

Reply on RC2

We have devoted our full efforts to researching and exploring solutions. Based on the reviewers' comments, we have conducted a comprehensive and in-depth review of the theoretical foundation, logical framework, and empirical research design of the submitted manuscript. In the revised version, we have thoroughly examined and revised the paper in accordance with the responsible and high-quality feedback from the reviewers. Some modified paragraphs and sentences are highlighted in red font for easy reference. Additionally, we have prepared a detailed point-by-point response in a Q&A format below, addressing each comment meticulously. We kindly ask the reviewers to provide further critique and suggestions:

1-4. **Response:** We sincerely thank the reviewers for their positive comments on our work. Their recognition of the novel ideas we presented, the relevant scientific issues, and the multidisciplinary context in which soil problems are addressed and the broad international significance of this paper is very encouraging and reinforces the value of this research, and the reviewers are sincerely thanked for their recognition of our work.

19 **5. Reviewer:** However, there are no clear hypotheses in the manuscript.
20 Adding hypotheses that drove the research approach would strengthen this
21 paper, and we suggest including the hypotheses that drove your research
22 questions and approach at the end of the introduction. For example, based
23 on precipitation or temperature differences across grassland types, did you
24 hypothesize differences in total MAOC or POC, or different necromass
25 contributions to MAOC or POC? What depth patterns did you expect?

26 **Response:** We agree with this suggestion and have now added
27 explicit hypotheses based on our research at the end of the introduction.
28 Our research focuses on four different grassland types, (1) investigate the
29 vertical distribution (0-100 cm) of SOC fractions and MNC across different
30 grassland types; (2) identify the relative contribution of fungi and bacteria
31 to SOC fractions; (3) elucidate the key drivers influencing MNC
32 contribution to MAOC and POC accumulation in deeper soil layers. We
33 hypothesized that (1) The contents of SOC fractions and MNC were
34 decreased with soil deep; (2) the contribution of fungal necromass carbon
35 is higher than that of bacteria; (3) Climatic factors and soil environment
36 influencing MNC contribution to MAOC and POC accumulation. By

elucidating the microbial mechanisms of SOC formation and accumulation in different grassland types and provides crucial insights for optimizing grassland management strategies and supporting regional carbon neutrality objectives, at the same time, it provides a theoretical basis and data support for the realization of the regional “dual-carbon” goal.

These hypotheses are now referenced in the Results and Discussion to frame interpretations.

6. **Reviewer:** The methods need to be significantly revised to include more details of procedures used, especially those used for soil physicochemical analyses. Either methodological details or citations that include such details should be added in the text. For example, what standard protocols did you use for your phosphorus and potassium measurements (Line 159)?

In the manuscript, it is written that soil was sieved through 2mm and 0.15 mm sieves, and then also separated by density fractionation. How do these methods relate to each other for determination of MAOC and POC?

Authors could go into greater detail about the distribution of the data and why parametric test and linear regression was most appropriate for the data.

55 Please include the significance level used for statistical tests (e.g., alpha =
56 0.05).

57 **Response:** We agree with the reviewer's observation and have revised
58 our methods. We have expanded the Methods section as follows:

59 2.3.1 Soil Physicochemical Properties

60 Soil bulk density (BD) was determined using the core method,
61 employing a 100 cm³ ring knife (5 cm height, 5.05 cm diameter) (Wang et
62 al. 2022b). Soil water content (SWC) was assessed via the oven-drying
63 method, where fresh soil was dried at 102°C until a constant weight was
64 achieved (Li et al. 2018). Soil pH was measured using a pH meter (pHS-
65 3C) with a soil-to-water ratio of 1:2.5 (w/v) (Roberts et al. 2007). Soil
66 organic carbon (SOC) was quantified using the K₂Cr₂O₇ external heating
67 method, followed by titration with 0.1 M FeSO₄ (Ding et al. 2019). Total
68 nitrogen (TN) was determined using the Kjeldahl method using the Kjeltac
69 8400 (FOSS, Denmark). Available nitrogen (AN) using alkaline hydrolysis
70 diffusion. Total phosphorus (TP) was measured by an ultraviolet
71 spectrophotometer (UV3200, Shimadu Corporation, Japan) after wet
72 digestion with H₂SO₄ and HClO₄. Available phosphorus (AP) using sodium

bicarbonate extraction. Available potassium (AK) was extract with 1mol/L ammonium acetate solution at pH 7.0 and subsequent determination by atomic absorption and emission spectrophotometry. Total carbon (TC) was analyzed using the potassium dichromate external heating method (Chai et al. 2024; Zhang et al. 2021). Soil electrical conductivity (EC) was measured using a conductivity meter.

2.3.2 MAOC and POC Measurement

The separation into the coarse fraction and mineral-associated fraction was achieved using the density-gravity method. Specifically, 20.00 g of air-dried soil (passed through a 2-mm sieve) was weighed into a conical flask, followed by the addition of 60 mL of sodium hexametaphosphate solution (5%, w/v). The mixture was shaken on an orbital shaker for 18 h (25°C, 180 r·min⁻¹), after which the suspension was passed through a 53-μm nylon sieve and rinsed with distilled water until the effluent became clear. The separated samples were oven-dried at 60°C and ground. The coarse fraction (particle size >53 μm) was designated as particulate organic matter (POM), while the fine fraction (particle size <53 μm) was classified as mineral-associated organic matter. The organic carbon content in each

fraction was determined using the potassium dichromate-external heating method(Sokol et al. 2019). Particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) were calculated according to Equations (1) and (2), with units expressed in $\text{g}\cdot\text{kg}^{-1}$.

$$\text{POC} = \frac{\Delta M_1}{M} \times C_{\text{POM}} \quad (1)$$

$$\text{MAOC} = \frac{\Delta M_2}{M} \times C_{\text{MAOM}} \quad (2)$$

Where ΔM_1 represents the oven-dry weight of the upper-layer soil sample after separation (g); ΔM_2 denotes the oven-dry weight of the lower-layer soil sample after separation (g); M is the total mass of the soil sample before separation (g); C_{POM} measured refers to the organic carbon content of the upper-layer soil sample determined by the potassium dichromate-external heating method ($\text{g}\cdot\text{kg}^{-1}$); and C_{MAOM} measured indicates the organic carbon content of the lower-layer soil sample determined by the potassium dichromate-external heating method ($\text{g}\cdot\text{kg}^{-1}$).

