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Contribution of soil Microbial Necromass Carbon to Soil Organic Carbon fractions and its influencing factors in different grassland types

Shenggang Chen[1,5,6], Yaqi Zhang[2,6], Jun Ma[1,5], Mingyue Bai[1,5], Jinxiao Long[2,6], Ming Liu[1,5], Yinglong Chen[4], Jianbin Guo[1,5]* & Lin Chen[3,6]*

[1] School of Soil and Water Conservation, Beijing Forestry University, Beijing 100083, China.

[2] School of Forestry and Prataculture, Ningxia University, Yinchuan, 750021, China.

[3] School of Ecology and Environment, Ningxia University, Yinchuan 750021, China.

[4] School of Earth and Environment (M087), The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

[5] Key Laboratory of State Forestry Administration on Soil and Water Conservation, Beijing Forestry University, Beijing 100083, China.

[6] Key Laboratory for Restoration and Reconstruction of Degraded Ecosystem in Northwest China of Ministry of Education, Ningxia University, Yinchuan 750021, China.

Shenggang Chen and Yaqi Zhang should be considered joint first author.

Corresponding author:

E-mail addresses: jianbinguo@bjfu.edu.cn (J. Guo), chenlin198388@163.com (Lin Chen)

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Abstract: Microbial necromass carbon(MNC) is a significant source of soil organic carbon(SOC), the quantitative contribution of MNC to distinct SOC fractions and its regulatory mechanisms across various grassland types remain largely unexplored. This study through a comprehensive investigation of soil profiles (0-20 cm, 20-40 cm, and 40-100 cm) across four grassland types in Ningxia, China, encompassing meadow steppe (MS), typical steppe (TS), desert steppe (DS), and steppe desert (SD). We quantified mineral-associated organic carbon (MAOC), particulate organic carbon (POC), and their respective microbial necromass components, including total microbial necromass carbon (TNC), fungal necromass carbon (FNC), and bacterial necromass carbon (BNC), and analyzed the contributions to SOC fractions and influencing factors. Our findings reveal three key insights. First, the contents of MAOC and POC in the 0-100 cm soil layer were in the following order of magnitude: Meadow steppe (MS) >Typical steppe (TS) > Desert steppe (DS) > Steppe desert (SD), with the average content of POC was 9.3 g/kg, which was higher than the average content of MAOC (8.73 g/kg). Second, the content of microbial TNC in MAOC and POC decreased with the depth of the soil layer, the average content of FNC was 3.02 g/kg and 3.85 g/kg, which was higher than the average content of BNC (1.64 g/kg and 2.08 g/kg). FNC dominated both MAOC and POC, and its contribution was higher than the contribution of BNC. Thid, through regression analysis and random forest modeling, we identified key environmental drivers of MNC dynamics: mean annual rainfall (MAP), electrical conductance (EC), and soil total nitrogen(TN) emerged as primary regulators in surface soils (0-20cm), while available potassium(AK), SOC, and mean annual temperature (MAT) dominated deeper soil layers (20-100 cm). This research by: 1) establishing the vertical distribution patterns of MNC and SOC fractions in soil profiles; 2) quantifying

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the relative contributions of MNC to SOC fractions across different grassland ecosystems soil profiles and elucidating their environmental controls, offering a deeper understanding of the mechanisms driving MNC to soc fractions accumulation in diverse grassland ecosystems, and provide data support for further research on the microbiological mechanisms of soil organic carbon formation and accumulation in arid and semi-arid regions.

Key words： Grassland types, Fungal necromass carbon (FNC), Bacterial necromass carbon (BNC), Mineral-associated organic carbon (MAOC), Particulate organic carbon (POC), Influencing factors

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1 Introduce

Soil organic carbon (SOC) is the largest carbon reservoir in grassland ecosystems and is strongly influenced by vegetation and microorganisms under certain conditions(Lehmann and Kleber, 2015). Microbial necromass carbon (MNC) is a crucial source of SOC, and its formation and accumulation processes vary significantly across different grassland types and SOC fractions, leading to varying contributions to SOC(Deng and Liang, 2022). The partitioning of SOC into particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) provides critical insights into carbon stabilization mechanisms.While POC primarily originates from plant residues, with a high C:N ratio and rapid turnover rate, represents the more labile carbon pool, MAOC is mainly derived from microbial sources, with a slower turnover rate, allowing it to persist in the soil for centuries, thus playing a vital role in long-term SOC stabilization(Angst et al., 2021; Wang et al., 2021b). The proportion of microbial and plant-derived necromass carbon varies due to microbial decomposition processes(Bölscher et al., 2024), leading to significant differences in the contribution of microbial-derived carbon to MAOC and POC formation. Recent advances in soil organic matter research have revealed that microbial-derived carbon contributes approximately 52% to MAOC, while plant-derived carbon contributes about 40% to POC(Liang et al., 2019). 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).

The accumulation of microbial necromass carbon in grassland ecosystems is governed by a complex interplay of biotic and abiotic factors(Li et al., 2017). Variations

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in plant biomass and diversity across different grassland types significantly influence soil physicochemical properties and microbial community structure, while land use patterns, soil depth, soil nutrients, and climatic conditions further influence the accumulation of MNC(Yang et al., 2024). Recent studies have provided the substantial contribution of mnc to SOC pools, Wang et al.(Wang et al., 2021a) found that nearly 47% contributes in the 0-20 cm soil layer of grasslands. Cotrufo et al.(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.(He et al., 2022) observed that the accumulation of MNC in alpine grasslands is closely related to soil depth. Liao et al.(Liao et al., 2023) found that necromass carbon content in the 0-5 cm and 5-20 cm soil layers of grasslands on the Loess Plateau ranges from 0.69 to 16.41 g/kg. Additionally, drought thresholds and soil stoichiometric ratios are critical factors influencing microbial necromass carbon accumulation in grasslands(Hao et al., 2021). Dou et al. highlighted that microbial necromass carbon is stored more in the MAOC fraction across different grassland types and soil layers, with soil bulk density, pH, and total organic carbon being the primary factors influencing its contribution to SOC accumulation. 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 microbial necromass carbon 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

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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 microbial necromass carbon 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.

