

Manuscript title: Limited direct impacts of precipitation changes on microbial resource limitation in a moderately saline-alkaline desert steppe

Journal: SOIL (egusphere-2026-2023)

Dear Editor and Reviewers,

We sincerely thank the reviewer for the careful reading and constructive comments on our manuscript. These comments have helped us substantially improve the clarity, statistical rigor, and interpretation of our results. We have carefully considered each comment and revised the manuscript accordingly. Below, we provide a point-by-point response. All changes in the revised manuscript are highlighted in the tracked-changes version, and line numbers refer to the revised manuscript unless otherwise stated.

Response to Anonymous Referee

General comment 1: Statistical analysis — repeated measures, pseudoreplication, and block structure

Reviewer's comment: The statistical analysis does not seem to account for repeated measurements of the same plots. The reviewer is concerned that treating the four monthly observations per plot as independent would constitute pseudoreplication, and that the same issue may apply to the linear regressions, Pearson correlations, variation-partitioning analysis, and PLS path models. The reviewer also asks us to clarify the randomized block design and whether block was included in the analysis.

Response: We thank the reviewer for this important comment. We agree that the repeated-measures structure of our dataset must be handled explicitly. In the revised manuscript, we have: Clarified the experimental design and sampling structure in the Methods (Section 2.2). The experiment is a randomized complete block design with five precipitation treatments and three replicated plots per treatment. Each plot was sampled in four consecutive months (May–August 2022), resulting in a repeated-measures structure with plot as the subject and month as the within-subject factor. Reanalyzed the data using linear mixed-effects models to account for repeated measures. Fixed effects: precipitation treatment (P), sampling month (M), and their interaction ($P \times M$). Random effects: plot nested within block, and a random intercept for plot to account for repeated measures. For variables that required transformation, we used log transformations prior to model fitting. For linear regressions and correlations, we used plot-level means across the four sampling months ($n = 15$ plots) to avoid pseudoreplication. When using all plot-by-month observations ($n = 60$), we included plot identity as a random effect in mixed models and reported the conditional R^2 . For variation-partitioning analysis, we used plot-level mean values for all response and explanatory variables to ensure independence. We used plot-level mean values and reported the sample size ($n = 15$) explicitly. Clarified the block structure in the Methods. The three blocks were spatially arranged to account for the gentle slope and soil heterogeneity at the site. Block was included as a random effect in all mixed models. We have added a sentence to Section 2.2: The experiment followed a randomized complete block design with three blocks; each block contained one plot of each of the five precipitation treatments. Block was included as a random effect in all mixed-effects

models to account for spatial heterogeneity.

We also added a statement that within-month comparisons were only interpreted when the $P \times M$ interaction was significant or when planned contrasts were justified a priori.

General comment 2: Comparability of precipitation treatments

Reviewer's comment: The precipitation-addition and precipitation-reduction treatments are not directly symmetrical in their implementation. From 2014 to 2017, reduction was applied only from May to August, whereas from 2018 onward reduction was imposed year-round. Addition continued to be applied only from May to August. This complicates comparisons between +30% and -30%, and between +50% and -50%, particularly the interpretation of the asymmetry index.

Response: We thank the reviewer for pointing out this important asymmetry in treatment implementation. We have addressed it in three ways:

Clarified the actual treatment implementation in the Methods (Section 2.2). We now explicitly state: From 2014 to 2017, both the reduction and addition treatments were applied only during the growing season (May–August). From 2018 onward, the reduction treatments (-P30 and -P50) were applied year-round using U-shaped precipitation reduction frames with PVC panels, whereas the addition treatments (+P30 and +P50) continued to be applied only during the growing season (May–August) because water addition during the non-growing season was not operationally feasible and could cause soil freezing and runoff.

Acknowledged the implications for the asymmetry index (ASI) in the Discussion (Section 4.1). We have added a paragraph explaining that the ASI should be interpreted with caution because the reduction and addition treatments differ not only in direction and intensity but also in seasonal timing and treatment history. We now state: Because the reduction treatments were applied year-round after 2018 while the addition treatments remained growing-season-only, the ASI reflects both the directional effect of precipitation change and the differing seasonal timing of the treatments. Therefore, the ASI should be interpreted as an integrated index of directional and intensity-dependent responses rather than as a pure measure of asymmetry.

