SOIL ECOLOGY LETTERS

SEL RESEARCH ARTICLE
Volume 6 / Issue 3 / 230216 / 2024
https://doi.org/10.1007/s42832-023-0216-0

Fungal residues were more sensitive to nitrogen addition than bacterial residues in a meadow grassland soil

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Received June 29, 2023; Revised October 26, 2023; Accepted November 2, 2023

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ABSTRACT

- Fungi are more sensitive to long-term nitrogen addition than bacteria.
- Long-term nitrogen addition significantly reduces fungal biomass, but promotes the accumulation of fungal residues in soil.
- Long-term nitrogen addition has no significant impact on bacterial biomass and residues.

Nitrogen (N) addition can significantly affect the amount of soil carbon (C) pools through biological routes, and microbial residues are important components of soil carbon pools. However, it remains unclear how N addition affects the accumulation of soil microbial residues in meadow grasslands. This study analyzed the effects of N addition on microbial residues in a meadow grassland soil, and the key factors affecting the accumulation of microbial residues under N addition were analyzed in combination with soil physicochemical properties and microbial community structure. The results showed that N addition significantly changed the structure of the microbial communities and the accumulation of microbial residues, mainly manifested by a significant decrease in fungal biomass and the fungal/bacterial ratio (F/B), but had no significant effect on bacterial or total microbial biomass (PLFAs). N addition significantly increased the accumulation of fungal residues (7.45%), but had no significant effect on the accumulation of bacterial residues or total amino sugar (TAS). We found that fungal residues were more affected by soil environmental factors than bacterial residues. The results of the random forest analysis showed that bacterial biomass under N addition was the most important predictor of soil bacterial residues, whereas total N (TN), pH and F/B were the most important predictors of soil fungal residue. In summary, our results indicate that fungal communities and residues accumulation play important roles in regulating the response of grassland soil C to N addition, further enhancing our understanding of the mechanisms of soil carbon pool to N addition.

Keywords nitrogen addition, microbial residues, microbial community composition, soil organic carbon

1 Introduction

Soil organic carbon (SOC) is a vital component of the terrestrial ecosystem C pool and plays an important role in regulating the concentration of carbon dioxide (CO_2) in the atmosphere and mitigating climate change (Stockmann et al., 2013; Lal et al., 2015; Yang et al., 2020). The accumulation of microbial residues and metabolites plays an important role in stabilizing SOC formation. Recent studies have shown that microbial residue C accounts for 30%−70% of the total SOC (Liang et al., 2017; Wang et al., 2021), indicating that it constitutes an important fraction of the soil carbon pool. At present, increasing atmospheric N deposition impact the SOC cycle in multiple ways, such as changing the aboveground and underground C allocation of plants, affecting litter decomposition and transformation, and promoting or inhibiting microbial C utilization efficiency (Liu et al., 2018; Yang et al., 2022a; Wu et al., 2023). These processes ultimately affect the formation and accumulation of microbial residues. Therefore, exploring the dynamic changes in microbial residues under N addition conditions is of great significance for elucidating the process and stable mechanisms of SOC accumulation under future climate

Cite this: Soil Ecol. Lett., 2024, 6(3): 230216

change.

Amino sugars have been widely used as important biomarkers of microbial residues in the soil to trace microbial-derived C (Glaser et al., 2004; Ding et al., 2023). The assimilation process of microorganisms in the soil can lead to the accumulation of microbial residues and metabolites, ultimately forming microbial-derived C in the soil (Liang et al., 2017; Camenzind et al., 2023). Due to the direct contact of microorganisms with the surface of soil minerals, microbial residues can selectively remain in the soil by being adsorbed onto the mineral surface or encapsulated by aggregates (Murugan et al., 2019; Chen et al., 2020). Studies have shown that microbial residues are key components of SOC accumulation in grassland (Ma et al., 2018). However, in the context of climate change (such as increased N deposition), there is still a limited understanding of the relationship between grassland soil microbial residue accumulation and SOC dynamics. Therefore, it is important to further explore the accumulation of microbial residues and their response to climate change, in order to accurately predict SOC dynamics in grassland.

