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

Responses to reviewer #1:
Reviewer 1
This manuscript presents a well-done designed and highly relevant study that investigates the effects of intensified freeze-thaw cycles (FTC) on winter nitrogen dynamics in temperate grasslands. The application of an in situ ^{15}N tracer approach across two contrasting grassland sites represents a significant methodological strength, providing direct insights into the fate of winter N sources. The central finding—that intensified FTC restructures winter N availability by enhancing microbial retention and altering plant competitive hierarchies, rather than causing simple N losses—is novel, compelling, and has important implications for predicting ecosystem responses to winter climate change. The study is timely, addresses a critical knowledge gap, and possesses innovation.

While the scientific foundation of the work is strong and the conclusions are broadly supported by the data, the manuscript in its current form requires significant revision to fully realize its potential. I am enthusiastic about the potential of this manuscript to make a valuable contribution to the field. The necessary revisions are needed and primarily focused on presentation and interpretation. I am confident that after a thorough revision addressing the points above and those detailed in the specific comments, this manuscript will be suitable for publication in BG journal.

Dear Editor and *Reviewer*:

We sincerely thank the reviewer for the thoughtful and constructive comments on our manuscript. Each suggestion has significantly enhanced the depth and clarity of our study. We have carefully addressed every point raised and made comprehensive revisions throughout the manuscript to incorporate the reviewer's valuable feedback. These suggestion have strengthened the overall quality and impact of our work.
Thank you once again for your time and expertise in helping us refine this research.

Best,
Linna Ma

Major concerns:
(1) Structural and Narrative Flow: The organization of the Results and Discussion sections could be optimized to create a more logical. Specifically, the order of presenting findings could be rearranged to better guide the reader from the ecosystem-level outcome (N retention) down to the underlying mechanisms

(soil processes, microbial uptake, plant competition).
We thank the reviewer for this excellent suggestion regarding the manuscript's narrative flow. We agree that reorganizing the presentation to follow a clear hierarchy from ecosystem-level outcomes down to underlying mechanisms significantly improves the logical progression and reader comprehension.

We have restructured both the Results and Discussion sections to create a more coherent narrative. The revised order now systematically guides the reader as follows:

(1) **Ecosystem-Level Outcome**: We first present the overarching finding regarding the fate of the winter-applied ^{15}N, specifically, the total ^{15}N recovery and retention at the ecosystem level, demonstrating that significant losses did not occur.

(2) **Underlying Mechanisms (Soil & Microbes)**: We then delve into the mechanisms responsible for this retention, first detailing the soil processes (e.g., ammonification, physical stabilization) and then the microbial response (e.g., shifts in biomass C and N, immobilization).

(3) **Plant-Level Consequences**: Finally, we present the consequences for the plant community, showing how the altered N availability restructured competitive hierarchies and species-specific ^{15}N acquisition.

We are confident that this reorganization has substantially improved the manuscript's clarity and impact, and we are grateful to the reviewer for this valuable recommendation.

(2) The Results section currently contains lengthy lists of percentage changes.
Thank you for your comment. We completely agree that this presentation style does not help to highlight the key findings and impairs the readability of the manuscript. We have rewritten the text in the Results section to be more narrative, guiding the reader to understand the meaning of the data rather than just the data itself. In the main text, we no longer list all data points one by one but instead focus on describing and emphasizing the most important and statistically significant trends and comparisons. We have retained only the most critical percentage values to support our core arguments.

(3) While generally clear, the manuscript requires a thorough proofread for grammatical consistency, conciseness, and precise scientific terminology.
We have undertaken a thorough revision of the entire manuscript with a specific focus on these aspects. This process included: comprehensive proofreading to correct grammatical errors and ensure consistency in tense, voice, and style throughout the text; a concerted effort to enhance conciseness by streamlining verbose sentences, removing redundant phrases, and improving the overall flow of the narrative.

