Reply on RC1

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. **Reviewer:** The overall writing should be carefully checked, e.g., Line 80, "xxx conservation initiatives.(Hou et al., 2024)." should be "xxx conservation initiatives (Hou et al., 2024).". Line 87, "Wang et al.(Wang et al., 2021a)" should be "Wang et al. (2021a)". Too many clerical errors show the MS was not well prepared.

Response: We sincerely apologize for the oversight in language editing. We have thoroughly revised the manuscript to correct all typographical and grammatical errors:

lines 77-80: Therefore, underscore the necessity to investigate the MNC contribution to SOC fractions, which is fundamental for accurately evaluating the environmental benefits and carbon sequestration potential of ecological conservation initiatives (Hou et al. 2024).

Lines 86-91: Recent studies have provided the substantial contribution of MNC to SOC pools, Wang et al.(2021) found that nearly 47% contributes in the 0-20 cm soil layer of grasslands. Cotrufo et al.(2019) demonstrated that MAOC contributes over 50% to SOC accumulation in grasslands, highlighting its critical role in carbon stabilization. Notably, He et al.(2022) observed that the accumulation of MNC in alpine grasslands is closely related to soil depth. Liao et al.(2023) found that…

Line 95: Dou et al.(2023) highlighted that…

Line 459: …with the findings of Hou et al.(2024) and Xue et al.(2023).

2. **Reviewer:** Lines 27–29, the authors stated that the quantitative contribution of MNC to distinct SOC fractions and its regulatory mechanisms across various grassland types remain largely unexplored.

However, mechanisms are also not involved in this study.

Response: We agree with the reviewer's observation and have revised to clarify that our study focuses on quantifying the contribution of microbial necromass carbon to SOC fractions: Lines 26–29: Microbial necromass carbon (MNC) is a significant source of soil organic carbon (SOC). However, the contribution of microbial necromass to different organic carbon fractions and their influencing factors in various soil layers under different various grassland types remain unclear.

3. **Reviewer: Line 52, SOC**

   Response: We have revised the errors, Line 50: offering a deeper understanding of the mechanisms driving MNC to SOC fractions accumulation in diverse grassland ecosystems.

4. **Reviewer:** The scientific significance is not clear based on the Introduction, e.g., why Ningxia is the representative research area.

Response: We have added justification for selecting Ningxia as the study area in the end of introduction and section 2(study area):

lines 101-109: 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 MNC to their accumulation in deeper soil layers (>60 cm) are not yet well understood (Wu et al., 2025).

Lines 124-148: 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 km^2, 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 its land area—encompassing nearly all major grassland types found in northern China. In the southern Loess Plateau, meadow steppe (MS) and typical steppe (TS) dominate, with the TS concentrated near Guyuan City (e.g., Yunwu Mountain Grassland Nature Reserve), belonging to the semi-arid climate, annual precipitation is generally in the range of 300–400 mm or so, with the dominance of drought-resistant perennial tufted grasses, the soil is dominated by black clay. In contrast, the MS is mainly distributed in the shady slopes and valleys of Liupan Mountains and other mountainous areas where the water conditions are better, the climate is more humid, and the annual precipitation is generally around 400–600 mm, which consists of perennial perennial and rhizomatous grasses in the middle of the arid zone, the soils are mainly mountain brown loam, mountain gray-brown soil and black clay. Desert steppe (DS) is distributed in the central and northern parts of Ningxia, which is the overland of grassland and desert, with arid climate, annual precipitation is generally around 200 - 300 mm, and the vegetation cover is 40-60%, with dry perennial grasses dominating and small dry shrubs participating; Steppe desert (SD) is distributed in the 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., 2025; Zhang et al., 2023). 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.

5. **Reviewer:** In Materials and Methods, please ensure the calculation of SOC fractions is reliable.

Response: We have added detailed steps and references for the SOC fractionation protocol to ensure reliability:

lines182-202: the separation into the coarse fraction and mineral-associated fraction was achieved using the density-gravity method (Lavallee et al. 2020). 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 $r \cdot min^{-1}$), 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 (MAOM). The organic carbon content in each fraction was determined using the potassium dichromate-external heating method (Bradford et al.

2008; 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 g·kg^{-1}.

$$\mathrm{POC} = \frac{\Delta \mathrm{M}_1}{\mathrm{M}} \times \mathrm{C}_{\mathrm{POM}} \tag{1}$$

$$\mathrm{MAOC} = \frac{\Delta \mathrm{M}_2}{\mathrm{M}} \times \mathrm{C}_{\mathrm{MAOM}} \tag{2}$$

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 (g·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 (g·kg^{-1}).

6. **Reviewer:** Line 317, Please revise "Zhang et al.(Zhang et al., 2024), Shen et al.(Shen et al., 2024), Ji et al.(Ji et al., 2020)."

Response: We have revised the errors: Line 411: In this study, the contents of mineral-associated organic carbon (MAOC) and particulate organic carbon (POC) were higher than those previously reported (Zhang et al., 2024; Shen et al., 2024; Ji et al., 2020).

7. **Reviewer:** In figure 3, POC, rather than ROC.