2 Materials and Methods

2.1 Study Area

The study area is located in Ningxia Hui Autonomous Region, China (35°14′–39°23′ N, 104°17′–107°39′ E), encompassing an area of 66,400 km². Situated in the northern part of China's geological "north-south central axis," Ningxia lies between the North China Plain, the Alxa Plateau, and the Qilian Mountains. The region experiences a typical continental semi-humid to semi-arid climate, with scarce and unevenly distributed precipitation, primarily concentrated from July to September. The average annual precipitation is 289 mm, with low temperatures (average annual temperature: 5–8°C), large temperature variations, long winters, and high evaporation rates (average annual evaporation: 1250 mm). Ningxia is one of the three pilot provinces for China's climate change adaptation research, with grasslands covering 47% of its land area, encompassing most of the northern Chinese grassland types(Zhang et al., 2025).

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2.2 Site Selection and Soil Sampling

To capture the ecological diversity along the precipitation gradient from south to north, four grassland types were selected: meadow steppe (MS), typical steppe (TS), desert steppe (DS), and steppe desert (SD) (Fig. 1). The number of sampling sites for each grassland type was proportional to their respective areas: CD (5 sites), HM (7 sites), DX (5 sites), and CH (5 sites). The latitude, longitude, and elevation of each site were recorded, and mean annual temperature (MAT) and annual precipitation (MAP) were obtained from global climate databases. At each site, three 20 × 20 m plots were established, with a minimum distance of 20 m between plots. Soil samples were collected from 0-20 cm, 20-40 cm, and 40-100 cm layers using a soil auger, mixed thoroughly, and air-dried in the laboratory. After removing plant roots and gravel, the soil was sieved through 2 mm and 0.15 mm sieves for MAOC, POC, and soil physicochemical property analyses. Additionally, 4-5g of soil was reserved for amino sugar analysis. Vegetation surveys were conducted in three randomly selected 1 × 1 m subplots within each plot (Table 1).

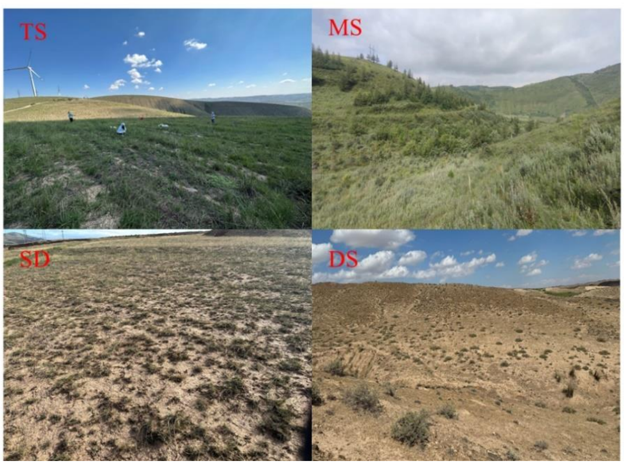

Fig.1 Distribution of the sampling sites in different grassland types. meadow steppe (MS), typical steppe (TS), desert steppe (DS), and steppe desert (SD)

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Table 1 Overview of sampling sites of grassland types

Grassland types	Longitude (E)	Latitude (N)	Elevation (m)	MAP (mm)	MAT (°C)	Domain vegetation
TS1	106°30′45.03″	36°45′6.57″	1973	380	7.0	Tansy(Tanacetum vulgare)
TS 2	106°16′3.48″	36°24′23.8″	1950	391	7.7	Altai Hawkweed(Aster altaicus)
TS 3	106°24′46.39″	36°12′17.30″	1859	432	7.0	Needlegrass(Stipa capillata)
TS 4	106°48′14.38″	36°1′8.24″	1679	470	7.7	Sedge (Carex)
TS 5	106°34′54.64″	36°13′56.39″	1743	444	7.2	
MS1	105°37′36.66″	36°27′6.49″	2653	384	4.6	Large-eared Saussurea
MS 2	105°37′6.92″	36°13′58.70″	2492	420	5.5	Baikal Needlegrass
MS 3	106°7′11.55″	35°54′56.04″	2246	457	5.1	White Wormwood (Stipa
MS 4	106°13′46.53″	35°29′47.80″	2486	563	4.4	baicalensis)
MS 5	106°14′15.35″	35°40′49.79″	2247	496	5.1	Meadow-rue (Thalictrum)
DS1	107°2′59.10″	38°4′58.89″	1474	285	7.6	Short-flowered Needlegrass (Stipa
DS2	106°59′50.93″	37°53′27.65″	1430	277	8.0	breviflora)
DS 3	106°28′44.93″	37°26′28.69″	1362	273	9.0	Seablite (Suaeda glauca)
DS 4	105°31′49.79″	36°44′59.79″	1807	290	8.0	Russian Thistle (Salsola collina)
DS 5	105°25′36.86″	37°8′51.70″	1720	252	7.9	Bush Clover (Lespedeza)
DS 6	105°1′40.06″	37°14′24.23″	1843	229	7.2	Intermediate Peashrub (Caragana
DS 7	105°44′38.66″	37°23′26.63″	1410	228	9.4	intermedia)
SD1	106°28′14.83″	38°20′38.9″	1169	197	8.5	Komarov's Swallowwort
SD 2	106°29′34.37″	38°6′44.67″	1228	220	9.2	(Cynanchum komarovii)
SD 3	106°5′16.86″	37°37′50.45″	1323	237	9.1	White Spiny Shrub
SD 4	105°57′47.86″	38°38′53.99″	1359	233	5.8	(Cynanchumkomarovii)
SD 5	104°41′47.20″	37°25′58.14″	1652	192	8.0	Pearl Russian Thistle (salsola passerina)

2.3 Measurement Methods

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). 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. Soil pH was measured using a pH meter (pHS-3C) with a soil-to-water ratio of 1:2.5 (w/v). Soil organic carbon (SOC) was quantified using the $K_2Cr_2O_7$

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external heating method, followed by titration with 0.1 M $FeSO_4$. Total nitrogen (TN) and available nitrogen (AN) were determined using the Kjeldahl method. Total phosphorus (TP), available phosphorus (AP), and available potassium (AK) were measured using standard protocols. 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

MAOC and POC were separated using a density fractionation method with a sodium hexametaphosphate solution (1.7 ± 0.02 g/cm³), followed by the removal of inorganic carbon using 0.5 mol/L HCl, and analyzed using a carbon-nitrogen analyzer(Sokol et al., 2019b).