Microorganisms, as decomposers of complex animal and plant residues, transport microbially-derived organic C to the soil through synthetic metabolic processes. (Liang et al., 2017; Bradford et al., 2021). The N addition can affect microbial activity by changing soil N availability and chemical properties. Microbial growth, reproduction, and community structure affect microbial residue accumulations (Zhou et al., 2017; Wang et al., 2018; Zhang et al., 2018). However, the response of microbial residues to N addition remains unclear. Studies have shown that high N addition reduces the contribution of tropical forest microorganisms to the SOC pool, mainly because of the negative effects of N addition on microorganisms (Zhang et al., 2016).

In contrast, other studies have shown that N addition can promote the accumulation of microbial residue C and is conducive to SOC sequestration (Chen et al., 2020). A regional-scale study showed that the contribution of microbial residue C to SOC was related to soil TN content, F/B and climate conditions (Ma et al., 2021). Therefore, there may still be significant uncertainty in the response of microbial residue C to N addition, possibly due to differences in the soil and climate.

The growth and reproduction of microorganisms are closely related to the accumulation of microbial residues. An increase in microbial growth can promote the accumulation of microbial metabolites and residues, and microbial residues as an available C source can promote the growth and reproduction of microorganisms and the cycling and renewal of SOC (Shao et al., 2021; Bhattacharyya et al., 2022). Many studies have shown that long-term N addition negatively affect soil microorganisms (Treseder 2008; Zhang et al., 2018; Han et al., 2020). In addition, microbial residue C accounted for approximately 50% of the SOC in grassland soil, the changes in microbial residues have an important impact on the composition and stability of grassland SOC. However, the impact of soil microorganisms and soil properties on microbial residues under N addition in grassland soil remains unclear. Therefore, this study aimed to explore the response and main driving processes of soil microbial residues in meadow grasslands to N addition. We hypothesized that: (1) The negative effect of long-term N addition on soil microbial biomass inhibits the accumulation of microbial residues; (2) Owing to the significant enrichment and acidification effects of soil inorganic nitrogen under nitrogen addition, inorganic nitrogen content and soil pH are important environmental factors affecting the accumulation of microbial residues.

2 Materials and methods

2.1 Site description and experimental design

The test site is in Yiminhe, Hulun Buir City, Inner Mongolia Autonomous Region, China. The geographical location is 48°27′–48°35′ N, 119°35′–119°41′ E. The sampling plot located on flat terrain at approximately 765 m above sea level. *Stipa baicalensis* was the group species, and *Leymus chinensis* was the dominant species. The climate in this area belongs to the temperate continental climate, with a large temperature difference between day and night. The annual temperature is −2.4 to 2.2°C, and the average annual precipitation is 350–400 mm. Precipitation is mainly concentrated in June–September, and the annual average frost-free period is approximately 100 days. The soil type is *Luvic-Kastanozem* (United Nations Classification). The basic physical and chemical properties of the 0–20 cm soil before the test were as follows: soil pH 7.07, soil organic carbon 27.92 g kg^{-1}, total nitrogen 1.85 g kg^{-1}, and total phosphorus 0.45 g kg^{-1}.

The test plot was enclosed in June 2010, and a simulated nitrogen deposition experiment was conducted. Considering the increasing trend of global atmospheric N deposition in the future, simulating the response of grassland ecosystems to future N deposition, a random block design was adopted, and five N application levels were set: 0 (CK), 30 (N30), 50 (N50), 100 (N100), and 150 (N150) kg N ha^{-1} yr^{-1}, with four replicates being set for each treatment. The plot area was 8 m × 8 m. Since 2010, the same amount of nitrogen fertilizer has been applied to the sample plots twice a year in mid-June and mid-July, and the nitrogen fertilizer was NH_4NO_3. To avoid volatilization, nitrogen fertilizer was dissolved in an appropriate amount of water and evenly sprayed into the plot with a watering can, with the same volume of water being sprayed in the control plot.