Response: We apologize for the error. The correct has been updated in lines 271-277 (Figure 3):

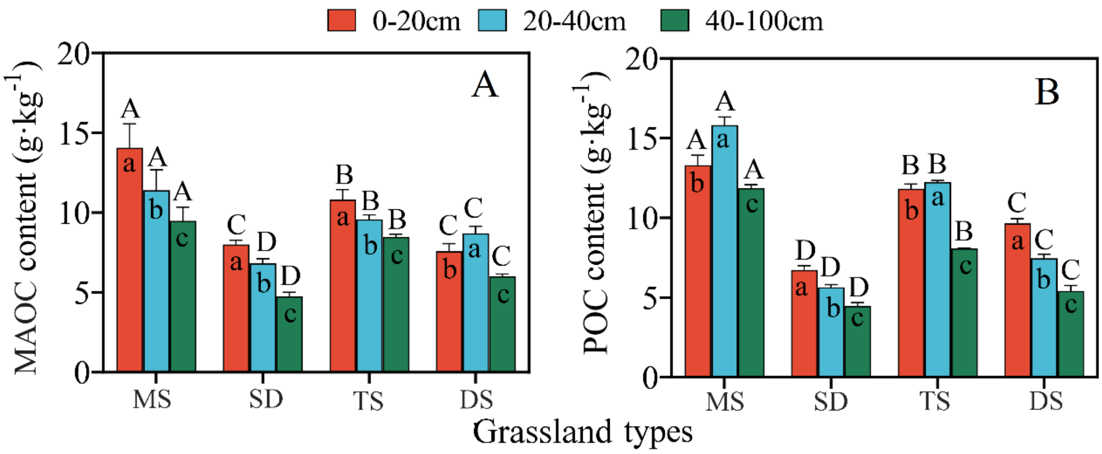


Fig.2 Contents of MAOC (A) and POC (B) in 0-100 cm soil layers under different grassland types. Different uppercase letters indicate significant differences in different grassland types in the same soil layer, and different lowercase letters indicate significant differences in different soil layers under 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.

8. **Reviewer:** In figure 10, R^2=83.4%?

Response: We apologize for the error. The correct R^2 value has been updated in lines 397-403 (Figure 8):

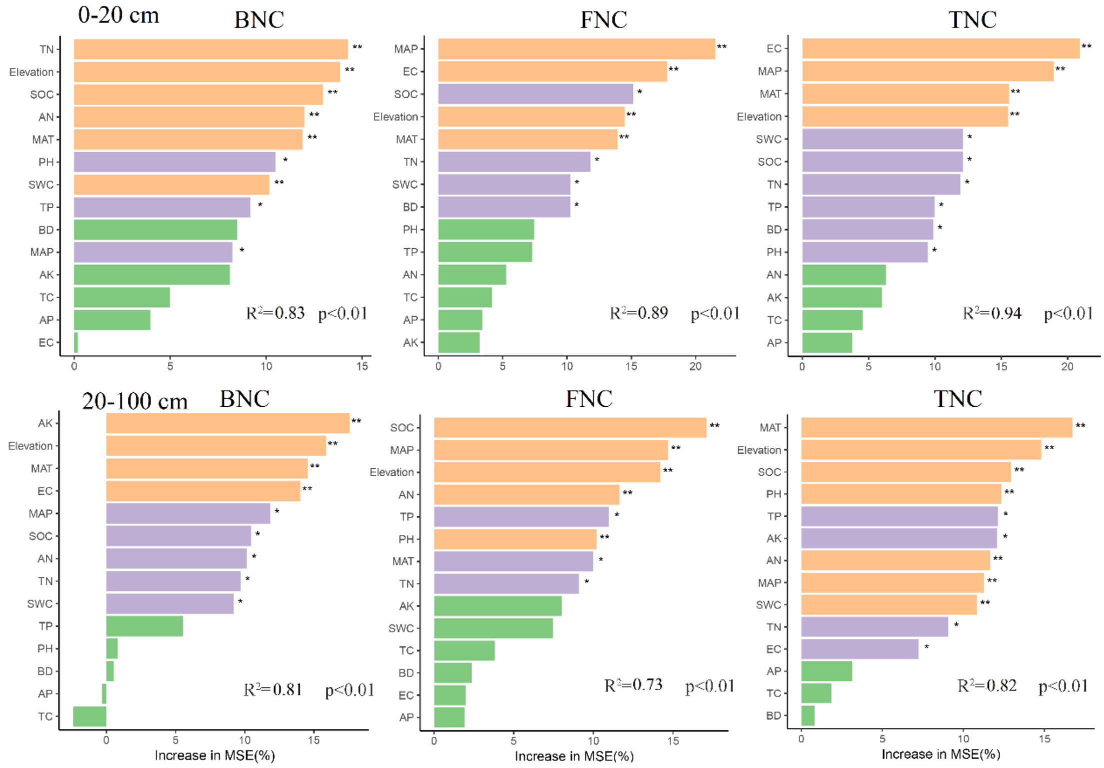


Fig. 8 Random forest analysis indicating the relative importance of soil properties to MNC. Colors represent significant correlations. MAT: mean annual temperature; MAP: mean annual precipitation; SWC: Soil water content; BD: Bulk density; TN: Total nitrogen; TC: Total carbon; TP: Total phosphorus; AK: 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).