2.4 Data Analysis

Before analysis, all variables were tested for normality and homogeneity of variances, and log-transformations were performed when necessary. Data were organized using Excel 2023 and Word 2023 and

109 statistical calculations (i.e., correlations and significant differences) were
110 conducted using the SPSS 20.0 statistical software package (SPSS Inc,
111 Chicago, USA). One-way, two-way ANOVA and LSD tests were used to
112 assess the differences of soil physicochemical properties, SOC fractions,
113 MNC among the different sampling sites, correlation analysis was
114 considered significant at $P < 0.05$. The relationship between BNC and FNC,
115 BNC/MAOC and FNC/MAOC, BNC/POC and FNC/POC were analyzed
116 by univariate linear regression. The use of Principal component (PC)
117 analysis to show that the soil properties of different grassland types were
118 significantly different between the 0-20 cm and the 20-40 cm and 40-100
119 cm soil layers. And using Spearman correlation to analysis the relationship
120 between environmental variables and MAOC, FNC/MAOC, BNC/MAOC,
121 TNC/MAOC, POC, FNC/POC, BNC/POC, TNC/POC. Subsequently, we
122 used a random forest model to predict the influences affecting the
123 accumulation of FNC, BNC, and TNC in different soil layers of different
124 grassland types. The random forest modeling was conducted using R
125 (version 4.3.1), with packages including "ggplot2," "tidyverse,"
126 "randomForest," "rfUtilities," and "rfpermute" (Liao et al. 2023).

127 7. **Reviewer:** The soil type/classification or geologic information was
128 not described. There was no mention of soil texture, or soil horizon but the
129 authors used the phrase soil profile throughout the manuscript. This paper
130 would greatly benefit from inclusion of soil type/classification into the
131 methods/sampling site section. Line 139. Why were these soil depths
132 chosen? Were there soil horizons or other soil characteristics unique at
133 these depths (e.g., pH, texture, mineral composition, organic matter, etc.)?

134 **Response:** We apologize for this oversight. We have added soil type
135 in study area, please see response 12. We classify soil layers according to
136 soil properties (An et al. 2010; Wu et al. 2025).

137 8. **Reviewer:** At first glance, analysis and assumptions appear valid but the
138 authors did not mention if the distribution of the data was normal or non-
139 normal, and why parametric test/linear regression was suited for the data.
140 If the data was normally distributed, the authors analysis is appropriate but
141 if the data is not normally distributed then alternative non-parametric
142 statistical analysis should be used.

143 **Response:** Regarding data analysis, we have already revised in our
144 answer to question 6.

145 **9. Reviewer:** However, most of the figures are lacking legends and clear
146 figure captions that explain abbreviations, symbols, or colors. Figure
147 captions for figures 3-10 all need to be updated to meet this requirement.
148 Additionally, figures 5, 6, 7, and 8 do not have the legend to differentiate
149 grassland type.

150 Further, do Figures 9 and 10 aggregate all grassland types? Please
151 include this information in the caption. Panel letters should be added to the
152 figures to allow easier reading.

153 Line 132-135: How do the four grassland types (MS, TS, DS, and SD)
154 relate to the abbreviations next to the number of sampling sites (CD, HM,
155 DX, CH)?

156 **Response:** We sincerely apologize for lacking legends and clear
157 figure captions. We have added legends, clear figure captions and panel
158 letters as follows. And we apologize for the writing error, the correct
159 grassland types have been updated MS, TS, DS, and SD not CD, HM, DX,
160 CH. The 20 - 100 cm layers were combined together in Figures 9 and
161 Figures 10, that's because **by principal component analysis, the soil**
162 **properties of same soil layers in four grassland types exhibited significant**

variations between the 0-20 cm soil layer and the deeper 20-40 cm and 40-100 cm layers. In different soil layers, the contribution values of PC1 and PC2 were 44.6% and 15.8%, respectively. Therefore, we analyzed the soil properties affecting MNC accumulation by dividing them into 0-20 cm and 20-100 cm groups.

We have added legends, clear figure captions and panel letters as follows:

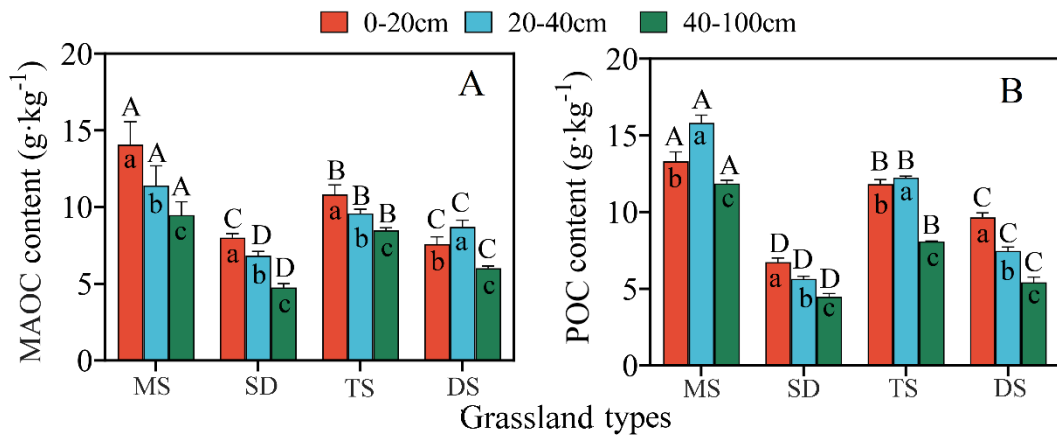


Fig.2 Contents of MAOC (A) and POC (B) in 0-100 cm soil layers under different grassland types. Different

uppercase letters indicate significant differences in different grassland types in the same soil layer, and different

lowercase letters indicate significant differences in different soil layers under the same grassland types ($p < 0.05$).