2.2 Soil sample collection

Soil samples were collected in August 2022, and 10 soil cores were randomly collected from each plot and evenly mixed. The diameter of the soil drill was 3.5cm, and the sampling depth was 0–20 cm. After the soil samples were collected, they were stored in an ice bag and returned to the room for treatment. After removing plant roots and other soil intrusions, the soil was sieved through a 2 mm mesh and divided into three parts. One part was dried and ground to determine pH, SOC, TN and amino sugars. Some samples were stored at −20°C for phospholipid fatty acid analysis. The remaining fresh samples were stored at −4°C to determine nitrate N and ammonium N in the soil.

2.3 Soil property analysis

SOC and TN were measured using an elemental analyzer (Vario El cube, German Elemental Company). Before sample determination, 0.1 M hydrochloric acid was used to remove inorganic C. Inorganic N was determined using a flow analyzer after extraction using 2 M potassium chloride with a solution to soil ratio of 10:1. Soil pH was measured using a pH meter (water to soil ratio of 5:1).

2.4 Amino sugar analysis

High-performance liquid chromatography (HPLC-FLD) was used to determine amino sugars in the soil. Approximately 0.5–1.0 g (containing 0.6 MGN) of the soil sample was added to the hydrolysis tube, 10 mL of 6 mol L^{-1} hydrochloric acids was added along the tube wall, and air was replaced with nitrogen for 2 min before sealing. This was hydrolyzed in the oven at 105°C for 8 h. After the hydrolysate had cooled to room temperature, the solution was shaken and allowed to stand for 10 min. The 2 mL supernatant was transferred into a 5 mL centrifuge tube, and 2 mL pure water was added. Then the solution was blow-dried with nitrogen at 40°C. Another 2 mL of water was added, mixed well and centrifuge at 8000 r min^{-1}. The supernatant was passed through a 0.45 μm membrane filter to obtain the test solution. An Agilent liquid chromatograph was used for analysis.

Total amino sugar (TAS) includes glucosamine (GluN), galactosamine (GlaN) and muramic acid (MurN). The ratio of GluN to MurN (GluN/ MurN) is often used to express the changes in the relative contribution of fungi and bacteria to soil microbial residues. MurN in the soil comes only from bacteria, whereas GluN mainly comes from fungi. Therefore, MurN has been identified as a biomarker of bacterial residues, and GluN has been identified as a biomarker of fungal residues. Fungal and bacterial residue C in the soil was estimated according to the following formula provided by Deng and Liang (2022).

$$Fungal\ residue\ C = \left[GluN - \left(2 \times MurN \times \frac{179.2}{251.2}\right)\right] \times 9 \quad (1)$$

$$Bacterial\ residue\ C = MurN \times 45 \quad (2)$$

Here, 179.2 and 251.2 are the molecular weights of GluN and MurN, respectively; and the molar ratio of GluN to MurN in the bacterial cells is assumed to be 2:1. Nine is the conversion coefficient of GluN to fungal residue C, and 45 is the conversion coefficient of MurN to bacterial residue C.