9. **Reviewer:** Regarding discussion, I recommend the authors present the key results first and discuss the results based on the published literature.

Response: We have restructured the discussion to first summarize key findings, lines 407-411: This study, encompassing the entire natural succession sequence in the Ningxia region, included a diverse array of plant types. Vegetation is a significant source of SOC, with the extent of root development and the composition of root exudates from diverse vegetation types exerting a direct influence on the content and distribution of SOC and its fractions (Shao et al. 2021; Zhao et al. 2023)… The experimental period experienced increased rainfall compared to previous years, coupled with enhanced vegetation diversity and density, which collectively contributed to a greater influx of organic carbon into the soil.

10. **Reviewer:**I recommend the authors carefully check English and improve writing quality.

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.

Reply on RC2

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.

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

Response: We agree with this suggestion and have now added explicit hypotheses based on our research at the end of the introduction: Lines 113-116: We hypothesized that: (1) the contents of SOC fractions and MNC would be decreased with soil deep; (2) The contribution of fungal necromass carbon is higher than that of bacteria; (3) The key factors influencing MNC contribution to MAOC and POC accumulation are mean annual precipitation and soil total nitrogen.

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.

Response: We agree with the reviewer's observation and have revised our methods. We have expanded the Methods section as follow lines 164-

202 and lines 219-236:

2.3.1 Soil Physicochemical Properties

Soil bulk density (BD) was determined using the core method, employing a 100 cm^3 ring knife (5 cm height, 5.05 cm diameter) (Wang et al. 2022b). Soil water content (SWC) was assessed via the oven-drying method, where fresh soil was dried at 102°C until a constant weight was achieved (Li et al. 2018). Soil pH was measured using a pH meter (pHS-3C) with a soil-to-water ratio of 1:2.5 (w/v) (Roberts et al. 2007). Soil organic carbon (SOC) was quantified using the $K_2Cr_2O_7$ external heating method, followed by titration with 0.1 M $FeSO_4$ (Ding et al. 2019). Total nitrogen (TN) was determined using the Kjeldahl method using the Kjeltec 8400 (FOSS, Denmark). Available nitrogen (AN) using alkaline hydrolysis diffusion. Total phosphorus (TP) was measured by an ultraviolet spectrophotometer (UV3200, Shimadu Corporation, Japan) after wet digestion with H_2SO_4 and $HClO_4$. 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 $r \cdot min^{-1}$), 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 $g \cdot kg^{-1}$.

$$POC = \frac{\Delta M_1}{M} \times C_{POM} \tag{1}$$

$$MAOC = \frac{\Delta M_2}{M} \times C_{MAOM} \tag{2}$$

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 ($g \cdot kg^{-1}$); and C_{MAOM} measured indicates the organic carbon content of the lower-layer soil sample determined by the potassium dichromate-external heating method ($g \cdot kg^{-1}$).

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

7. **Reviewer:** The soil type/classification or geologic information was not described. There was no mention of soil texture, or soil horizon but the authors used the phrase soil profile throughout the manuscript. This paper

would greatly benefit from inclusion of soil type/classification into the methods/sampling site section. Line 139. Why were these soil depths chosen? Were there soil horizons or other soil characteristics unique at these depths (e.g., pH, texture, mineral composition, organic matter, etc.)?

Response: We apologize for this oversight. We have added soil type in study area:

Lines 133-134: …with the dominance of drought-resistant perennial tufted grasses, the soil is dominated by black clay.

Line 138: …which consists of perennial perennial and rhizomatous grasses in the middle of the arid zone, the soils are mainly mountain brown loam, mountain gray-brown soil and black clay.

Lines 146-147: …The soil is predominantly light gray calcareous in DS and SD.

Lines 158-159: We classify soil layers according to soil properties (An et al. 2010; Wu et al. 2025).

8. **Reviewer:** At first glance, analysis and assumptions appear valid but the authors did not mention if the distribution of the data was normal or non-normal, and why parametric test/linear regression was suited for the

data. If the data was normally distributed, the authors analysis is appropriate but if the data is not normally distributed then alternative non-parametric statistical analysis should be used.

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

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

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

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

Response: We apologize for the writing error, the correct grassland types have been updated MS, TS, DS, and SD not CD, HM, DX, CH (lines

152-153).

The 20 - 100 cm layers were combined together in Figures S3 and Figures 8, that's because (lines 384-390):

By principal component analysis, the soil 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.

Further, Fig. S3 and Fig. 8 aggregate all grassland types in the same soil layer, we have added this information in the caption.