MAOC: mineral-associated organic carbon; POC: particulate organic carbon. The bars in red, blue and green

represent the soil layers 0–20 cm, 20–40 cm and 40–100 cm respectively. MS: meadow steppe; SD: steppe desert;

TS: typical steppe; DS: desert steppe.

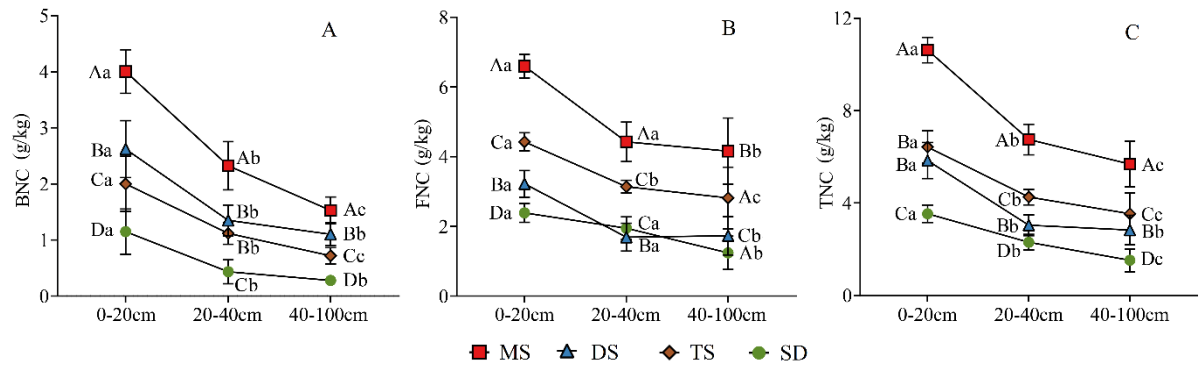


Fig. 3 Contents of BNC、FNC and TNC in MAOC in different soil layers under different grassland types.

Different uppercase letters indicate significant differences in different grassland types in the same soil layer ($p < 0.05$), and different lowercase letters indicate significant differences in different soil layers under the same grassland types ($p < 0.05$). The red square, blue triangle, brown rhombus and green circle represent: meadow steppe (MS); desert steppe (DS); typical steppe (TS); steppe desert (SD). FNC: fungal necromass carbon, BNC: bacterial necromass carbon, TNC: total necromass carbon.

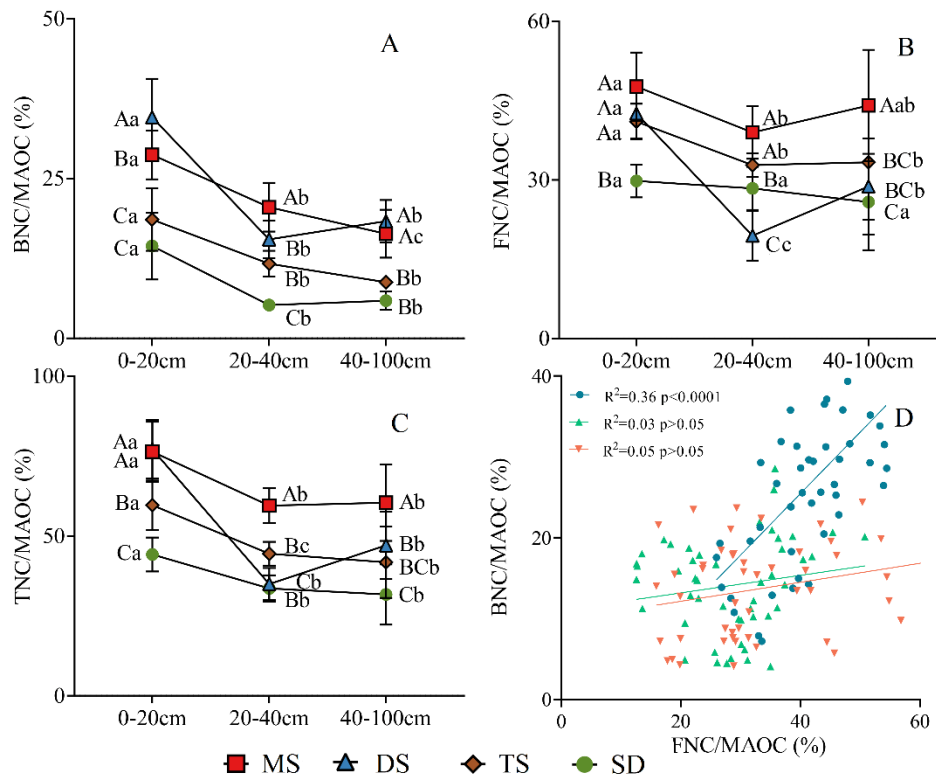


Fig. 4 The contribution of BNC, FNC and TNC in MAOC in different soil layers under different grassland types. (A) The contribution of BNC to MAOC; (B) the contribution of FNC to MAOC; (C) the contribution of TNC to MAOC. Different uppercase letters indicate significant differences in different grassland types in the same soil layer ($p<0.05$), and different lowercase letters indicate significant differences in different soil layers under the same grassland types ($p<0.05$). The red square, blue triangle, brown rhombus and green circle represent: meadow steppe (MS); desert steppe (DS); typical steppe (TS); steppe desert (SD). (D) the relationship between BNC/MAOC and FNC/MAOC. The blue circle, green triangle and orange inverted triangle represent the soil layers 0–20 cm, 20–40 cm and 40–100 cm respectively. FNC: fungal necromass carbon, BNC: bacterial necromass carbon, MAOC: mineral-associated organic carbon.