2.5 Phospholipid fatty acid (PLFA) analysis

Phospholipid fatty acid (PLFA) analysis was performed according to (Bossio et al., 1998). A 4 g sample of freeze-dried soil was taken, 23 mL extraction solution was added, the cover was tightly sealed, shaken and mixed, and oscillated for 2 h (oscillating frequency ⩾ 250 r min^{-1}), then centrifuged at 25°C and 7000 r min^{-1} for 10 min, and the supernatant was transferred into 100 mL test tubes. Then, 23 mL of the extract was added, centrifuged, and the supernatant was poured into a test tube. Trichloromethane (10 mL) and citric acid buffers (10 mL) were then added. After being left overnight, the chloroform layer was transferred into a test tube and dried in a 30°C water bath with high-purity nitrogen. The concentrated fatty acids were transferred into the active silica gel column with chloroform, rinsed with chloroform, acetone and methanol respectively, collected in a test tube, mixed with 1 mL methanol toluene (1:1) mixed solution and 1 mL 0.2 mol L^{-1} KOH methanol solution, and placed in a 37°C water bath for 15 min. In succession, 0.3 mL 1 mol L^{-1} acetic acid solution, 2 mL *n*-hexane and 2 mL ultra-pure water were added, and the upper *n*-hexane solution was moved into a vial at low speed for 10 min (repeated oscillation extraction), N_2 was dehydrated and dried to obtain methyl ester fatty acid samples. The sample was dissolved in 200 μL n-hexane and analyzed using a gas chromatograph (Agilent 7890, USA). The phospholipid fatty acids were identified using the MIDI Sherlock system.

Based on current literature, the representative biomarkers of Gram-positive bacteria (G+) are: 20:0 iso, 19:0 iso, 18:0 iso, 17:0 iso, 17:0 anteiso, 16:0 iso, 16:0 anteiso, 15:0 iso, and 15:0 anteiso (Helfrich et al., 2015; Francisco et al., 2016). The representative biomarkers of Gram-negative bacteria (G−) are: 20:1 w9c, 19:1 w9c, 18:1 w7c, 18:1 w5c, 17:1 w8c, 16:1 w7c/16:1 w6c, 16:1 w6c/16:1 w7c, 16:0 2OH, 15:1 w8c, 15:1 w6c, 15:1 w5c, 14:1 w5c, 12:0 2OH, 10:0 2OH 18: 1 w9c, representing fungal biomarkers (Welc et al., 2012). 18:1 w7c 11-methyl, 17:0 10-methyl, and 16:0 10-methyl are actinomycete biomarkers (Chowdhury and Dick, 2012; Welc et al., 2012). Total bacteria was represented by the sum of G+ and G− bacteria, and the total PLFAs was the sum of all PLFAs.

2.6 Statistical analysis

Before data analysis, a normality test was conducted, and the test results showed that all data had a normal distribution. Duncan's univariate analysis of variance was used to test the changes in soil chemical properties, microbial biomass and amino sugar concentration under different N addition treatments. Correlation analysis between bacterial and fungal residues and soil chemical properties and microbial compositions was conducted to further analyze the main driving factors of the changes in microbial residues under N addition. Combined with random forest prediction, the importance of the overall environmental factors was ranked, and the most important environmental factors affecting bacterial and fungal residues were screened out (Ge et al., 2022). Finally, based on the screening results of the driving factors, a structural equation model was established to analyze the main mechanisms driving the changes in fungal residues under N addition. The possible interaction pathways between environmental factors including TN, pH, F/B and fungal residues, are based on experience and background knowledge. The Chi-square test, degree of freedom, probability level, goodness of fit index, RMS of the standard residual and approximate RMS error were used to evaluate the overall model (Fox, 2006; Eisenhauer et al., 2015).

3 Results

3.1 Soil chemical properties and PLFAs

The analysis of variance results showed that the addition of N had no significant effect on SOC and TN, however, compared to the CK, N100 and N150 treatments, soil C/N was significantly reduced ($P < 0.05$; Table 1). The addition of N significantly reduced soil pH and inorganic N content ($P < 0.05$; Table 1). The addition of N did not significantly affect the biomass of soil bacteria and PLFAs, but significantly reduced the biomass of soil fungi, actinomycetes and F/B ($P < 0.05$; Table 1).