2.3.3 Amino Sugar Measurement

Amino sugars were measured according to the method described by Indorf et al.(Indorf et al., 2011). Soil samples underwent hydrolysis, purification, and derivatization, followed by gas chromatography (GC) analysis to determine four amino sugar derivatives: glucosamine (GlcN), mannosamine (ManN), galactosamine (GalN), and muramic acid (MurA). Microbial necromass carbon was calculated using the optimized formulas by Hu et al. (Hua et al., 2024)：

$$\mathrm{BNC} = \mathrm{MurA} \times 31.3 \qquad (1)$$

$$\mathrm{FNC} = (\frac{GlcN}{179.17} - 1.63 \times \frac{MurA}{251.23} \times 179.17 \times 10.8$$

$$= (\mathrm{GlcN} - 1.16 \times \mathrm{MurA}) \times 10.8 \qquad (2)$$

$$\mathrm{TNC} = \mathrm{FNC} + \mathrm{BNC} \qquad (3)$$

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Where FNC is fungal necromass carbon, BNC is bacterial necromass carbon, TNC is total necromass carbon, and GlcN and MurA are the concentrations of glucosamine and muramic acid in the soil, respectively. 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.

2.4 Data Analysis

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 significance of the differences among the different sampling sites. The liner regression analysis and graphs were created using Origin 2021. Principal component (PC) analysis and random forest modeling were conducted using R (version 4.3.1), with packages including "ggplot2," "tidyverse," "randomForest," "rfUtilities," and "rfpermute".

3 Results and Analysis

3.1 Soil Physicochemical Properties Across Different Grassland Types

Significant vertical variations in soil properties were observed across the 0-100 cm soil profile among different grassland types (Fig.2). In meadow steppe (MS) and typical steppe (TS), the 0-20 cm layer showed significantly higher SWC compared to the 20-40 cm and 40-100 cm layers. Conversely, desert steppe (DS) and steppe desert (SD) displayed an inverse trend, with lower SWC in the upper layers. SOC and TN were markedly higher in MS and TS than in SD and DS, with no significant differences observed among the three soil layers in DS and SD ($p>0.05$). TC and AK exhibited

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significant differences between the 0-20 cm and 40-100 cm layers across all grassland types, though no notable differences were found between the 20-40 cm and 40-100 cm layers. Notably, TC was lowest in the 0-20 cm layer, while AK peaked in this layer. BD and available AP showed minimal variation across grassland types, whereas EC varied significantly within the same soil layer across different grasslands.

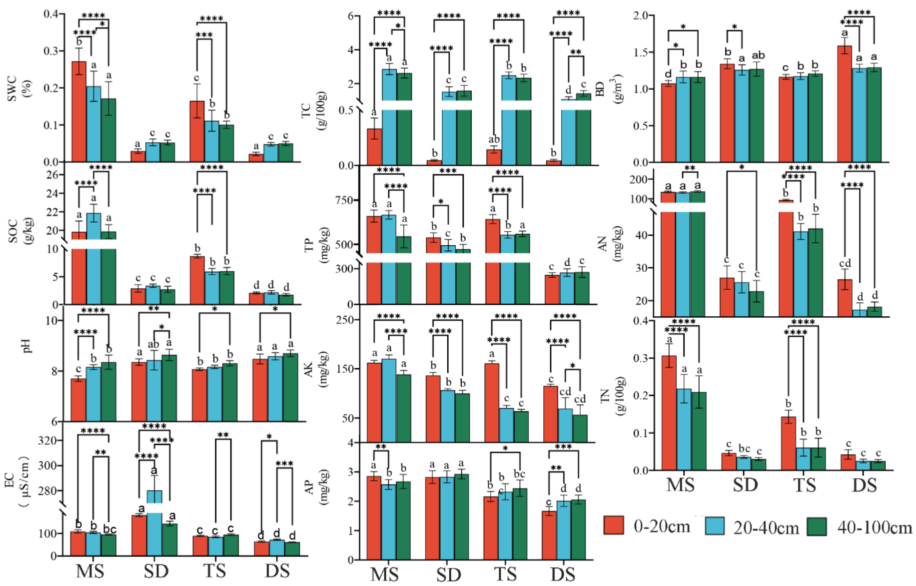

Fig.2 Characteristics of Soil Physicochemical Properties in 0-100 cm Under Different Vegetation Types

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. Significant differences of the same grassland types in different soil layers *:p<0.05； **:p<0.01; ***: p<0.001; Different lowercase letters indicate significant differences between different grassland types in same soil layer ($p < 0.05$).

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3.2 Variations in MAOC and POC content across different grassland types

MAOC and POC contents were highest in MS, followed by TS and DS, with SD having the lowest content. MAOC content decreased with soil depth, except in DS. POC content in MS and DS was higher in the 20-40 cm layer than in the 0-20 cm layer, with significant differences among the three soil layers. In the 0-20 cm layer, MAOC content showed significant differences among grassland types, except between SD and HM. In the 20-40 cm and 40-100 cm layers, MAOC and POC contents varied significantly across grassland types. In HM, MAOC content was higher in the 20-40 cm layer than in the 0-20 cm layer, while POC content showed the opposite trend. The 40-100 cm layer had the lowest MAOC and POC contents across all grassland types (Fig. 3).