We sincerely apologize for lacking legends and clear figure captions. We have added legends, clear figure captions and panel letters as follows:

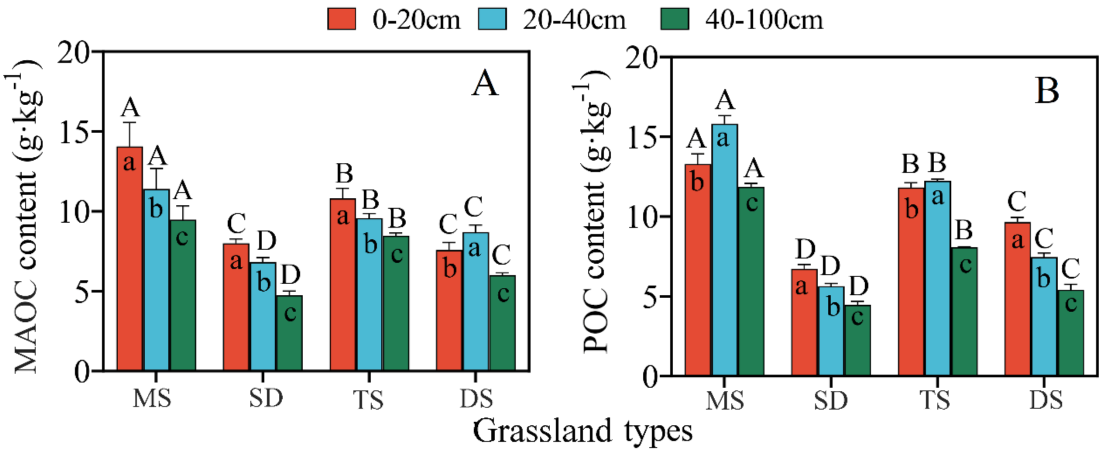


Fig.2 Contents of MAOC (A) and POC (B) in 0-100 cm soil layers under different grassland types. Different uppercase letters indicate significant differences in different grassland types in the same soil layer, and different lowercase letters indicate significant differences in different soil layers under 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.

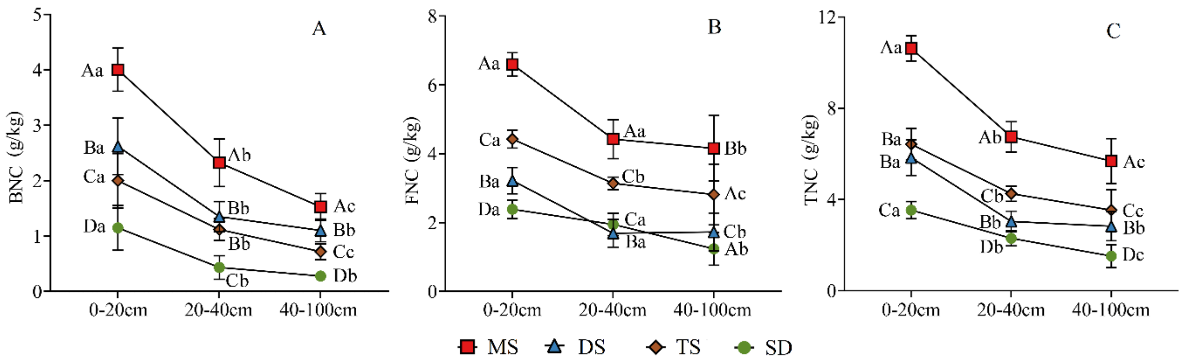


Fig. 3 Contents of BNC、FNC and TNC in MAOC in different soil layers under different grassland types. Different uppercase letters indicate significant differences in different grassland types in the same soil layer ($p<0.05$), and different lowercase letters indicate significant differences in different 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.