3.2 Soil amino sugar

N addition had no significant effects on soil TAS and MurN, but significantly affected GalN and GluN (Table 2). Compared with CK, the N100 treatment significantly decreased soil GalN (10.51%), the N150 treatment significantly increased soil GluN (7.45%), and different N levels increased soil GluN/MurN compared with CK ($P < 0.05$; Table 2).

3.3 Contribution of microbial residues to SOC

N addition had no significant effect on total microbial residue C and bacterial residue C, but significantly affected fungal residue C (Fig. 1). Compared with the CK, the N150 treatment significantly increased soil fungal residue C (8.61%), and different N application levels significantly increased fungal residue C/ bacterial residue C ($P < 0.05$; Fig. 1B, D). Under different N application levels, microbial residue C accounted for 64.4%–68.7% of SOC, and fungal residue C accounted for 41.32%–45.35% of SOC (Fig. 2A, B). However, there were no significant changes in the contributions of fungi,

Table 1 Soil chemical properties and microbial community composition under different nitrogen application levels. The value is the mean ± standard error (n = 4). Duncan's test was used to analyze the differences between nitrogen application levels. Different lowercase letters in a row represent significant differences among nitrogen treatments.

Treatment	CK	N30	N50	N100	N150
SOC (g kg^{-1})	31.79+0.19a	32.01+1.28a	31.34+0.82a	30.00+1.50a	31.53+1.18a
TN (g kg^{-1})	2.88+0.01a	2.91+0.09a	2.88+0.05a	2.82+0.14a	2.98+0.09a
C/N	11.02+0.08a	10.96+0.12a	10.85+0.09ab	10.61+0.04bc	10.54+0.06c
pH	6.93±0.06a	6.48±0.11b	6.40±0.03b	6.41±0.09b	5.66±0.08c
NH_4^+-N (mg kg^{-1})	0.51±0.05c	2.28±0.59b	2.57±0.25b	2.80±0.37b	5.44±0.81a
NO_3^--N (mg kg^{-1})	2.72±0.32c	4.91±1.03c	4.82±0.56c	9.03±1.11b	13.42±1.91a
PLFAs (nmol g^{-1})	143.23+2.07a	130.81+8.42a	137.80+9.40a	145.36+6.41a	126.22+3.58a
Bacteria (nmol g^{-1})	81.38+0.24a	73.80+5.30a	83.44+5.97a	87.47+4.08a	75.39+2.52a
Fungi (nmol g^{-1})	13.37+0.17a	11.37+0.76ab	11.47+0.91ab	10.78+0.62bc	9.13+0.47c
Actino. (nmol g^{-1})	15.77+0.44a	11.53+0.83bc	11.85+0.66bc	13.41+0.67b	10.71+0.29c
F/B	0.16+0.00a	0.16+0.00a	0.14+0.01b	0.12+0.00c	0.12+0.00c
G+ (nmol g^{-1})	27.07+0.98a	25.29+1.75a	28.00+1.64a	29.65+1.41a	25.45+0.83a
G− (nmol g^{-1})	28.51+1.12a	25.71+1.77a	30.00+2.39a	30.89+1.30a	26.03+0.85a

Note: Actino., actinomycete; G−, Gram-negative bacteria; G+, Gram-positive bacteria; F/B, the ratio of fungi to bacteria.

Table 2 Total amino sugar (TAS), muramic acid (MurN), galactosamin (GalN), glucosamine (GluN) and galactosamin/muramic acid under different nitrogen application levels.

Treatment	TASs (mg kg^{-1})	MurN (mg kg^{-1})	GalN (mg kg^{-1})	GluN (mg kg^{-1})	GluN/MurN
CK	2871+39.8a	163.1+2.6a	1016.2+13.8a	1692+26.3b	10.38+0.19b
N30	2846+83.2a	156.5+4.6a	978.2+27.6ab	1711+63.0ab	10.94+0.25a
N50	2856+45.0a	156.9+2.8a	965.3+36.8ab	1734+11.6ab	11.05+0.14a
N100	2767+46.2a	153.6+2.2a	909.4+21.7b	1704+23.1ab	11.10+0.03a
N150	2944+48.0a	163.2+2.6a	964.7+11.6ab	1818+34.5a	11.14+0.05a

The value is the mean ± standard error (n = 4). Duncan's test was used to analyze the differences between nitrogen application levels. Different lowercase letters in a row represent significant differences among nitrogen treatments.