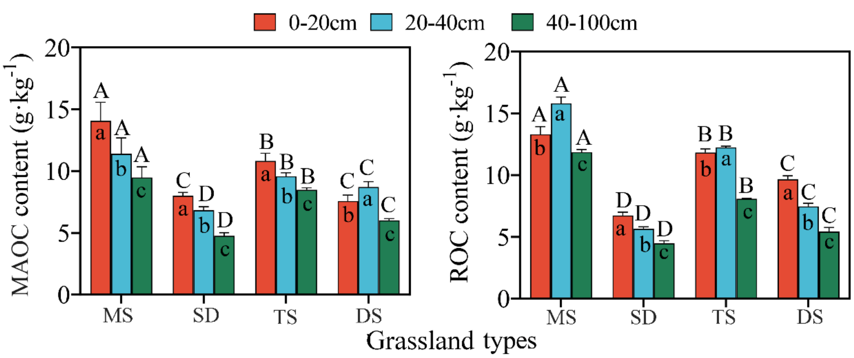

Fig.3 Contents of MAOC and POC in 0-100 cm soil layers under different grassland types

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 ($p<0.05$).

3.3 Characteristics of changes in MNC and proportion of MAOC and POC in different grassland types

3.3.1 Characterization of changes in the content and proportion of MNC in MAOC

In the 0-20 cm soil layer, the contents of BNC, FNC, and TNC within MAOC ranged from 1.5–4.0 g/kg, 4.2–6.6 g/kg, and 3.5–10.6 g/kg, respectively. These values

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were significantly higher than those observed in the 20-40 cm and 40-100 cm layers ($p < 0.05$). In the 20-40 cm layer, CH exhibited the lowest BNC content (0.43 g/kg), while HM recorded the lowest FNC content (1.69 g/kg). The TNC content across the 0-100 cm layer followed the order: MS > TS > DS > SD, with MS showing significant differences compared to other grassland types ($p < 0.05$, Fig. 4). The FNC/BNC ratio, which reflects the relative contributions of fungi and bacteria to MNC, demonstrated a significant positive correlation across all soil layers (0-20 cm: $R^2 = 0.79$, $p < 0.0001$; 20-40 cm: $R^2 = 0.63$, $p < 0.0001$; 40-100 cm: $R^2 = 0.43$, $p < 0.001$). Notably, SD had a significantly higher FNC/BNC ratio than other grassland types ($p < 0.05$, Fig. 4). FNC contributed 2.65–4.63 times more to MNC than BNC in the 0-20 cm layer, 2.06–10.17 times in the 20-40 cm layer, and 2.30–8.03 times in the 40-100 cm layer (Fig. 4).

In the 0-20 cm layer, DS exhibited a higher BNC/MAOC ratio (35%) compared to MS (28%), TS (18%), and SD (14%), with significant differences ($p < 0.05$, Fig.6). The FNC/MAOC ratio for DS (42%) was lower than that of MS (48%) but higher than TS (41%) and SD (29%), with SD showing significant differences from other grassland types. DS and MS had similar TNC/MAOC ratios (77%), which were higher than those of TS (66%) and SD (44%), with significant differences observed ($p < 0.05$). In the 20-40 cm layer, MS had a higher BNC/MAOC ratio (21%) than DS (15%), TS (12%), and SD (5%), with significant differences among grassland types ($p < 0.05$). The 40-100 cm layer exhibited trends similar to those in the 0-20 cm layer. The contributions of BNC and FNC to MAOC were positively correlated in the 0-20 cm layer ($R^2 = 0.36$, $p < 0.001$), but no significant correlations were found in the 20-40 cm and 40-100 cm layers ($R^2 = 0.03$ and 0.05, respectively, $p > 0.05$) (Fig. 6).

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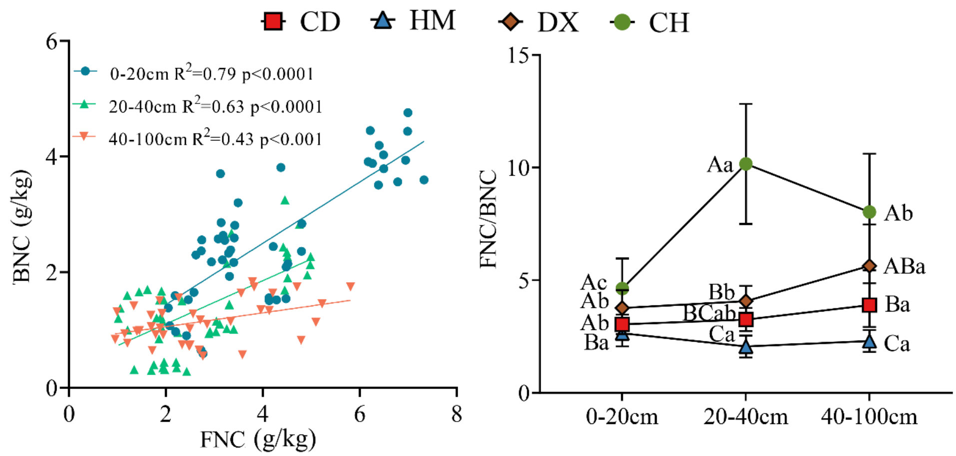

Fig.4 Contribution of FNC, BNC in TNC at 0-100 cm under different grassland types

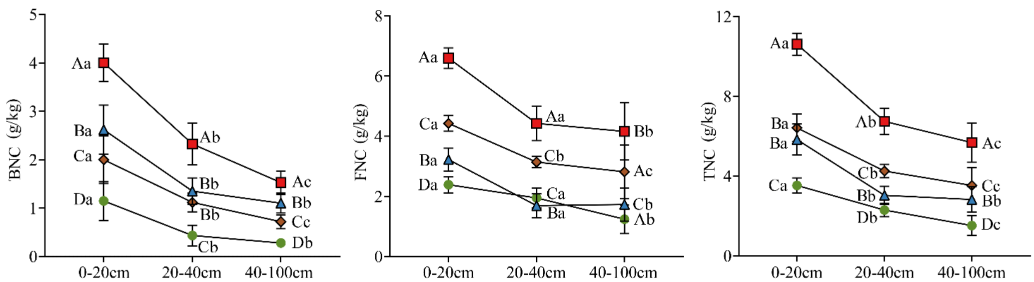

Fig.5 Contents of BNC、BNC and FNC in MAOC at 0-100 cm under different grassland types