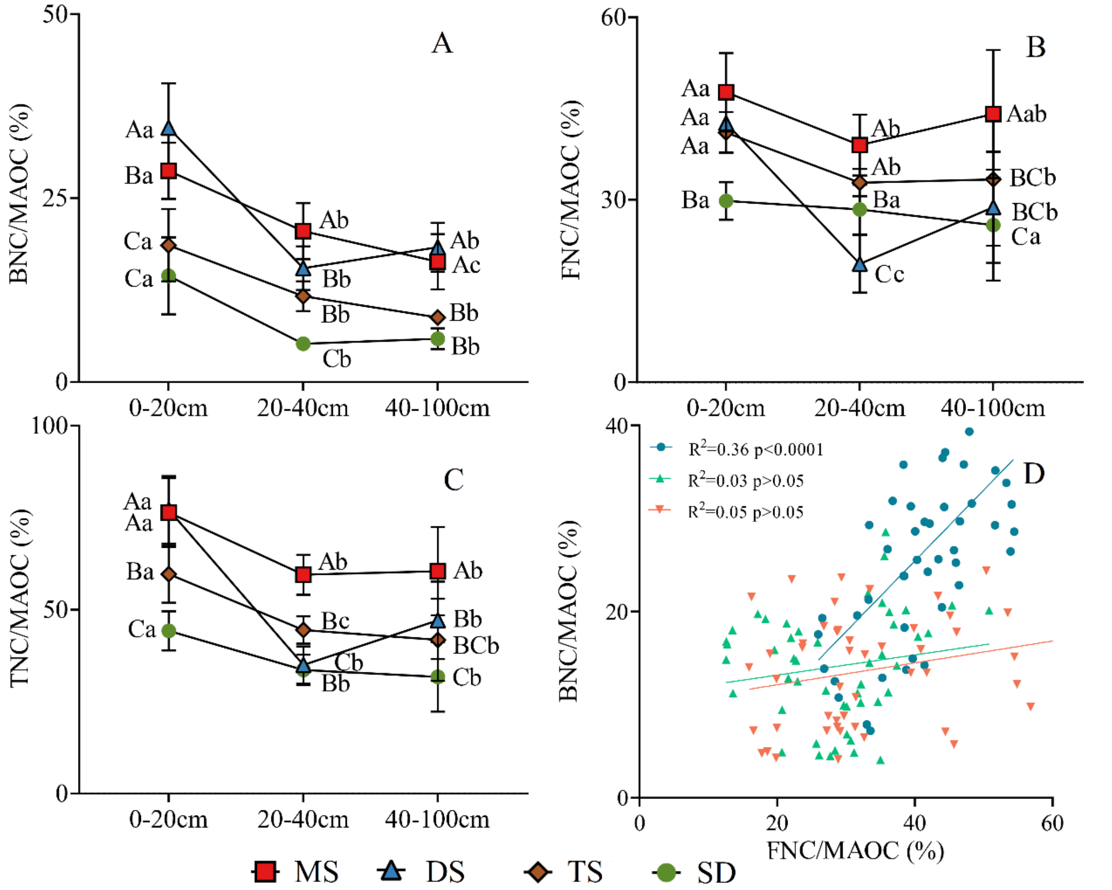


Fig. 4 The contribution of BNC, FNC and TNC in MAOC in different soil layers under different grassland types. **(A)** The contribution of BNC to MAOC; **(B)** the contribution of FNC to MAOC; **(C)** the contribution of TNC to MAOC. Different uppercase letters indicate significant differences in different 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.

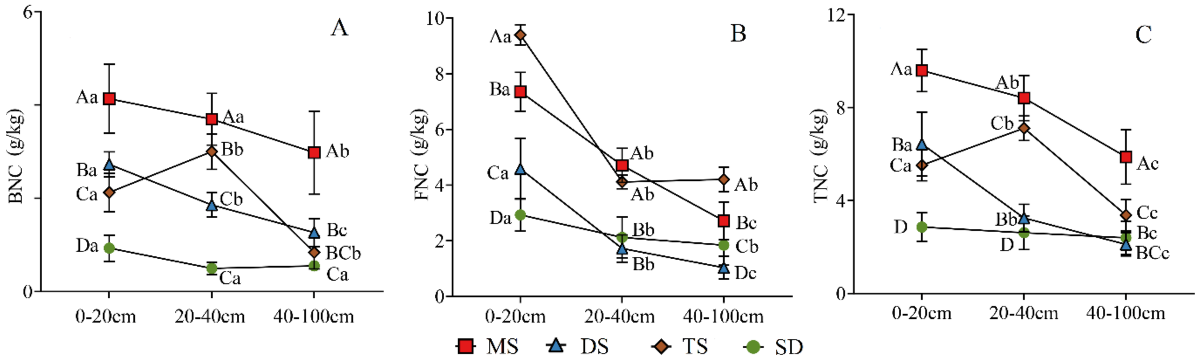


Fig. 5 Contents of BNC, FNC and TNC in POC in different soil layers under different grassland types. Different uppercase letters indicate significant differences in different grassland types in the same soil layer ($p<0.05$), and different lowercase letters indicate significant differences in different 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.

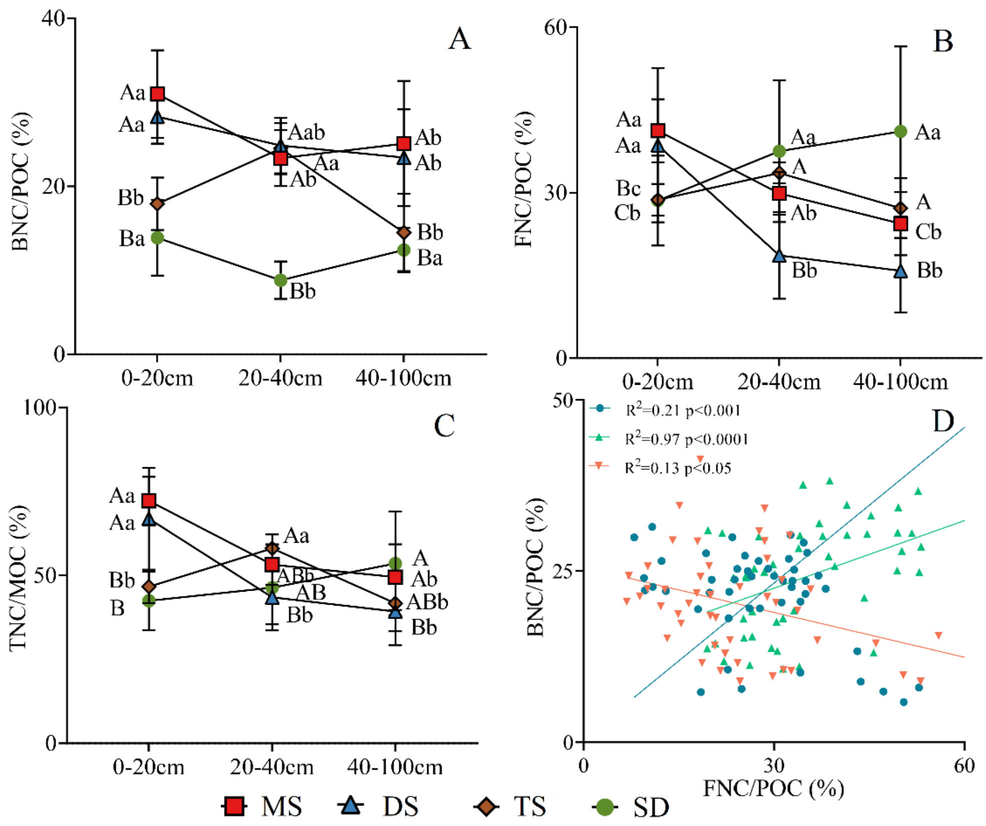


Fig.6 The contribution of BNC, FNC and TNC in POC in different soil layers under different grassland types.

