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, line 77-80: Therefore, underscore the necessity to investigate the microbial necromass carbon 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); Line 86-88: Recent studies have provided the substantial contribution of mnc to SOC pools, Wang et al.(Wang et al. 2021) found that nearly 47% contributes in the 0-20 cm soil layer of grasslands.

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 rather than mechanistic exploration, Lines 27–29: 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 52: 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, emphasizing its represents a transitional zone between the Loess Plateau and the Mongolian Plateau, encompassing nearly all major grassland types found in northern China, lines 99-116: 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, such as Ningxia, which encompasses diverse grassland types representative of northern Chinese ecosystems.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), critical knowledge gaps persist regarding the dynamics of MAOC and POC fractions, particularly the contribution of microbial necromass carbon to their accumulation. Therefore, this study addresses these gaps by investigating the vertical distribution (0-100 cm) of SOC fractions and MNC across different grassland types, while identifying the key drivers 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 118-141: 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. Ningxia belongs to typical continental semi-humid semi-arid climate. This area is one of the three pilot provinces of "Research on climate change adaptation in China", 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. 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. 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. 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 reproducibility, lines163-166: 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 $g \cdot kg^{-1}$.

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

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

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

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 315-317: In this study, the contents of mineral-associated organic carbon (MAOC) and particulate organic carbon (POC) were higher than those reported by Zhang et al.(Zhang et al. 2024), Shen et al.(Shen et al. 2024), Ji et al.(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 Figure 3:

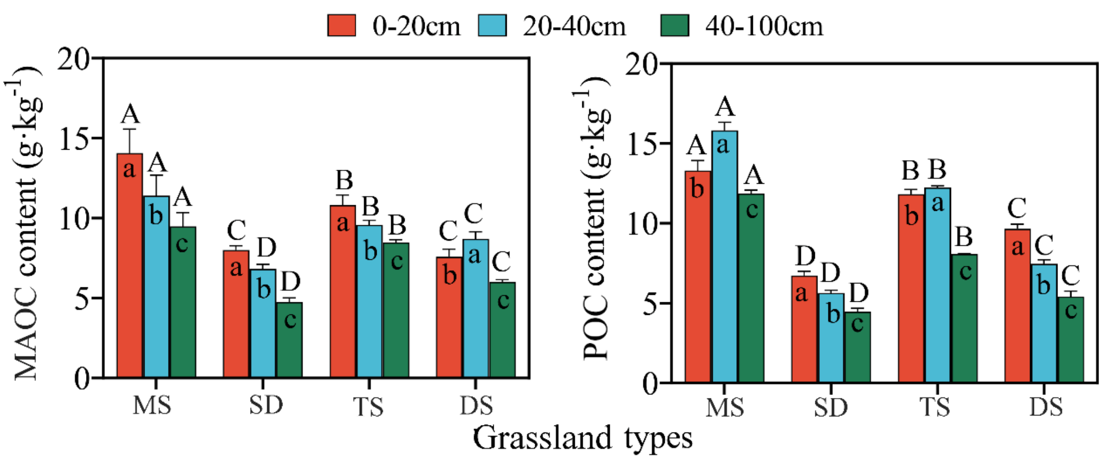

Fig.3 Contents of MAOC and POC in 0-100 cm soil layers under different grassland types. Different uppercase letters indicate significant differences in different vegetation types in the same soil layer, and different lowercase letters indicate significant differences in different soil layers under the same vegetation type ($p<0.05$).

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

Response: We apologize for the error. The correct R^2 value has been updated in Figure 10:

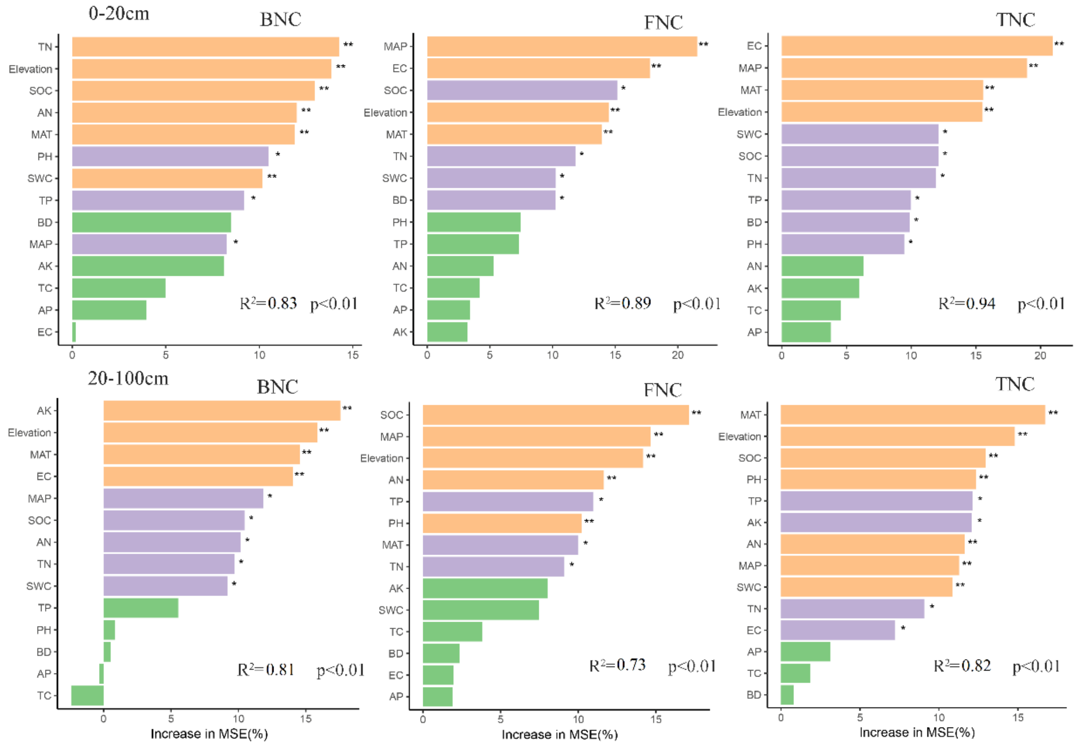

Fig.10 The relative importance of envieonmental and soil factors on MNC.

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 312-328: 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. In this study, the contents of mineral-associated organic carbon (MAOC) and particulate organic carbon (POC) were higher than those reported by Zhang et al.(Zhang et al. 2024), Shen et al.(Shen et al. 2024), Ji et al.(Ji et al. 2020). This divergence can be attributed to variations in the input and output of organic carbon fractions, driven by differing hydrothermal conditions that affect aboveground vegetation. Additionally, researchers have noted that climate, soil, and vegetation factors significantly influence soil carbon content, with vegetation factors accounting for up to 55% of the variation in SOC accumulation (Huang et al. 2024).

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.

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