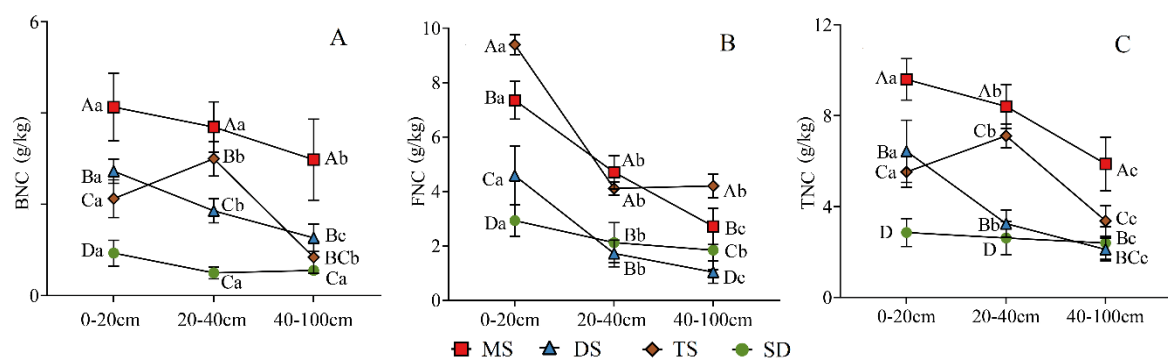


Fig. 5 Contents of BNC, FNC and TNC in POC in different soil layers under different grassland types. Different uppercase letters indicate significant differences in different grassland types in the same soil layer ($p<0.05$), and different lowercase letters indicate significant differences in different soil layers under the same grassland types ($p<0.05$). The red square, blue triangle, brown rhombus and green circle represent: meadow steppe (MS); desert steppe (DS); typical steppe (TS); steppe desert (SD). FNC: fungal necromass carbon, BNC: bacterial necromass carbon, TNC: total necromass carbon.

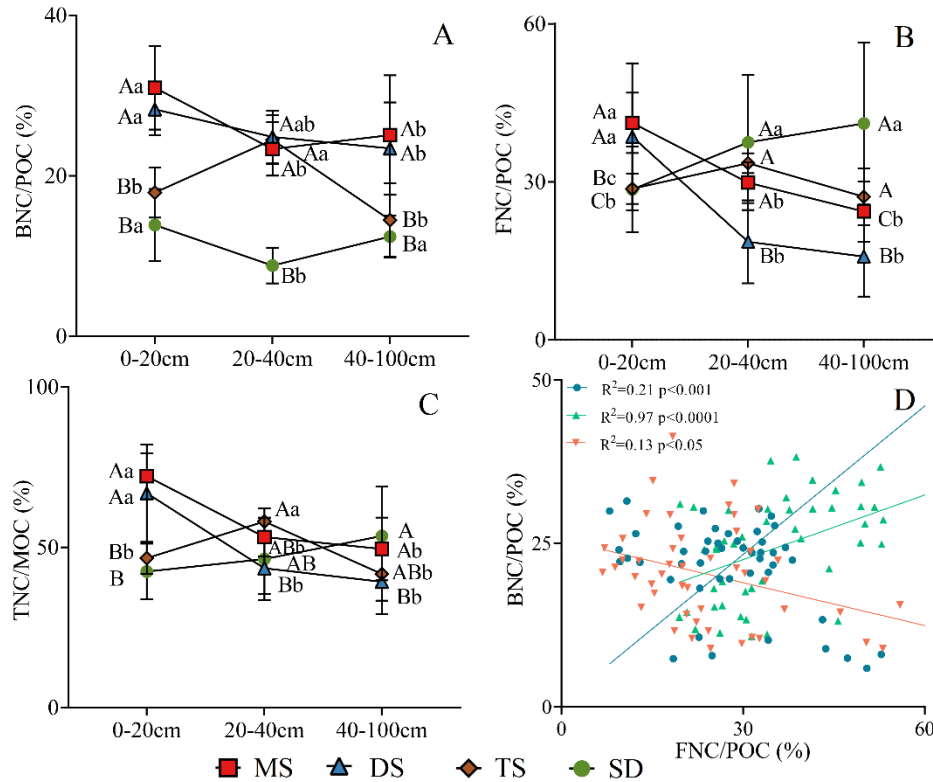


Fig.6 The contribution of BNC, FNC and TNC in POC in different soil layers under different grassland types.

(A) The contribution of BNC to POC; **(B)** the contribution of FNC to POC; **(C)** the contribution of TNC to POC.

Different uppercase letters indicate significant differences in different grassland types in the same soil layer ($p < 0.05$),

and different lowercase letters indicate significant differences in different soil layers under the same grassland types

($p < 0.05$). The red square, blue triangle, brown rhombus and green circle represent: meadow steppe (MS); desert

steppe (DS); typical steppe (TS); steppe desert (SD). **(D)** the relationship between BNC/POC and FNC/POC. The

blue circle, green triangle and orange inverted triangle represent the soil layers 0–20 cm, 20–40 cm and 40–100 cm

respectively. FNC: fungal necromass carbon, BNC: bacterial necromass carbon, POC: particulate organic carbon.