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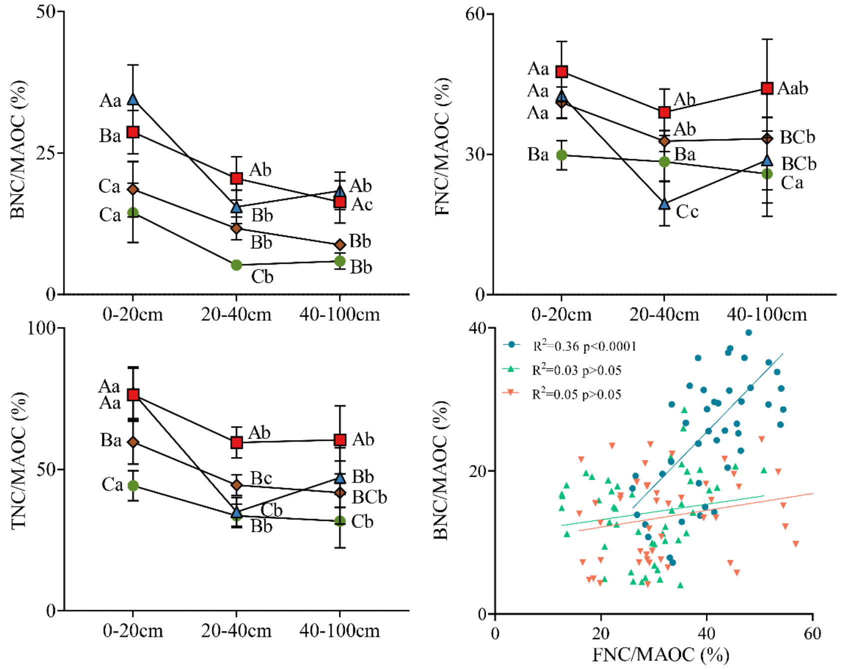

Fig.6 Contribution of BNC,FNC and TNC in MAOC at 0-100 cm soil layer under grassland types

3.3.2 Characterization of changes in the content and proportion of MNC in POC

In the 0-100 cm soil layer, the contents of BNC and TNC in POC exhibited similar trends across various grassland types. Specifically, MS, DS, and SD showed a gradual decrease in POC content with increasing soil depth, whereas TS displayed an initial increase followed by a subsequent decline. In contrast, FNC consistently decreased with soil depth. Within the 0-20 cm layer, the contents of BNC, FNC, and TNC in POC ranged from 0.9–4.1 g/kg, 2.9–9.4 g/kg, and 2.9–9.6 g/kg, respectively. In the deeper 40-100 cm layer, these values decreased to 0.5–3.0 g/kg, 1.0–4.2 g/kg, and 2.1–5.9 g/kg, respectively, with significant differences observed between the 0-20 cm and 40-100 cm layers ($p < 0.05$, Fig. 7). The contributions of BNC to POC were 13%–31%, 9%–24%, and 12%–25% in the 0-20 cm, 20-40 cm, and 40-100 cm layers, respectively (Fig.8). Similarly, FNC contributed 29%–41%, 19%–38%, and 16%–41%, while TNC contributed 42%–72%, 43%–58%, and 39%–54% to POC in the respective layers.

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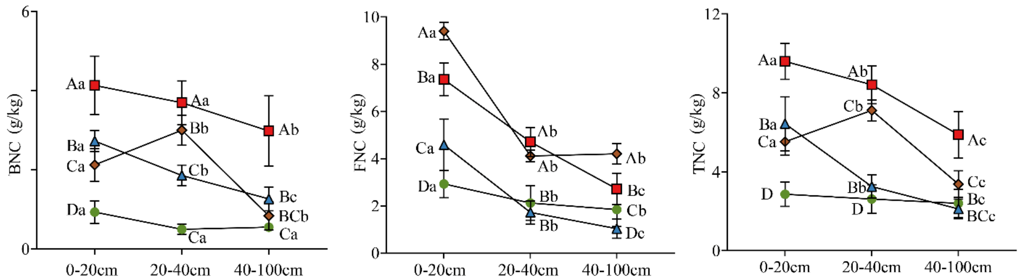

Fig.7 Contents of BNC, FNC and TNC in POC at 0-100 cm under different grassland types

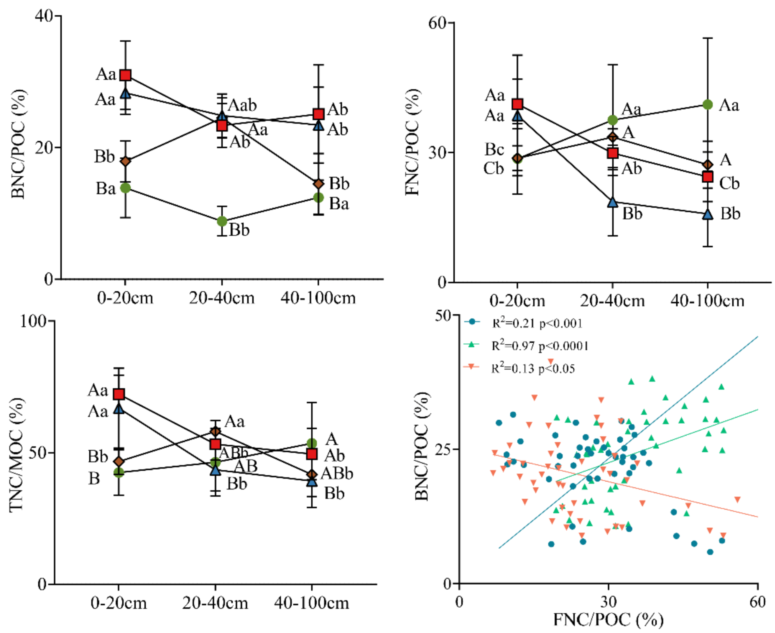

Fig.8 Contribution of BNC, FNC and TNC in POC at 0-100 cm under different grassland types