(4) Discussion: The Discussion would benefit from stronger integration between sections (e.g., explicitly linking microbial decoupling to plant responses) and a more focused interpretation of the statistical analyses (e.g., highlighting the key drivers from the random forest analysis, rather than listing correlations).
We agree that strengthening the mechanistic narrative and providing a more focused interpretation of the statistical analyses are crucial for elevating the impact of our Discussion.

We have restructured the Discussion to create a logical causal chain and strengthen integration between sections and explicitly linking microbial processes to plant responses. In the newly structured section 4.2, we have proposed a clear, causal narrative that integrates the fate of nitrogen across the ecosystem components. From ecosystem outcome to underlying mechanisms, the section now begins by establishing the key ecosystem-level finding: the enhanced but transient total ^{15}N recovery under HFTC. We then explicitly introduce the two primary retention mechanisms responsible for this pattern: the soil pool as a physical sink and microbial biomass as a biological buffer. We have directly addressed the link between microbial immobilization and subsequent plant availability as suggested, providing a more nuanced mechanistic understanding.

Regarding the random forest analysis, we have completely rewritten the relevant section (in 3.5) to move beyond a mere listing of correlations. We now clearly highlight and interpret the dominant predictors identified by the model. We discussed the potential biological and ecological reasons why these specific factors are the key drivers of the observed patterns. This provides a much clearer and more insightful interpretation of our statistical results.

Specific comments:

1. **Abstract Line 34: Modify to read "employing the dual-labeled isotope".**
   We don't see this on line 34, and we guess maybe line 36 needs to be changed. This has been revised as suggested.

2. **Introduction Line 37: Change the period (.) before "while" to a comma (,).**
   Revised as suggested.

3. **Introduction Line 57: Modify to read "FTC results in distinct competitive environments".**
   Revised as suggested.

4. **Line 58: Change "present" to "exhibit".**
   Revised as suggested.

5. **Line 59: Change "," to "-".**
   Revised as suggested.

6. **Line 82: Modify to read "(1) intensified FTCs reduce winter N retention through three primary mechanisms: (a) physical disruption of soil aggregates that enhances N mobility, (b) root damage that impairs plant N uptake capacity, and (c) microbial cell lysis that leads to N leaching and denitrification losses;".**

Revised as suggested.

7. **Line 82: Modify to read "intensified FTC would cause differential utilization of winter N sources among plant species. This effect is mediated by interspecific differences in three key traits: competitive ability, root system architecture (particularly rooting depth and winter root activity), and growth phenology (temporal niche partitioning)."**

Revised as suggested.

8. **Line 112: Modify to read "During the study period, the meadow steppe had a persistent snow cover that reached a depth of 20-25 cm in late winter".**

Revised as suggested.

9. **Line 114: Replace "exhibited" by "had".**

Revised as suggested.

10. **Line 117: Replace "underwent" by "experienced".**

Revised as suggested.

11. **Line 121: Modify to read "This contrast enables a comprehensive assessment of...".**

Revised as suggested.

12. **Line 125: Modify to read "...the predominant soil type is loam in the meadow steppe and sandy loam in the sandy steppe...".**

Revised as suggested.

13. **Line 132: Replace "(Trin)." by "(Trin.)" and replace "(L)." by "(L.)".**

Revised as suggested.

14. **Line 133: It seems that there is extra space between the figure (70) and the unit (%).**

Revised as suggested.

15. **Line 155: Is it wrong here? If the amount of solution added is 120 mg ^{15}N m^{-2}, then it should be 600 mg ^{15}N L^{-1}.**

Thanks for pointing this mistake. We have replaced “120” by “600”.

16. **Line 226: Replace “contrast” by “difference”.**

Revised as suggested.

17. **Line 227: Replace “are” by “were”.**

The words “are” used instead of “were”.

18. **Line 231: Supplement the molar concentration of the K_2SO_4 solution, modify to read “...by shaking with 60 mL of a figure M K_2SO_4…”.**

We have corrected these words.