(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.

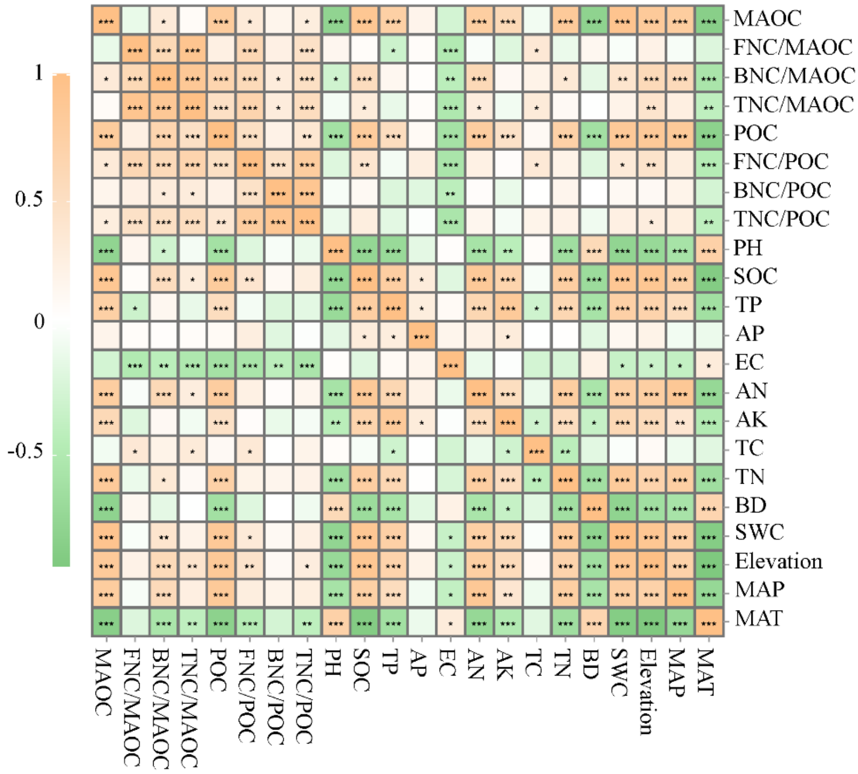


Fig.7 The Spearman correlation of MAOC、POC and MNC with environmental factors in 0-100cm soil layer under different grassland types. Colors represent Spearman correlations; MAT: mean annual temperature; MAP: mean annual precipitation; SWC: Soil water content; BD: Bulk density; TN: Total nitrogen; TC: 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.