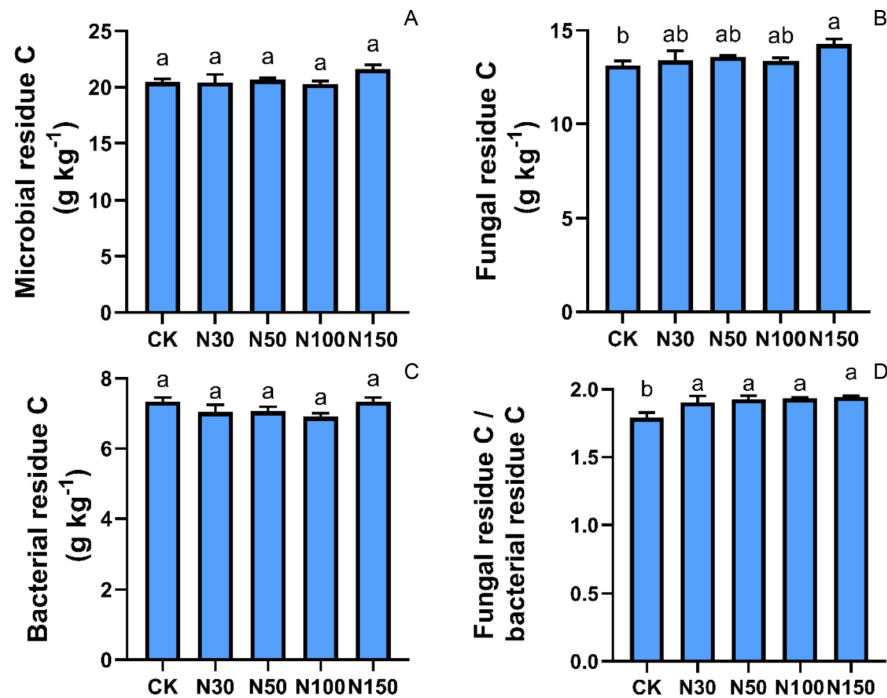

Fig. 1 Soil microbial residue carbon (A), fungal residue carbon (B), bacterial residue carbon (C), and fungal residue carbon/ bacterial residue carbon (D) under different nitrogen application levels. The value is the mean ± standard error (n = 4). Different lowercase letters represent significant differences among nitrogen treatments. CK, no fertilization; N30, 30 kg N ha^{-1} yr^{-1}; N50, 50 kg N ha^{-1} yr^{-1}; N100, 100 kg N ha^{-1} yr^{-1}; N150, 150 kg N ha^{-1} yr $^{-1}$.

bacteria or total microbial residue C to SOC under different N levels (P > 0.05; Fig. 2).

3.4 The main driving factors and pathways affecting microbial residues

The correlation analysis showed that soil fungal residues under N addition were more affected by soil physical and chemical factors than by bacterial residues (Fig. 3). The prediction results of the random forest showed that the accumulation of soil bacterial residues under N addition was mainly driven by bacterial biomass (Fig. 4B), whereas the accumulation of soil fungal residues was mainly driven by soil TN, pH and F/B (Fig. 4B).

The results of the structural equation model showed that TN had a significant and positive effect on fungal residue accumulation, whereas soil pH had a significant and negative effect (P < 0.05; Fig. 5). Under N addition, the accumulation of fungal residues was mainly affected by soil pH (P < 0.05; Fig. 5).

