurement and implications of differences:
While soil moisture data were recorded using data loggers (line 205), the manuscript does not specify the sensor types, calibration methods, or how values (in $m^3\ m^{-3}$) were derived. The presence of negative soil moisture values suggests possible measurement or calculation errors that should be addressed. Additionally, there is an inconsistency between the text (lines 280–284), which states that elevated soil moisture occurred only in early spring, and Figure 2b, which appears to show sustained increases under LFTC and HFTC throughout much of the season. It should be included in the discussion how treatment-induced changes in soil moisture may have influenced nitrogen dynamics and plant ^{15}N uptake, as it was a significant predictor in the correlation and random forest analyses.
We apologize for the absence of these essential methodological details. We have now revised the Methods section to include the following information:
Soil volumetric water content (VWC, m^3/m^3) and temperature were monitored using a HOBO H21-002 data logger (Onset Computer Corporation, USA) coupled with 10HS soil moisture sensors. The 10HS sensor estimates VWC by measuring the soil dielectric permittivity at a frequency of 70 MHz. The sensors were deployed with their factory-predefined standard calibration equation, which directly converts the measured dielectric readings into volumetric water content values (m^3/m^3). Therefore, for the conventional soils in our study, the data logger directly outputs the final VWC values, and no further calculations were required by us. The negative values occurred primarily in cold and frozen soil conditions and are a known artifact of the sensor's calibration at the extremely lower end of its measurement range. In our revised dataset, all negative VWC values have been set to 0 m^3/m^3, reflecting that the liquid water content was at or below the sensor's effective detection limit. This is a standard

data-cleaning practice, and we have added a note in the methods section to state this correction. The number and magnitude of these values were negligible and did not influence the statistical outcomes or overall conclusions.

Upon re-examination, we confirm that Figure 2b is accurate: the LFTC and HFTC treatments led to a sustained increase in soil moisture over much of the seasons. We have therefore corrected the text in the Results section to accurately reflect the figure.

We fully agree that exploring the mechanistic link is crucial. We have now added a dedicated paragraph in the Discussion section to elaborate on this. In this new paragraph, we explicitly state that soil moisture was a key predictor in our statistical models, discuss how the treatment-induced increases in soil moisture could have created conditions that enhanced microbial activity and nitrogen mineralization.

13. Restructure Chapter 2.3 Sampling and Processing:
This section would benefit from restructuring to more clearly distinguish which subsamples were used for which analyses and to detail the analytical procedures more consistently. We would recommend to reorganizing this section to clearly present the workflows for each measured variable (e.g., soil mineral N, DOC, microbial C and N, soil/plant/microbial ^{15}N, soil moisture and temperature) to improve readability and reproducibility.

Further, it is unclear whether the described K_2SO_4 extraction and analysis refer only to microbial biomass C and N samples or if the same procedure was used for soil mineral N (NH_4^+ and NO_3^-) and DOC analyses as well (lines 231-233). Please clarify whether different extraction procedures were used for mineral N and DOC, and if so, provide the details (e.g., solution type, shaking duration, soil-to-solution ratio).

Regarding the ^{15}N measurements, plant and soil ^{15}N were measured with an elemental analyzer coupled to IRMS — was the same system used for total microbial ^{15}N, or was a different method used? Please also clarify whether the same elemental analyzer (Elementar Vario Max CN) was used for all C/N and ^{15}N analyses, or if multiple instruments were involved.
(1) We have thoroughly restructured and revised Chapter 2.3 to present a clear, workflow-based structure according to your recommendations. The revised section now contains dedicated sub-sections for each major group of analyses, as follows:
2.3.1. Soil moisture and temperature
2.3.2. Soil and plant properties
2.3.3. Soil Microbial Biomass
2.3.4 ^{15}N level in soil, plant and microbe

This new structure explicitly outlines which subsamples were used for each analysis and details the analytical procedures in a logical sequence, significantly enhancing

readability and reproducibility.

(2) Different extraction procedures were indeed used for different analyses, and we have now provided distinct details for each in their respective sub-sections.