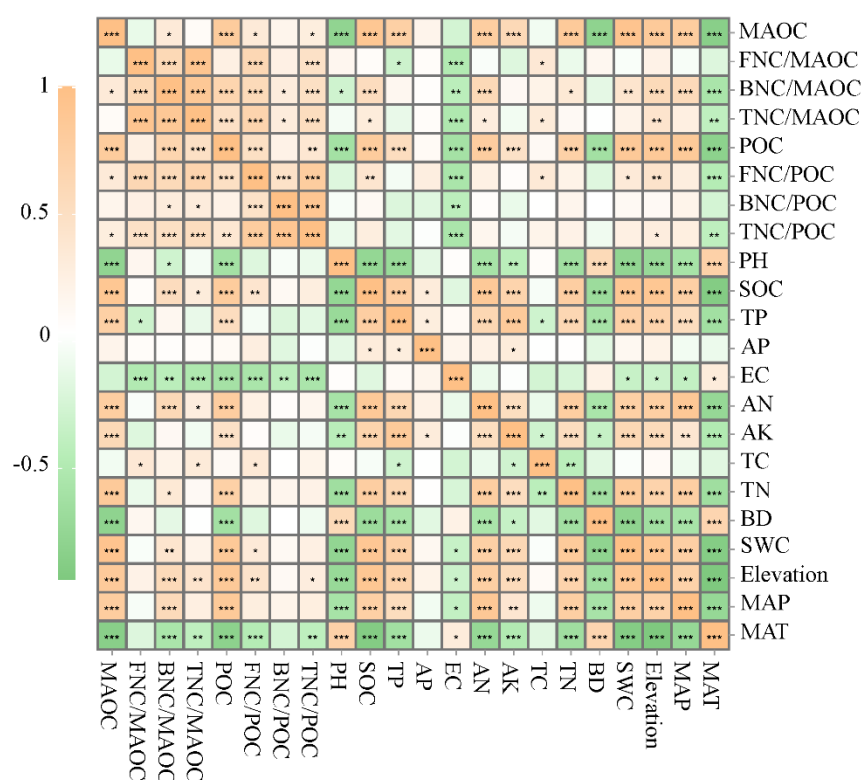


Fig.7 The Spearman correlation of MAOC、POC and MNC with environmental factors in 0-100cm soil layer under different grassland types. Colors represent Spearman correlations; MAT: mean annual temperature; MAP: mean annual precipitation; SWC: Soil water content; BD: Bulk density; TN: Total nitrogen; TC: Total carbon; TP: Total phosphorus; AK: Available potassium; AP: Available phosphorus; AN: Available nitrogen; EC: Electrical conductance; SOC: soil organic carbon. FNC (BNC, TNC)/MAOC: the ratio of fungal necromass carbon(bacterial necromass carbon, total necromass carbon) to mineral-associated organic carbon; FNC (BNC, TNC)/POC: the ratio of fungal necromass carbon(bacterial necromass carbon, total necromass carbon) to particulate organic carbon; * represents the Spearman correlation importance *:p<0.05; **: p<0.01; *: p<0.001.**

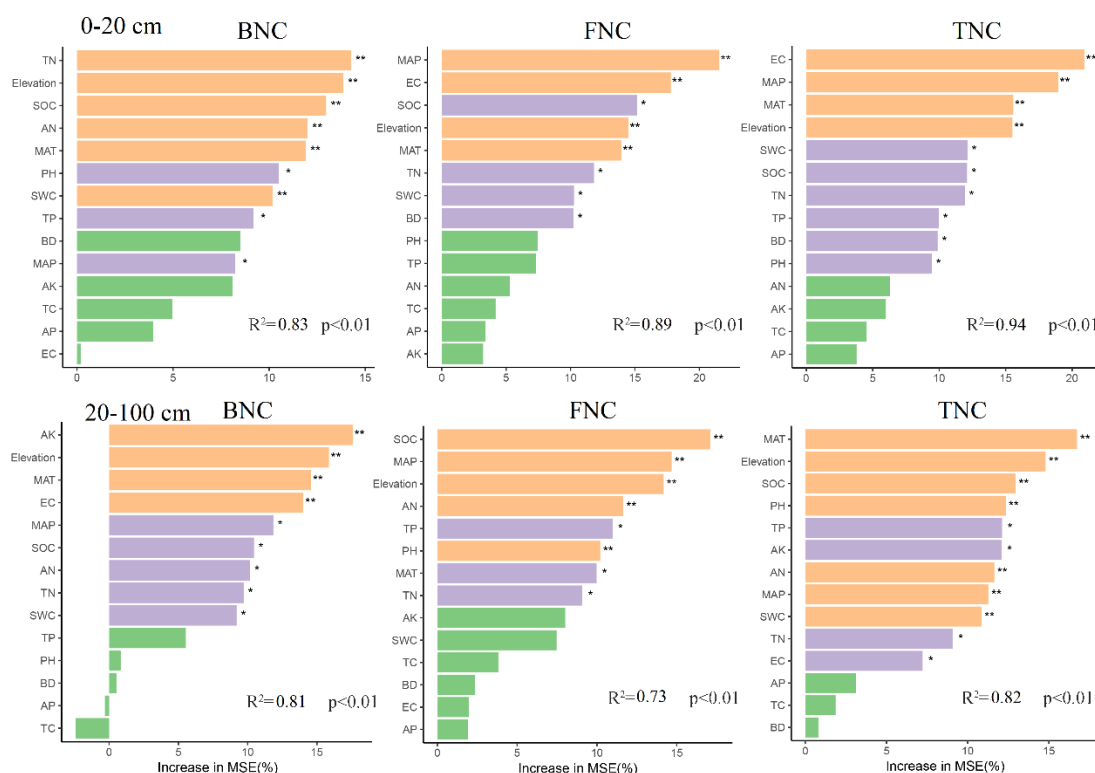


Fig. 8 Random forest analysis indicating the relative importance of soil properties to MNC. Colors represent significant correlations. MAT: mean annual temperature; MAP: mean annual precipitation; SWC: Soil water content; BD: Bulk density; TN: Total nitrogen; TC: Total carbon; TP: Total phosphorus; AK: Available potassium; AP: Available phosphorus; AN: Available nitrogen; EC: Electrical conductance; SOC: soil organic carbon. FNC: fungal necromass carbon, BNC: bacterial necromass carbon, TNC: total necromass carbon. *: $p<0.05$; **: $p<0.01$; *: $p<0.001$ (This analysis aggregated four grassland types in same soil layer).**