3.4 Relationship between MNC in SOC fractions and environmental and soil factors

Correlation analysis shows that MAOC, BNC/MAOC and TNC/MAOC, POC have a significant positive correlation with environmental factors (MAP, Elevation), SWC, TN, TC, AK, AN, SOC, TP, AP. There is a significant negative correlation with MAT. There is a negative correlation with BD, but the correlation in some cases is not significant. Among them, MAT and DB are significantly negatively correlated with FNC/POC and TNC/POC respectively; EC has a significant negative correlation with

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FNC/MAOC and FNC/POC; pH has a significant negative correlation with MOC, POC, BNC/MAOC, TNC/MAOC, TNC/POC (Fig. 9).

The soil properties of different grassland types exhibited significant variations between the 0-20 cm soil layer and the deeper 20-40 cm and 40-100 cm layers. The contribution values of PC1 and PC2 were 44.6% and 15.8%, respectively. Consequently, we conducted a random forest model prediction and analysis on the soil properties influencing residue carbon accumulation, stratified into the 0-20 cm and 20-100 cm soil layers (Fig. 9).

The random forest model (Fig. 10) predicted the key factors affecting the accumulation of FNC, BNC, and TNC in different soil layers across various grassland types. Environmental factors such as MAP, Elevation, SOC, SWC, and EC were identified as significant influencers for the accumulation of FNC, BNC, and TNC in both the 0-20 cm and 20-100 cm soil layers. Additionally, AN and AK were important factors in the 0-20 cm layer, while AN and pH played crucial roles in the 20-100 cm layer..

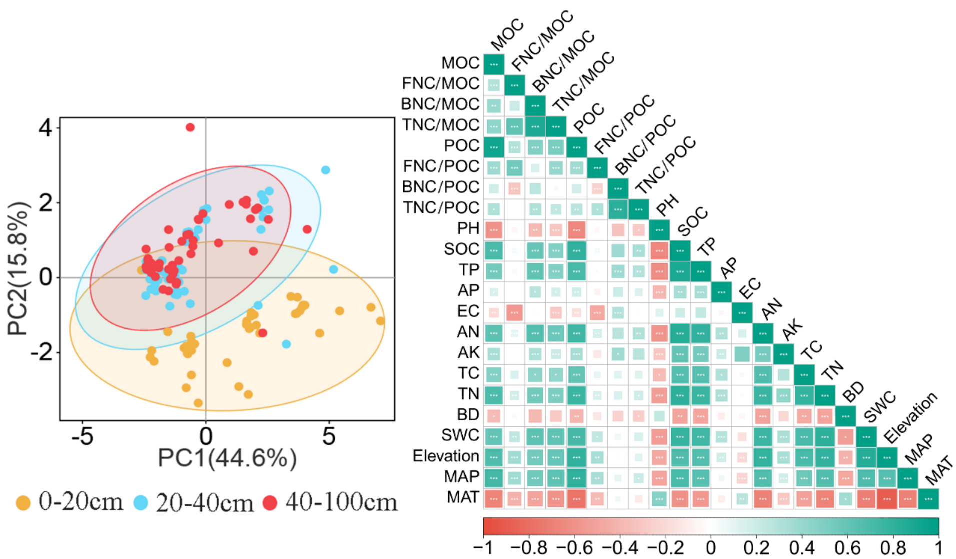

Fig.9 Correlation of MAOC、POC and MNC with environmental factors and PC analysis of soil properties in 0-100cm soil layer under different grassland types

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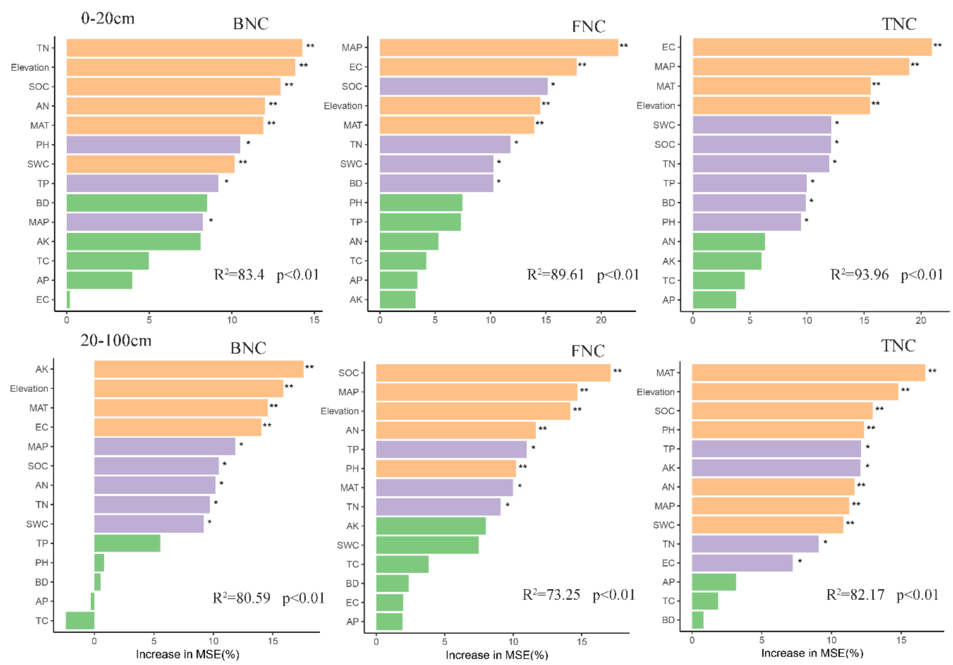

Fig.10 The relative importance of envieonmental and soil factors on MNC

4 Discussion

4.1 Distribution characteristics of SOC fractions contents in different grssland 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(Zhao et al., 2023; Shao et al., 2021). 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). This study, encompassing the entire natural succession sequence in the Ningxia region, included a

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diverse array of plant types. 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. Consequently, this led to elevated levels of SOC and its fractions, particularly MAOC and POC.