Soil inorganic N and DOC: fresh soil samples were extracted with 2 M KCl at a 1:5 soil-to-solution ratio (10 g fresh soil with 50 mL KCl) by shaking for 1 hour on a mechanical shaker. The extract was then filtered and used for the determination of NH_4^+ and NO_3^-, as well as for DOC analysis. All plant and soil elemental (C/N) were performed using elemental analyzer (Elementar analyzer Vario MAX CN, Germany).

Microbial biomass C and N: the chloroform fumigation-extraction method was employed. Both fumigated and non-fumigated soils were extracted with 0.5 M K_2SO_4 at a 1:4 soil-to-solution ratio (15 g fresh soil with 60 mL K_2SO_4) by shaking for 30 minutes and then filtered.

^{15}N analyses: ^{15}N analyses for plant and soil samples were performed using elemental analyzer (Elementar analyzer Vario MAX CN, Germany) coupled in continuous flow mode to a Isoprime Precision isotope ratio mass spectrometer (IRMS) (Isoprime, USA). ^{15}N analyses of microbial biomass was determined using an modified diffusion method (with slight heating and acid-soaked glass fiber filters as the trap), and the filters containing the absorbed N were then measured using the same EA-IRMS system.

14. Correlation analysis and random forest:
The manuscript includes correlation and random forest (RF) analyses to identify key predictors of plant 15N acquisition, incorporating variables such as DOC, microbial carbon, and microbial community composition (bacterial vs. fungal biomass). However, the rationale for including both statistical approaches is not clearly explained, and the results from these analyses are not sufficiently integrated into the discussion. It remains unclear how the outputs of both approaches complement each other, and what ecological insights they offer regarding nitrogen dynamics under freeze-thaw conditions. Additionally, the manuscript does not explain the relevance of DOC and microbial biomass C to nitrogen cycling or FTC effects. Similarly, the ecological importance of differentiating microbial groups (bacteria vs. fungi) is not introduced and not interpreted in the results or discussion. Lastly, it is unclear why these analyses were performed only for the two FTC treatments and not for the control. To improve coherence, we recommend explaining the rationale for including both correlation and RF approaches and discussing the ecological relevance of the identified predictors.
We thank the reviewer for these insightful comments regarding our statistical approaches. We have partly revised the relevant sections to address these concerns as follows:

1. Clarified rationale for using both correlation and random forest analyses:
In the revised Methods section (Statistical Analysis), we now explicitly explain the complementary purposes of these two approaches:
(1) Correlation analysis was used as an initial screening tool to identify potential relationships between environmental factors and plant ^{15}N acquisition across different treatments.
(2) Random Forest analysis was then employed as a more robust machine learning method that can handle high-dimensional data and minimize overfitting, while effectively ranking variable importance and handling collinearity among predictors.

2. Enhanced ecological interpretation of key predictors
We have significantly expanded the Discussion to provide proper ecological context for the identified predictors:
(1) The relevance of DOC and microbial biomass C to N cycling under FTC conditions is now explicitly discussed, particularly their roles in microbial metabolism and N immobilization processes.
(2) We have removed soil bacterial and fungal biomass from the revised MS, as these variables were found to be less important predictors in our Random Forest analysis and did not contribute significantly to explaining plant ^{15}N acquisition patterns.

3. Separate analyses for all treatments
Following the reviewer's suggestion, we have now performed and presented separate correlation and Random Forest analyses for the Control, LFTC, and HFTC treatments in the revised Figures 7 and 8. This approach allows for clearer comparison of how key drivers of plant ^{15}N acquisition change across different treatment intensities.

4. Improved integration of statistical results
The Results section now more clearly presents the outputs of both analyses, while the Discussion section provides a synthesized interpretation of what these statistical approaches collectively reveal about the mechanisms controlling plant N acquisition under FTC conditions.

We believe these revisions have significantly improved the coherence and ecological insight derived from our statistical analyses, and we thank the reviewer for these valuable suggestions.

15. Discussion on the relevance and magnitude of observed differences:
While the manuscript highlights statistically significant differences between treatments, it does not sufficiently address the ecological or functional relevance of these differences. A more in-depth discussion is needed on the magnitude of the changes observed. Please consider discussing whether the observed changes are likely to have substantial impacts on grassland resilience, nutrient cycling, or plant community structure in the context of winter climate change.