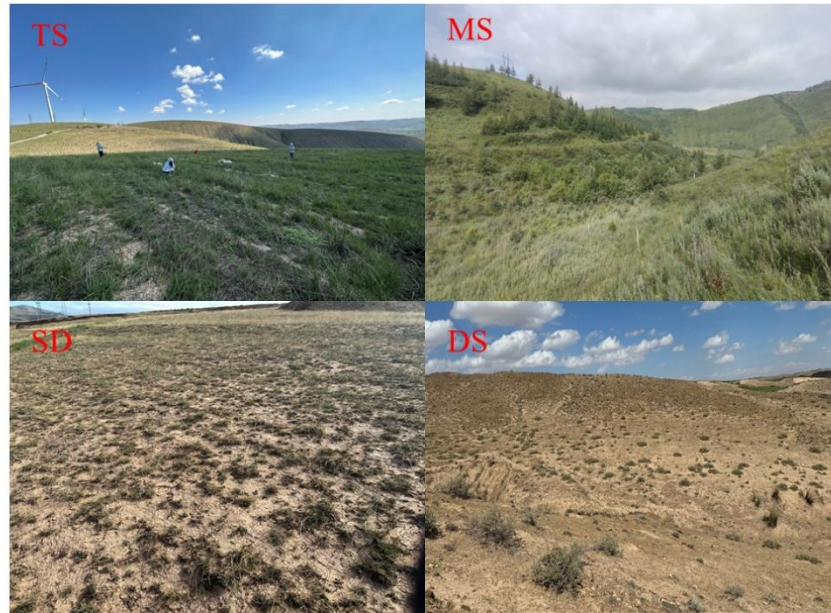


Fig. S1 Distribution of the sampling sites in different grassland types. typical steppe (TS), meadow steppe (MS), and steppe desert (SD), desert steppe (DS).

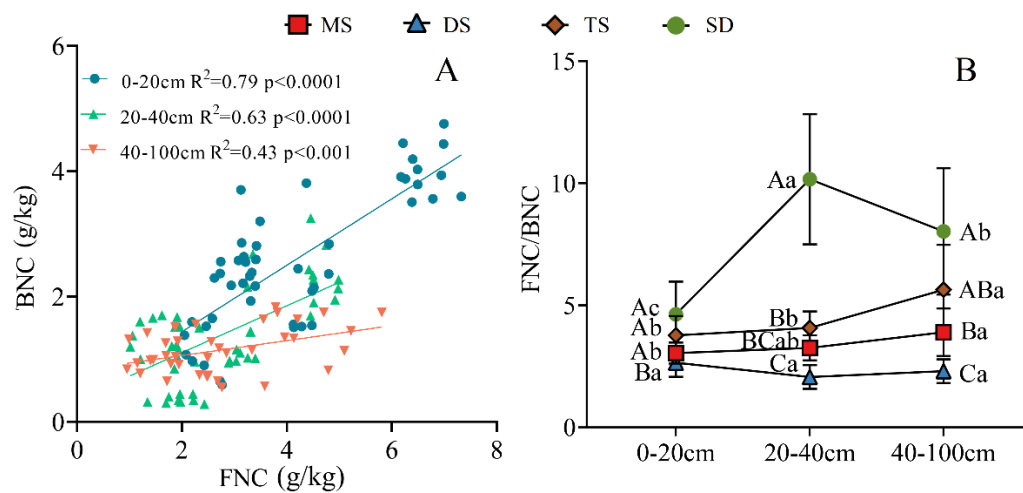


Fig. S2 The correlation of BNC and FNC in different soil layers among four grassland types (A); The blue

circle, green triangle and orange inverted triangle represent the soil layers 0–20 cm, 20 –40 cm and 40 –100 cm

respectively. The ratio of FNC/BNC in different soil layers among different grassland types (B); Different

uppercase letters indicate significant differences in different grassland types in the same soil layer ($p < 0.05$), and

different lowercase letters indicate significant differences in different soil layers under the same grassland types

($p < 0.05$). The red square, blue triangle, brown rhombus and green circle represent: meadow steppe (MS); desert

steppe (DS); typical steppe (TS); steppe desert (SD). FNC: fungal necromass carbon, BNC: bacterial necromass carbon, FNC/BNC: the ration of FNC to BNC.

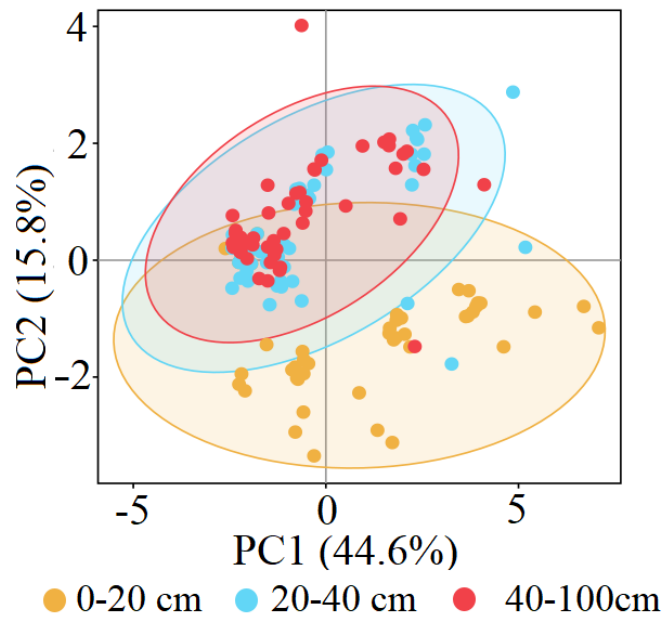


Fig. S3 Principal component analysis of soil properties in different soil layers of four grassland types. Yellow, green and red circles represent 0-20cm, 20-40cm, 40-100cm soil layers (This analysis aggregated four grassland types in same soil layer).