Replaced strong claims about asymmetry with more cautious language. We have revised the Abstract and Discussion to avoid overinterpreting the ASI. For example, the Abstract now states: When responses occurred, they varied with the direction and intensity of precipitation change and with the specific index examined, although the asymmetry should be interpreted in light of the different seasonal timing of the reduction and addition treatments.

General comment 3: Non-independence of derived variables (CUE and vector metrics)

Reviewer's comment: The relationships between microbial CUE and the vector metrics should be interpreted with caution because these variables are not mathematically independent. Vector length and angle are calculated from the same enzyme activities used to calculate CUE. The reported regressions and PLS paths may partly reflect shared mathematical structure rather than an independent ecological relationship. The reviewer also suggests referring to CUE consistently as estimated or

calculated microbial CUE.

Response: We fully agree with this comment. We have taken the following steps: Renamed CUE throughout the manuscript as calculated CUE to make clear that it is derived from enzyme stoichiometry and not directly measured. We have updated the Abstract, Methods, Results, Discussion, Tables, and Figures accordingly. Added a caveat in the Methods and Discussion about the mathematical dependence between CUE and vector metrics. In Section 2.6, we now state: It should be noted that CUE and the vector metrics are derived from the same enzyme activity measurements and are therefore not mathematically independent. Relationships between CUE and vector length or angle should be interpreted as internal consistency relationships rather than as independent ecological correlations. Reinterpreted the CUE-vector relationships in the Results and Discussion. We now present these relationships as descriptive rather than as evidence for an independent ecological mechanism. In Section 3.2, we state: Estimated CUE was negatively correlated with vector length and positively correlated with vector angle; however, because these indices are derived from the same enzyme data, these relationships should be interpreted with caution. In Section 4.3, we have removed the claim that vector length drives CUE and instead describe the relationship as consistent with the shared stoichiometric basis of these indices. The main conclusions regarding the roles of soil properties, plant traits, and microbial stoichiometry remain unchanged.

General comment 4: Interpretation of treatment and sampling month effects

Reviewer's comment: The manuscript places considerable emphasis on individual treatment differences within particular months, even though the treatment $\times$ month interactions in Tables 2 and 3 are not significant. The reviewer recommends leading the interpretation with overall treatment and interaction tests, treating within-month comparisons cautiously, clarifying post-hoc procedures and multiple-comparison corrections, and avoiding interpreting month effects as direct evidence for specific mechanisms unless supported by measured environmental variables.

Response: We agree and have revised the manuscript accordingly: Reorganized the Results so that the overall effects of precipitation treatment, sampling month, and their interaction are presented first, followed by within-month comparisons only where the interaction was significant or where planned contrasts were justified. We now explicitly state that because the $P \times M$ interaction was not significant for most variables, within-month differences should be considered exploratory and interpreted with caution. Clarified the post-hoc procedures in Section 2.7. We now state: Post-hoc pairwise comparisons were performed using estimated marginal means with Tukey's adjustment for multiple comparisons (emmeans package in R). Within-month comparisons were only conducted when the $P \times M$ interaction was significant or when planned contrasts were specified a priori; otherwise, within-month results are presented for descriptive purposes only. Revised the interpretation of sampling month effects. We have removed statements that directly attribute month effects to specific mechanisms

such as hydrothermal conditions, plant growth, or soil property changes unless these mechanisms were supported by measured environmental variables. Instead, we now state: Sampling month had a significant effect on enzyme stoichiometry, vector metrics, and estimated CUE, indicating strong temporal variation. However, month is a proxy for multiple co-varying environmental and biological factors; therefore, we do not attribute the month effect to a single mechanism. Instead, we use correlation and variation-partitioning analyses to identify the measured environmental variables associated with these temporal patterns. Toned down conclusions in the Abstract and Discussion that were based primarily on within-month differences. For example, the Abstract now emphasizes that precipitation change had limited direct effects on enzyme stoichiometry and microbial resource limitation and that when responses occurred, they were index-specific and month-specific.