In the 0-100 cm soil layer, the average contents of MAOC and POC across different grassland types followed the order: meadow steppe (MS) > typical steppe (TS) > desert steppe (DS) > steppe desert (SD). Significant differences were observed between soil layers and grassland types ($p < 0.05$ Fig2). 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). This study also found that the average POC content across different grassland types was higher than that of MAOC. While MAOC content decreased with soil depth, POC content in MS and TS initially increased and then decreased. Possible reasons for this include: 1) The theoretical upper limit of mineral binding with carbon in the soil, as total organic carbon increases, MAOC may reach saturation, reducing its proportion and thereby increasing the relative proportion of POC. Thus, in grasslands or soil layers with higher total organic carbon, POC content tends to be higher than MAOC(Cotrufo et al., 2019; Zhou et al., 2024; Zhou et al., 2023). 2) Compared to DS and SD, MS and TS experience higher rainfall intensity and a more humid, colder climate(He et al., 2022; Jiang et al., 2024). Increased rainfall enhances vegetation biomass and carbon input into the soil, promoting POC formation and causing MAOC to leach into deeper layers. This explains the observed decrease in MAOC content with depth and the higher POC content in surface layers. 3) Increased plant biomass input has been shown to elevate POC content (Zhou et al., 2024). Given that MS and TS exhibit higher vegetation biomass per unit area compared to SD and DS, and considering that surface POC lacks physical protection, it is more susceptible to microbial decomposition (Liao et al., 2022).

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Therefore, in MS and TS, POC accumulation in the 20-40 cm layer is higher than in the 0-20 cm layer ($p<0.05$, Fig3). 4) Carbon inputs originate from both aboveground and belowground sources. In the 0-20 cm layer, dense surface vegetation, abundant litter, and extensive root systems contribute to increased dead root biomass, favoring POC accumulation(Liu et al., 2018). This also significantly influences soil aggregate formation and internal pore structure, enhancing POC quality(Zhang et al., 2020; Rocci et al., 2021), while having little effect on MAOC and SOC. Additionally, the desert shrub Caragana intermedia typically has a high root-to-shoot ratio(Table 1), with well-developed root systems, which also contributes to higher POC content. In deeper soil layers, where nutrient availability is significantly reduced, the rhizosphere priming effect theory suggests that microorganisms may secrete enzymes and release metabolites such as amino acids to accelerate turnover rates, leading to the utilization of MAOC and POC by microorganisms(Dijkstra et al., 2021; Cui et al., 2023). As a result, carbon fraction content in deeper layers is lower than in surface and subsurface layers, consistent with the findings of Hou and Xue et al.(Hou et al., 2024; Xue et al., 2023).

4.2 Contribution of MNC to different carbon fractions in different grassland types

Microbial necromass is also a key source of stable SOC pools(Min et al., 2024). The proportion of MNC in both MAOC and POC serves as a key indicator of its contribution to these carbon fractions(Hu et al., 2022). Our findings reveal that the average content of TNC in MAOC and POC across different grassland types within the 0-100 cm soil layer follows a distinct order: MS > TS > DS > SD, with significant differences ($p < 0.05$, Fig. 5 and 7). Notably, TNC content generally decreased with soil depth, except in TS, where POC-associated TNC exhibited an initial increase followed by a decline. This result partially aligns with the findings of Wang et al. and Qin et al.(Wang et al., 2021a; Hou et al., 2024), suggesting that the gradient in carbon input quantity and quality from SD to MS may enhance the efficiency of the "microbial

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carbon pump"(Zhu et al., 2020). Furthermore, the 0-20 cm soil layer, characterized by higher nutrient availability and more diverse microbial communities compared to deeper layers(He et al., 2024b), supports elevated microbial metabolic activity and turnover rates, thereby generating greater necromass production(Spohn et al., 2020; Li et al., 2024), This explains the observed decline in TNC within MAOC as soil depth increases. Furthermore, the ratio of FNC to BNC can indirectly reflect the stability of the microbial environment (Fig. 4). A higher ratio indicates slower soil nutrient cycling and lower microbial nutrient utilization, making deeper soil layers less susceptible to external disturbances(Camenzind et al., 2021). Consequently, total organic carbon in soil fractions decreases with soil depth.

We also found that FNC content in MAOC and POC was consistently higher than BNC in the 0-100 cm soil layer (Fig. 5 and 7), and FNC's contribution to MAOC and POC was also consistently higher than BNC (Fig. 6 and 8). This disparity can be attributed to the superior microbial utilization efficiency and higher carbon-to-nitrogen ratios of fungi, which facilitate more efficient necromass production compared to bacteria. Griepentrog et al.(Griepentrog et al., 2014) found that newly formed FNC is 2.6–4.5 times that of BNC. Structural and compositional differences between bacterial and fungal cell walls further influence their stability. Fungal cell walls, predominantly composed of chitin and melanin, degrade more slowly, conferring greater stability(Zhao et al., 2025), Moreover, the thicker cell walls and hyphae of fungi, coupled with their smaller surface area-to-volume ratios, promote the formation of large molecular polymers (Villarino et al., 2021), and enhance physical protection, leading to greater fungal necromass accumulation in soils. In contrast, bacteria are more likely to serve as nutrient sources under substrate-limited or nitrogen-limited conditions, rendering them more susceptible to microbial decomposition(Fernandez et al., 2019; Jia et al., 2017), which explains the lower BNC content. The importance of FNC has been emphasized in farmlands, grasslands and forests globally(Wang et al., 2021a), especially grasslands, where the chemical composition of BNC is more easily decomposed compared to

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FNC(Chen et al., 2021).