4 Discussion

4.1 Effect of N addition on soil microbial community composition

This study showed that the addition of N did not significantly affect soil PLFAs and bacterial biomass, but significantly reduced soil fungal and actinomycete biomass (Table 1). This was similar to the results of localized experiments on typical grasslands, where N addition significantly reduced

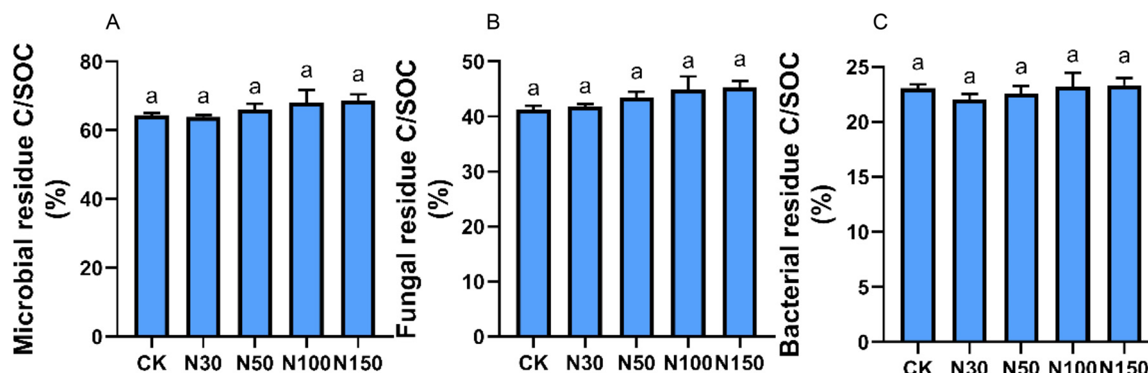

Fig. 2 The contribution of soil microbial residues to SOC under different nitrogen application levels. The value is the mean ± standard error (n = 4). Different lowercase letters represent significant differences among nitrogen treatments. CK, no fertilization; N30, 30 kg N ha^{-1} yr^{-1}; N50, 50 kg N ha^{-1} yr^{-1}; N100, 100 kg N ha^{-1} yr^{-1}; N150, 150 kg N ha^{-1} yr^{-1}.

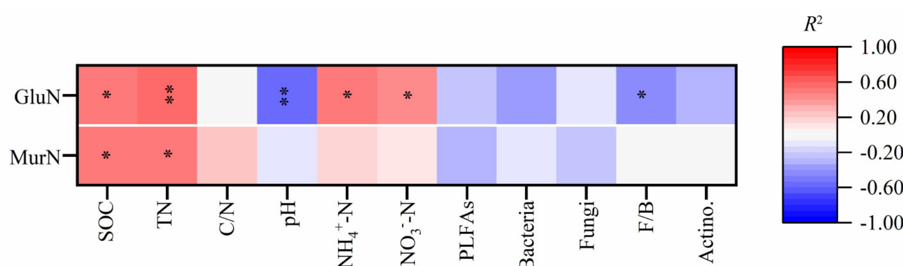

Fig. 3 Relationship between soil amino sugar, soil chemical properties and microorganisms. Actino., actinomycete; F/B, the ratio of fungi to bacteria.