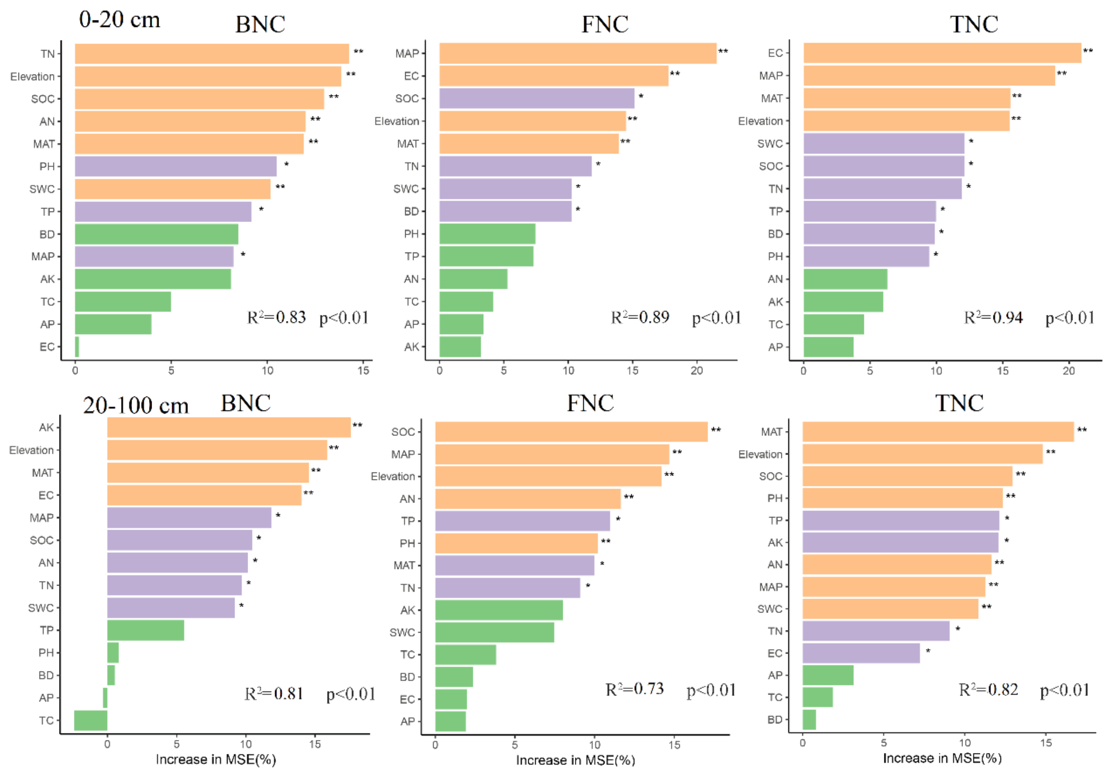
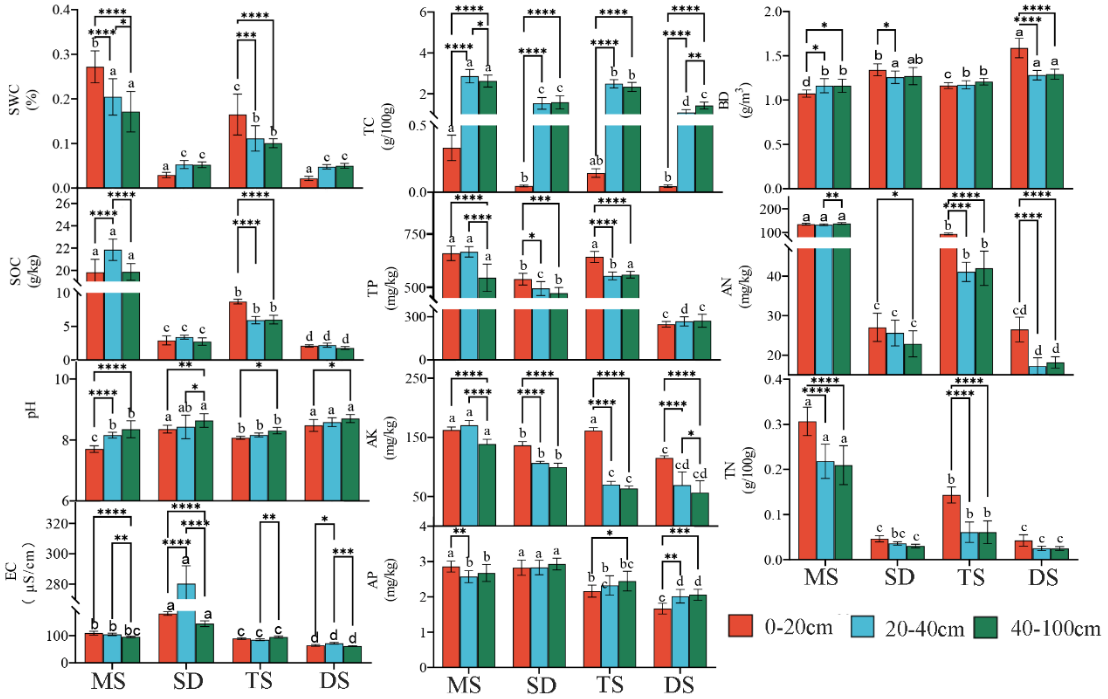


Fig. 8 Random forest analysis indicating the relative importance of soil properties to MNC. Colors represent significant correlations. MAT: mean annual temperature; MAP: mean annual precipitation; SWC: Soil water content; BD: Bulk density; TN: Total nitrogen; TC: Total carbon; TP: Total phosphorus; AK: 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).



386

387 **Fig. S1 Characteristics of Soil Physicochemical Properties in 0-100 cm Under Different Grassland Types.** SWC:

388 Soil water content; BD: Bulk density; TN: Total nitrogen; TC: Total carbon; TP: Total phosphorus; AK: Available

389 potassium; AP: Available phosphorus; AN: Available nitrogen; EC: Electrical conductance. Significant differences

390 of the same grassland types in different soil layers *:p<0.05； **: p<0.01; ***: p<0.001; Different lowercase letters

391 indicate significant differences between different grassland types in same soil layer (p < 0.05). The bars in red, blue

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

393 desert; TS: typical steppe; DS: desert steppe.