In summary, the average content of total microbial necromass carbon in MAOC and POC across different grassland types within the 0-100 cm soil layer was 4.73 and 4.96 g/kg, respectively, with FNC content and contribution dominating. In the 0-20 cm layer, FNC and BNC contributed more significantly to MAOC, while their contributions shifted toward POC in the 20-40 cm and 40-100 cm layers as soil nutrient levels declined (Fig. 6 and 8). These results partially align with those of Sokol et al.(Sokol et al., 2019a; Sokol et al., 2022). In the 0-20 cm layer, MNC primarily enters POC through "extracellular modification," whereas in the 20-100 cm layer, it tends to integrate into the more stable MAOC via "intracellular turnover."

4.3 Factors influencing the accumulation of MNC in different carbon fractions

Biological and abiotic factors were the primary influences on the accumulation of MNC in soil(Chen et al., 2020b; Wang et al., 2021c). In this study, key environmental and soil properties—such asMAP, elevation, SWC, TN, TC, AK, AN, TP, and SOC—were significantly correlated with mineral-associated organic carbon (MAOC), particulate organic carbon (POC), and the ratios of BNC/MAOC and TNC/MAOC ($p < 0.05$, Fig. 8). The relationship between MAP and SWC is particularly critical, as these factors are integral to soil nutrient cycling and energy flow(Mou et al., 2021), water availability enhances microbial growth and directly affects MNC accumulation (Hu et al., 2023). The increase in SWC can stimulate microbial activity, thereby promoting the formation and accumulation of MNC in the soil(Bell et al., 2009), On the other hand, the increase in SWC promotes plant growth and the input of organic matter into the soil, which increases the substrates available for microbial use, thus fostering microbial growth and the accumulation of MNC(Sokol et al., 2019a; Maestre et al., 2015). Compared to SD and DS, the relatively higher SWC in MS and TS to some extent explains the greater contribution of MNC in MS and TS to the organic carbon components. Higher levels of TN, TC, and TP promote microbial biomass growth,

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indirectly facilitating the formation of microbial necromass(Wang et al., 2021a). Moreover, BNC and FNC, as well as microbial functions, are closely related to the dynamics of C pool and N pool. The microbial demand for N can lead to the reuse of MNC by soil microbial communities, and AN can prevents the decomposition of microbial necromass(Wang et al., 2024b). As elevation increases, the decomposition rate of organic matter decreases, resulting in the accumulation of organic carbon at higher altitudes, with MNC emerging as a significant contributor to SOC. POC, being more susceptible to environmental factors, likely reflects its plant-derived origin(Soong et al., 2020). Additionally, the correlation with AP may be explained by phosphorus deficiency in soil promoting the secretion of organic acids by plant roots, which can disrupt MAOC(Ding et al., 2021)， However, this conclusion requires further analysis and verification. Furthermore, MAT, EC, BD, and pH were significantly negatively correlated with MAOC, POC, and some necromass carbon fractions consistent with the findings of Wang et al.(Wang et al., 2024a) and Zhu et al.(Zhu et al., 2024). This may be because temperature affects soil microbial respiration. He et al.(He et al., 2011) found that the accumulation of MNC decreases with increasing temperature, while He et al.(He et al., 2024a) concluded through meta-analysis that both aboveground and belowground plant carbon inputs increase with temperature, influencing soil microbial community structure and ultimately leading to increased MNC accumulation. Soil BD reflects changes in soil structure and aeration; lower BD improves soil permeability, enhancing microbial turnover and carbon use efficiency(Spohn et al., 2016), thereby promoting MNC accumulation. Lower soil pH has been shown to facilitate the accumulation of FNC and BNC(Wang et al., 2021a), consistent with the findings of Liu et al.(Liu et al., 2024)、Cui et al.(Cui et al., 2023) and Gavazov et al.(Gavazov et al., 2022). Additionally, lower pH environments is associated with higher MNC concentrations(Li et al., 2023), likely due to the ability of plant roots to acidify the soil by releasing small molecular organic acids(Fujii, 2014; Chen et al., 2020a), which in turn stimulates MNC accumulation(Wang et al., 2021b; Wang et al., 2021a). Conversely,

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elevated pH limits MNC accumulation in SOC fractions, likely due to slower microbial turnover rates and reduced carbon use efficiency in alkaline soils (Malik et al., 2018; Tao et al., 2023). The influencing factors predicted by the random forest model were validated through correlation analysis.

5 Conclusion

This study provides a comprehensive analysis of the contribution of MNC to SOC fractions and its driving factors across diverse grassland types and soil layers. In the 0-100 cm soil profile, the contents of MAOC and POC exhibited a consistent order across grassland types: MS > TS > SD > DS, with POC content generally higher than MAOC. MNC was dominated by FNC, which contributed more to MAOC and POC accumulation than BNC. A striking divergence was observed in the contribution of MNC to MAOC and POC accumulation between soil layers. In the 0-20 cm layer, FNC and BNC contributed more to MAOC accumulation than POC, while in the 20-100 cm layer, FNC and BNC contributed more to POC accumulation, showing an opposite trend between surface and deeper layers. Furthermore, the key drivers of MNC accumulation exhibited pronounced stratification across soil depths. In the 0-20 cm layer, the most influential factors on MNC accumulation were TN, MAP, and EC, while in the 20-100 cm layer, AK, SOC, and MAT. These findings not only elucidate the complex interplay between environmental factors and soil nitrogen-carbon dynamics but also provide a nuanced understanding of how these interactions vary with soil depth and grassland type, offering valuable implications for ecosystem management under changing environmental conditions.

Code and data availability.

The R code and datasets generated during this current study are available from the corresponding author upon reasonable request.

Author contributions:

Conceived : SGC and YQZ

Writing—original draft: SGC and YQZ

Review & editing: YLC, JBG and LC

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Analyzed the data: LM, MYB and JM

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The authors declare that they have no competing interests.

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