19. **Line 233: Replace “elemental analyzer” by “total organic carbon analyzer”.**

Thanks. We have corrected these words.

20. **Line 234: The conversion coefficient is abrupt, and it is integrated with line 225 to change: Microbial biomass C and N were calculated by dividing the differences in extractable C and N between fumigated and non-fumigated samples by a conversion factor of 0.45.**

We have corrected these words.

21. **Line 264: R package “rcorr” should be combined with version information. It is recommended to read: “Spearman correlation coefficients between variables were calculated using the rcorr function (in the Hmisc R package).”.**

Thanks. We have corrected these words.

22. **Line 268: Replace “above mentioned” by “above-mentioned” and replace “analysis” by “analyses”.**

We have corrected these words.

23. **Line 271: Replace “SigmaPlot, 14.0” by “SigmaPlot 14.0” and replace “R Studio” by “RStudio”.**

We have replaced “SigmaPlot, 14.0” by “SigmaPlot 14.0” and replaced “R Studio” by “RStudio”.

24. **Line 277: Replace “lowest” by “minimum”.**

The words “lowest” used instead of “minimum”.

25. **Line 297: Modify to read “In contrast, neither LFTC nor HFTC significantly affected NO_3^--N concentrations or net nitrification rates at either site during the study...”.**

We have corrected these words.

26. Line 302: Replace "LFTC" by "HFTC".
The words "LFTC" used instead of "HFTC".

27. Line 311: Modify to read "In contrast to the significant effects of HFTC, intensified LFTC had no significant impact on the shoot or root biomass N of the selected plant species at either site (Fig. 5a-f).".
We have corrected these words.

28. Line 315: The result section can be more concise and incorporate duplicate structures. Modify to read "HFTC significantly reduced biomass N in the perennial rhizome grass...(shoot: ...; root: ....)..." The sandy grassland is also changed in this way.
We have corrected these words.

29. Line 325: Replace "was highest" by "peaked".
The words "peaked" was used in the MS.

30. Line 338: Modify to read "In contrast to the positive effects on microbial recovery, HFTC significantly reduced plant ^{15}N acquisition in both grasslands. LFTC had no significant effect on plant ^{15}N recovery ...".
Revised as suggested.

31. Line 353: This paragraph also simplifies the language.
We have corrected these words.

32. Line 363: This section can be more concise and incorporate duplicate structures.
We have corrected these words.

33. Line 364: Modify to read "In the meadow steppe".
The word "the" was added in this sentence.

34. Line 367: Is it net nitrification rate or net nitrification rates? The whole article should be unified.
We have corrected these words. We also checked this across the whole MS.

35. Line 369: Modify to read "plant ^{15}N acquisition showed the strongest positive correlation with soil temperature, while bacterial biomass, MBC, soil DOC, soil moisture, soil NO_3^--N levels, and soil total C also exhibited significant positive correlations.".
We have corrected these words.

36. Line 370: Replace "dissolved organic C" by "DOC" and replace

"microbial biomass C" by "MBC".

Revised as suggested.

37. **Line 372: Modify to read "…soil total N content and net ammonification rate displayed...".**

Revised as suggested.

38. **Line 375: Modify to read "In the sandy steppe".**

The word "the" was added in this sentence.

39. **Line 375: According to correlation, rank the indicators from largest to smallest and long indicators are replaced with abbreviations.**

Revised as suggested.

40. **Line 377: It seems that a space is lacking between the "C" and the "and".**

Revised as suggested.

41. **Line 418: Replace "with" by "as".**

Revised as suggested.

42. **Line 421: Replace "."by ",".**

Revised as suggested.

43. **Line 440: Replace "luxuriously utilize" by "engage in the luxury consumption of".**

Revised as suggested.

44. **Line 446: Replace "triggers shift" by "triggers a shift".**

Revised as suggested.