10. Reviewer: Also, the authors infer that MAOC is being leached into deeper soil layers (line 346), are there any findings or relevant citations that back this up?

Response: We have added the citation. Increased rainfall enhances vegetation biomass and carbon input into the soil, promoting POC formation and causing MAOC to leach into deeper layers (Chen et al. 2020;

Wang et al. 2022a). Higher soil moisture can not directly lead to a large amount of acid inputs into soil. However, higher soil moisture is indicative of the higher leaching conditions due to higher rainfall, and can stimulate plant growth and subsequent root respiration, enhance the inputs of H⁺ ions from carbonic acid, and thus lead to a low soil pH. On the contrary, the decrease of soil pH can protect the leaching of soil base cations such as Ca²⁺, which stabilizes organo-mineral associations, thus increasing MAOC.

11. Reviewer: however it would be useful to have a clearer discussion of how the grasslands are different from each other and how this impacts potential grassland management strategies for carbon sequestration. Are there implications for how grasslands should be managed for MAOC or POC pools, or to mitigate C losses in the subsoil?

Response: We've discussed the grasslands are different from each other in lines 329-335: In the 0-100 cm soil layer, the contents of MAOC and POC across different grassland types followed the order: MS > TS > DS > SD. Significant differences were observed between soil layers and grassland types ($p < 0.05$ Fig 1). This is because MS, compared to the other three grassland types, has higher vegetation coverage, greater root density and

more abundant nutrient conditions, resulting in higher total organic carbon content and, consequently, higher soil carbon fractions (Hu et al. 2025). In the revised manuscript, we have added to explicitly address how this impacts potential grassland management strategies for carbon sequestration: In understanding the distribution and accumulation of soil carbon fractions across different grassland ecosystems, which highlight the intricate relationships between environmental variables, soil properties, and microbial necromass, offering a deeper understanding of the factors driving MNC accumulation in diverse grassland ecosystems. This study not only advances our knowledge of soil carbon dynamics but also provides a foundation for future research aimed at optimizing soil carbon sequestration strategies in response to changing environmental conditions, and have implications for soil carbon management and climate change mitigation strategies.

12. Reviewer: Although there were sections in the introduction we do believe should have a citation:

Response: In order to response reviewer's 5, We have added explicit hypotheses based on our research at the end of the introduction. The new

section as follow:

Lines 99-109 we added citations: However, our understanding of MNC dynamics remains incomplete, most studies focus on the 0-20 cm and 20-40 cm soil layers, with limited research on MNC in deeper soil layers (>60 cm), this knowledge gap is particularly pronounced in ecologically transitional zones(An et al. 2010; Du et al. 2021). Particular in Ningxia, which is one of the three pilot provinces of “Research on climate change adaptation in China”, and encompasses diverse grassland types representative of northern Chinese ecosystems: meadow steppe, typical steppe, desert steppe and steppe desert. While previous research in Ningxia has primarily focused on conventional SOC parameters (e.g., soil carbon density, storage, and spatial distribution of water-soluble organic carbon), regarding the dynamics of MAOC and POC fractions, particularly the contribution of microbial necromass carbon to their accumulation in deeper soil layers (>60 cm) are not yet well understood (Wu et al. 2025). To fill this gap, our research focuses on four different grassland types, (1) investigate the vertical distribution (0-100 cm) of SOC fractions and MNC across different grassland types; (2) identify the relative contribution of

fungi and bacteria to SOC fractions; (3) elucidate the key drivers influencing MNC contribution to MAOC and POC accumulation in deeper soil layers. We hypothesized that (1) The contents of SOC fractions and MNC were decreased with soil deep; (2) the contribution of fungal necromass carbon is higher than that of bacteria; (3) Climatic factors and soil environment influencing MNC contribution to MAOC and POC accumulation. By elucidating the microbial mechanisms of SOC formation and accumulation in different grassland types and provides crucial insights for optimizing grassland management strategies and supporting regional carbon neutrality objectives, at the same time, it provides a theoretical basis and data support for the realization of the regional “dual-carbon” goal.

Lines: 119 - 127: According to other reviewer’s suggestion, we have restructured the study area and added citations: The study area (35°14′–39°23′ N, 104°17′–107°39′ E) of the Ningxia Hui Autonomous Region, China, with a total area of 66,400 km², represents a transitional zone between the Loess Plateau and the Mongolian Plateau (Ji et al. 2023). Ningxia belongs to typical continental semi-humid semi-arid climate, and hosting a remarkable diversity of grassland ecosystems that cover 47% of