We thank the reviewer for this important comment regarding the ecological relevance of our findings. We have substantially revised the Discussion to provide a more in-depth analysis of the potential ecological consequences of the observed changes, focusing specifically on their implications for grassland resilience and community structure.

In the revised MS (Section 4.3), we now explicitly discuss the functional significance of the species-specific shifts in N acquisition. While the absolute magnitude of changes in ^{15}N uptake for individual species might appear limited in the short term, we argue that their "directional consistency" is ecologically meaningful. The amplified competitive advantage for cold-adapted species like *S. baicalensis* and *H. mongolicum*, coupled with the simultaneous suppression of subordinate species, represents a redistribution of N resources that could alter plant community composition if sustained over multiple years. This is particularly relevant in the context of winter climate change, as repeated FTC events may cumulatively favor stress-tolerant species, thereby reducing functional diversity and potentially shifting the community toward a new state.

Furthermore, we link these plant-level responses to ecosystem resilience. The overall reduction in community-level ^{15}N acquisition under high-frequency FTC, despite the stability of the soil and microbial N pools, suggests a potential decoupling between ecosystem N retention and plant N utilization. This indicates that resilience, defined as the capacity to maintain both structure and function, may be challenged, as the ecosystem's ability to conserve N does not directly translate to unchanged plant resource acquisition.

By framing our results in terms of these longer-term, cumulative ecological processes, we have strengthened the discussion of the functional relevance of our findings beyond immediate statistical significance.

16. Future research directions and limitations of the study:
The future research section outlines useful directions, particularly regarding microbial functional traits and long-term ^{15}N fate. However, other potentially impactful avenues are overlooked. For instance, it would be valuable to differentiate between the origins of newly available nitrogen during freeze-thaw cycles, specifically, whether it stems from microbial cell lysis, root mortality, or physical disruption of soil aggregates. Distinguishing these sources could significantly enhance mechanistic understanding of nitrogen retention and loss pathways. Additionally, the study briefly references nitrogen losses but does not address gaseous emissions. Monitoring greenhouse gases (e.g., N_2O, CO_2) during FTC events could offer further insight, especially given that FTC induced N_2O peaks often occur without corresponding CO_2 increases, which could have a relation to your results e.g. on increases in microbial biomass N and decreases in microbial biomass C.

We have completely rewritten and expanded the "Limitations and future work" section (now Section 4.4) to incorporate these specific suggestion.

Our revision as follows:

Limitations and future work.

(1) Methodological constraints:

First, while our ^{15}N tracer approach precisely tracked the fate of winter inorganic N, it cannot account for the dynamics of the native soil N pool, including mobilization and loss pathways of unlabeled N. Second, the temporal resolution of our sampling, while appropriate for quantifying seasonal patterns of plant N uptake, was insufficient to capture rapid microbial N transformations and gaseous fluxes occurring within days following FTC events. Third, our 20 cm soil sampling depth, though capturing the majority of root activity in these grasslands, may not fully reflect N dynamics in deeper layers where deep-rooted species access resources and nitrate leaching could occur.

(2) Experimental design considerations include potential microclimatic effects of our tent-based FTC manipulation. While we employed mesh windows to minimize CO_2 accumulation and implemented short-duration treatments, we did not monitor humidity, photosynthetically active radiation, or precise CO_2 levels, which could have provided additional context for interpreting plant responses.

(3) Contextual limitations should be noted. Our findings from two contrasting temperate grassland types provide important insights but may not be directly transferable to other ecosystems with different soil properties, microbial communities, or plant functional compositions. Additionally, the single-year duration of our experiment limits our ability to assess long-term ecosystem adaptations to repeated winter climate perturbations.

(4) Differentiation of N sources. We now highlight the importance of distinguishing the specific origins of newly available N, whether derived from microbial cell lysis, root mortality, or physical disruption of soil aggregates, as a key priority for future research. We acknowledge that such differentiation would substantially enhance our mechanistic understanding of nitrogen retention and loss pathways during FTC events.

(5) Gaseous emission monitoring. We have incorporated the reviewer's valuable suggestion regarding simultaneous monitoring of greenhouse gases (particularly N_2O and CO_2) with high temporal resolution during FTC events. We specifically note that this approach would help elucidate the relationships between microbial C and N cycling, especially relevant given the decoupled responses of microbial biomass C and N observed in our study.