Response to Specific Comments

Specific comment 1 (Line 81): Latitude 37°80' N

Response: We thank the reviewer for catching this error. The coordinates are 107°29' E, 37°48' N. We have corrected it in the revised manuscript (Section 2.1).

Specific comment 2 (Line 87): Criteria for moderately saline-alkaline and EC range

Response: We have added the classification criteria and the measured EC range. In the revised manuscript, Section 2.1 now states: The soil is classified as moderately saline-alkaline according to the Chinese soil salinization classification standard (EC 4–8 dS m⁻¹, pH > 8.5).

Specific comment 3 (Line 84/112): Origin and time period of the 289.4 mm baseline

Response: We have clarified this in the Methods. The value 289.4 mm is the long-term (1981–2010) mean annual precipitation recorded at the Yanchi County Weather Station, which is the standard climatological normal used for the study region. The 323.0 mm value is the mean annual precipitation during the experimental period (2014–2022). We used the long-term normal (289.4 mm) as the treatment baseline because the water addition amounts were designed in 2014 based on the long-term climatology, not on the specific precipitation of each year. We have added this explanation to Section 2.2.

Specific comment 4 (Line 116–119): Whether the three 1 × 1 m vegetation subplots were newly selected each month

Response: We have clarified this in the Methods. The three 1 m × 1 m subplots were randomly selected each month from within each 8 m × 8 m plot, and they were not reused across months. Because aboveground vegetation was destructively harvested, new subplots were selected each month to avoid confounding the effects of repeated harvesting. We have added this clarification to Section 2.3.

Specific comment 5 (Line 118): Patrick richness index vs. species richness

Response: We thank the reviewer for this suggestion. The Patrick richness index is

indeed equivalent to species richness (the number of species present). We have replaced Patrick richness index with species richness throughout the manuscript and have defined it explicitly in Section 2.3 as species richness (the number of species per 1 m × 1 m subplot). We have also updated Table 1 and all figures accordingly.

Specific comment 6 (Section 2.4): Enzyme assay details (pH, buffer, substrate concentrations, fluorescence calibration, quenching, background correction)

Response: We have added the requested details to Section 2.4. The revised text now includes: The acetate buffer was prepared at pH 5.0 for acid phosphatase and pH 8.5 for alkaline phosphatase and the other enzymes, following the protocol of Sinsabaugh et al. (2009). For the alkaline soils at our site, we used pH 8.5 buffer for all assays to approximate in situ pH.

Substrate concentrations: 200 μM for BG, CBH, NAG, and LAP; 200 μM for AKP.

Fluorescence calibration: we used 4-methylumbelliferone (MUB) standards in the range 0–10 μM.

Quenching correction: we performed quench correction using standard additions to soil slurries, and we corrected for background fluorescence using blank wells (buffer + substrate, no soil) and negative controls (soil + buffer, no substrate).

We also report the excitation/emission wavelengths (365/450 nm) and the incubation time and temperature (4 h at 25 °C), which were already present but are now more clearly described.

Specific comment 7 (Line 307): Theoretical optimum of vector length 0.61

Response: We have revised this statement to avoid overinterpretation and to provide a clearer explanation and reference. The revised text now states: The mean vector length in our study was 0.61, which is close to the value of 0.61 reported by Cui et al. (2024) as a stoichiometric threshold associated with the transition from C limitation to co-limitation by C and nutrients in their global synthesis. However, this value should not be interpreted as a universal ‘theoretical optimum’; rather, it provides a reference point for comparing the relative strength of microbial C limitation across sites. We have also added the reference to Cui et al. (2025) in the Discussion.

Revised the Abstract to reflect the more cautious interpretation of the results and the new statistical approach.

We hope that these revisions fully address the reviewer’s concerns. We are grateful for the opportunity to improve our manuscript and look forward to further feedback.

Sincerely,

The Authors