321 its land area—encompassing nearly all major grassland types found in
322 northern China. In the southern Loess Plateau, meadow steppe (MS) and
323 typical steppe (TS) dominate, with the TS concentrated near Guyuan City
324 (e.g., Yunwu Mountain Grassland Nature Reserve), belonging to the semi-
325 arid climate, annual precipitation is generally in the range of 300–400 mm
326 or so, with the dominance of drought-resistant perennial tufted grasses, the
327 soil is dominated by black clay. In contrast, the MS is mainly distributed in
328 the shady slopes and valleys of Liupan Mountains and other mountainous
329 areas where the water conditions are better, the climate is more humid, and
330 the annual precipitation is generally around 400–600 mm, which consists
331 of perennial perennial and rhizomatous grasses in the middle of the arid
332 zone, the soils are mainly mountain brown loam, mountain gray-brown soil
333 and black clay. Desert steppe (DS) is distributed in the central and northern
334 parts of Ningxia, which is the overland of grassland and desert, with arid
335 climate, annual precipitation is generally around 200 - 300 mm, and the
336 vegetation cover is 40-60%, with dry perennial grasses dominating and
337 small dry shrubs participating; Steppe desert (SD) is distributed in the
338 northern and northwestern parts of Ningxia, adjacent to the Tengger Desert

and Mao Wusu Desert, the climate is extremely arid, the annual precipitation is usually less than 200 mm, the vegetation is sparse (<30%), and super-arid shrubs and small half-shrubs are dominant (Zhang et al. 2023; Zhang et al. 2025). The soil is predominantly light gray calcareous in DS and SD. This diversity offers a unique natural laboratory to investigate how varying ecosystems respond to climatic shifts.

Line 143: Can you cite or expand on the vegetation survey method used? The names, levels, average and maximum heights, cover and abundance of species in the sample plots were recorded in detail, so that the relative cover and abundance could be calculated to reflect the range and density of species in the sample plots.

Other responses please see we response 6 (section 2.3.1 and 2.4) 13-16 . **Response:** We sincerely thank the reviewers for their positive comments on our work. Their recognition of our study title, abstract, and overall structure is very encouraging and reinforces the value of this study. We sincerely thank the reviewers for recognizing our work. For the discussion section, I have divided it into clear paragraphs in the revised manuscript.

17. **Reviewer:** Overall, we recommend the text be revised for grammar and clarity. Several suggestions are noted below in the minor items list below, but the paper should be thoroughly edited for similar issues throughout.

Response: We sincerely apologize for the oversight in language editing. We have thoroughly revised the manuscript to correct all typographical and grammatical errors. The text has also been polished by a professional English editing service to improve clarity and readability.

18. **Reviewer:** Figures and tables are useful, but the figure captions for many of the figures could be more informative as to what is it we are looking at and what is significant versus not significant. The figure caption for Figure 2 is excellent and explanatory, but many of the other captions lack important details.

Response: We have added legends, clear figure captions and panel letters, please see response 9.

19-22. **Reviewer:** Abbreviations need some more clarification and consistency. Overall, there were many abbreviations and some of these abbreviations were not used many times in the paper and could be omitted. The discussion section reiterates much of the results; We

recommend moving some of the 10 figures to supplemental, in order to streamline the presentation of the results, this text could be streamlined to reference rather than repeat the results.

Response: Many abbreviations have been omitted from the revised version, and the discussion section has been revised. We have moved some of the 10 figures to supplemental (Fig.S1, Fig.S2, Fig.S3 and Table S1), please see response 9.

MINOR ITEMS:

Line116: What is the “dual-carbon” goal?

Response: The dual-carbon target is China's two-stage emission reduction commitment to combat climate change, carbon peaking and carbon neutrality.

Line 137: Add reference to Table 1.

Response: The latitude, longitude, and elevation of each site were recorded, and mean annual temperature (MAT) and annual precipitation (MAP) were obtained from the databases (<http://www.worldclim.org/>).

Line 182: Add citation for the conversion factor.

Response: The molecular weights of GlcN and MurA are 179.17 and

393 251.23, respectively, and 31.3 is the conversion factor for bacterial
394 muramic acid to bacterial necromass carbon (Liang et al. 2019).

395 **Line 188:** Define LSD, and add citation.

396 **Response:** A least significant difference (LSD) was performed to assess
397 the differences at the significance level of 0.05 (Wu et al. 2025).

398 **Line 283:** Are these correlations performed by grassland type or do they
399 aggregate grassland types together?

400 **Response:** These correlations performed by aggregate grassland types
401 together.

402 **Line 295:** How do you define “residue carbon accumulation?”

403 **Response:** We apologize for the error (residue carbon accumulation), the
404 correct (microbial necromass carbon accumulation) have been updated.

405 **Line 440:** You state that as elevation increases the rate of SOM
406 decomposition decreases. But does the input of SOM also decrease? Both
407 are key to determining overall accumulation of organic matter.

408 **Response:** Altitude exerts a multifaceted influence on SOM content,
409 primarily mediated through its interplay with climatic and edaphic factors.
410 As elevation increases, higher precipitation and enhanced soil moisture

create favorable conditions for plant productivity and microbial activity. This stimulates the mineralization of soil elements, accelerating nutrient cycling and ultimately enriching SOM content (Cotrufo et al. 2019; Guo et al. 2025). However, this positive correlation is counterbalanced by another critical altitudinal effect: declining temperatures and intensified UV radiation at higher elevations suppress plant growth and impede microbial decomposition. Consequently, organic matter breakdown slows, leading to the accumulation of recalcitrant carbon pools (Hernandez et al. 2021). This dualistic mechanism—where altitudinal gradients simultaneously promote SOM turnover and preservation.

We have revised the other minor items in manuscript.

References:

An SS, Huang YM, Liu MY (2010) Soil Organic Carbon Density and Land Restoration: Example of Southern Mountain Area of Ningxia Province, Northwest China. *Communications in Soil Science and Plant Analysis* 41(2): 181-189

Chai J, Ling ZB, Wang Y, Dong R, Zheng YH, Qi JT (2024) A method for measuring soil water content based on principal component analysis. *Review of Scientific Instruments* 95(2):

Chen JG, Xiao W, Zheng CY, Zhu B (2020) Nitrogen addition has contrasting effects on particulate and mineral-associated soil organic carbon in a subtropical forest. *Soil Biology & Biochemistry* 142:

Cotrufo MF, Ranalli MG, Haddix ML, Six J, Lugato E (2019) Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience* 12(12): 989-+

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