These limitations, however, clearly define valuable avenues for future research, including high-resolution tracking of coupled C-N gas fluxes, molecular characterization of microbial functional traits, differentiation of N sources from various soil pools, and long-term ^{15}N tracing across multiple freeze-thaw seasons. We believe this comprehensive revision significantly strengthens the forward-looking

impact of our manuscript, and we are grateful for the reviewer's guidance in helping us identify these key research gaps.

Technical Corrections

1. **Line 273-395 Results: We suggest removing redundant "p < 0.05" and instead define significance threshold in the Methods or where relevant, report actual p-values.**

We have removed redundant "$p < 0.05$" and defined significance threshold in the Methods.

2. **Figure 2: We recommend to make the figure broader to better see the single FTCs. The labeling of the dates is unclear. The legend for samplings looks like another sampling event which is confusing.**

Revised as suggested. We have made the figure broader, added the label of dates and changed the legend for samplings to better show our data.

3. **Figure 3, 4 and 6: The dots for single measurements are not necessary and just confusing if they overlap – having error bar should be enough.**

Revised as suggested. Please Figure3, 4 and 6.

4. **Figure 5 and 7: Both are exactly the same plot, figure 5 doesn´t fit to the description – the figure showing plant biomass N is missing.**

We apologized for our mistake. We have upload a new Figure 5 in the revised MS.

5. **Figure 3 to 7: please including the exact dates of sampling in the legend.**

Revised as suggested. We added the exact dates of sampling in the legend. Please see Figure 3 to 7. And we have indicated the sampling time in Section 2.3 Sampling and Processing.

6. **Introduction line 37: replace "while" with "While"**

Revised as suggested.

7. **Line 106: does -2-1°C mean -2.1 °C?**

It means -2 °C to 1 °C.

8. **Line 125: replace "the predominant soil type in meadow grassland is loam soil, and which in sandy grassland is sandy loam soil." With "the predominant soil type in meadow grassland is loam, while in sandy grassland it is sandy loam."**

Revised as suggested.

9. **Line 131: add space "78 %"**

Revised as suggested.

10. Line 161: There is a verb missing: ", and no significant differences in plant/microbial N concentrations when compared to the 15N treatments."

Revised as suggested.

11. Line 172: add space "15 cm "

Revised as suggested.

12. Line 231: add space "60 ml "

Revised as suggested.

13. Line 232: How much Molar was the K_2SO_4 solution?

"0.5 Molar", we have added this.

14. Line 232: add space "30 min"

Revised as suggested.

15. Line 234: please state for what the conversion coefficient is used.

The conversion coefficient is 0.45.

16. Line 244: replace "as well as microbial community structure in situ soils" with "as well as the microbial community structure of in situ soils"

Revised as suggested.

17. Line 250: The part with the calculation of ^{15}N acquisition/recovery is not statistical analysis.

Thanks for pointing out. We have changed this section to 2.3 Sampling and Processing.

18. Line 253: replace ";" with ","

Revised as suggested.

19. Line 258: add space "x V x"

Revised as suggested.

20. Line 259: start new paragraph

Revised as suggested.

21. Line 264: Is "rcorr" from R or SPSS?

"Rcorr" is from R.

22. Line 266: randomForest and rfPermute packages from R or SPSS?

"RandomForest" and "rfPermute" packages are from R.

23. Line 271: remove comma “SigmaPlot 14.0”
Revised as suggested.

24. Line 271: Origin 14.0 is not existing
Thanks for pointing out. We have changed this to “Origin 2021”.

25. Line 335: replace “^{5}N” with “^{15}N”
Revised as suggested.

26. Line 377: add space “C and” and replace “Under” with “under”
Revised as suggested.

27. Line 388: replace “plants” with “plant”
Revised as suggested.

28. Line 400: subsequent growing seasons would mean several years as there is one growing season per year
Thank you for this suggestion. The text has been modified to improve clarity.

29. Line 414: Abbreviation of MBN was already introduced
Revised as suggested.

30. Line 421: remove “.”
Revised as suggested.

31. Line 436: specify that you mean plant water uptake
Revised as suggested.

32. Line 457: A verb is missing: “indicating that effective ecosystem-level N retention mechanisms.”
Revised as suggested.