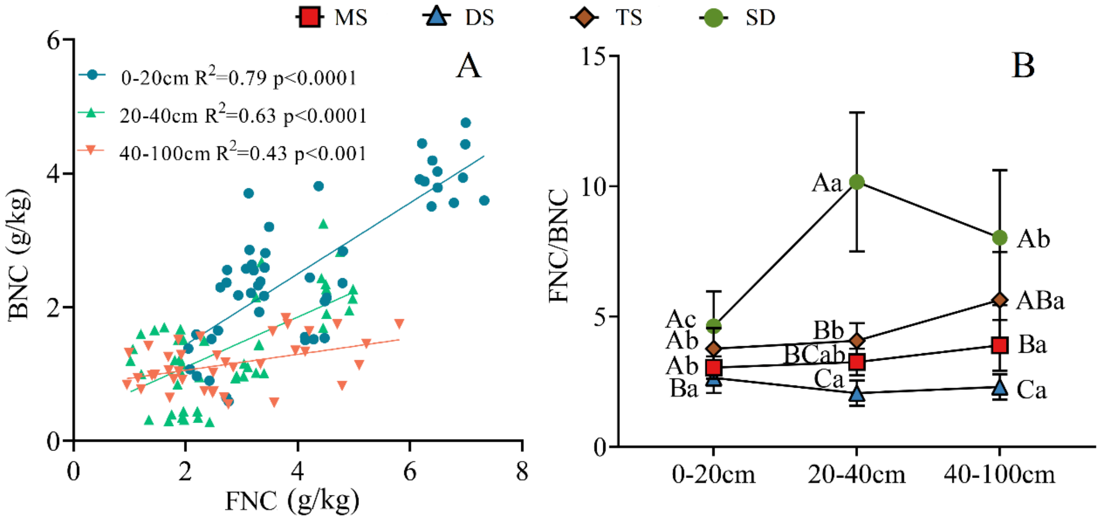


Fig. S2 The correlation of BNC and FNC in different soil layers among four grassland types (A); The blue circle, green triangle and orange inverted triangle represent the soil layers 0–20 cm, 20 –40 cm and 40 –100 cm respectively. **The ration of FNC/BNC in different soil layers among different grassland 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.

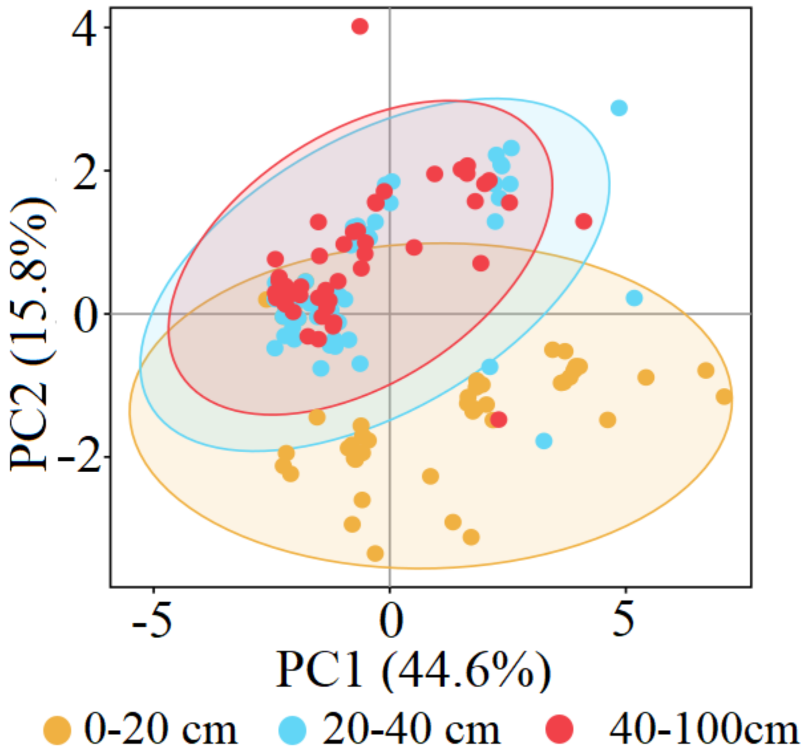


Fig. S3 Principal component analysis of soil properties in different soil layers of four grassland types. Yellow, green and red circles represent 0-20cm, 20-40cm, 40-100cm soil layers (This analysis aggregated four grassland types in same soil layer).

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). Hgher 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 Ca^{2+}, 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 423-428: 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 (lines 560-567): 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:

Lines 99 - 109: Here the authors identify a major knowledge gap about microbial necromass dynamics, but do not have any corresponding citations.

Lines 119 - 127: Sampling site description has no citations.

Line 143: Can you cite or expand on the vegetation survey method used?

Line 151: Citation for the core method.

Line 155: Citation for SOC $K_2Cr_2O_7$ external heating method. Also, it would help to list the chemical compound name (Potassium dichromate) before listing the formula, Line 157: Citation for Kjeldahl method, Line 159: Citation and name for the “standard protocols”.

Lines 191 - 192: R packages should have citations.

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 fellow:

Lines 98-113: 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 MNC 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…

Lines 124-148: we have added citations, please see our response 4 (Reply on RC1).

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 our response 6 (section 2.3.1 and 2.4) (Reply on RC2)

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 (Reply on RC2).

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 (Reply on RC2).

MINOR ITEMS:

Line121: 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: line 155, 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: line 218, the molecular weights of GlcN and MurA are 179.17 and 251.23, respectively, and 31.3 is the conversion factor for bacterial muramic acid to bacterial necromass carbon (Liang et al. 2019).

Line 188: Define LSD, and add citation.

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

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

Response: These correlations performed by aggregate grassland types together.

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

Response: line 389, We apologize for the error (residue carbon accumulation), the correct (microbial necromass carbon accumulation) have been updated.

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

Response: Altitude exerts a multifaceted influence on SOM content, primarily mediated through its interplay with climatic and edaphic factors. 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.

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