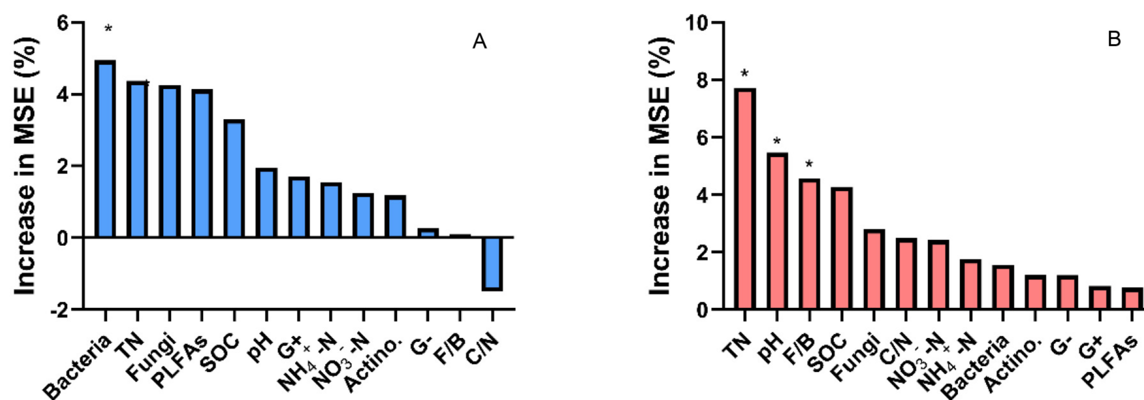

Fig. 4 Importance ranking of environmental factors affecting bacterial residues (A) and fungal residues (B). The random forest algorithm calculates each prediction variable's MSE (root mean square error). The percentage of MSE increase in the variable is used to characterize the importance of factor prediction, and a higher MSE% value means a more important prediction factor. * P < 0.05, * * P < 0.01. Actino., actinomycete; G− , Gram-negative bacteria; G+ , Gram-positive bacteria; F/B, the ratio of fungi to bacteria.

the relative abundance of fungi and F/B (Wang et al., 2020). This may be related to microbial nutrient survival strategies. Studies have shown that the enrichment of N leads to soil microorganisms toward eutrophication, and the addition of N promotes the abundance of eutrophic bacteria and reduces the abundance of oligotrophic microorganisms such as fungi and actinomycetes (Yang et al., 2017; Wang et al., 2020; He et al., 2022). In addition, this may be related to the changes in soil environmental factors. N addition not only negatively affects microorganisms by reducing soil pH, but also regulates nutrient acquisition strategies of soil microorganisms by changing the stoichiometric characteristics of carbon and nitrogen, ultimately impacting microbial activity (Chen et al., 2019; Yuan et al., 2020; Li et al., 2021b). The findings of a study conducted on typical grassland revealed that the main factor inhibiting microbial activity under high N addition was the increase in carbon restriction, followed by a decrease in pH (Wang et al., 2020; Ning et al., 2021). This significant decrease in fungal biomass may be related to changes in specific populations of the fungal communities.

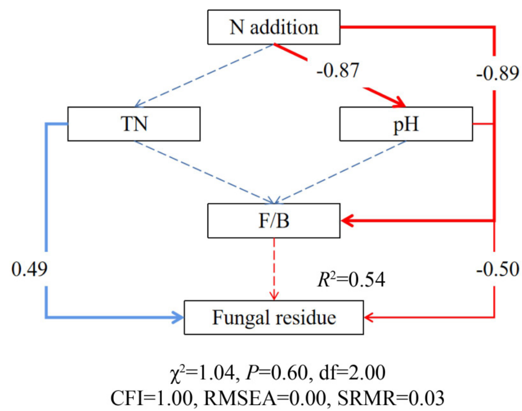

Fig. 5 The structural equation model shows that TN, pH, and F/B regulate fungal residue pathways under nitrogen addition. The blue and red arrows represent positive and negative relationships, respectively. The line's thickness represents the relationship's size, and the numbers adjacent to the arrows represent the normalized path coefficients. The solid line indicates a significant relationship at P < 0.05, while the dotted line indicates non-significant relationships.

Some studies have shown that with an increase in N application, the proportion of the Basidiomycota community in the soil decreases, and the proportion of the Ascomycota community increases (Xiao et al., 2011; Zhang and Han, 2012). N enrichment can select fungal species that are more tolerant to N (Moore et al., 2021; Zhou et al., 2021; Andrew et al., 2022).

The addition of N reduced the biomass of soil actinomycetes, which is consistent with the results of a global scale meta-analysis (Wang et al., 2018). Actinomycetes are saprophytic bacteria that play an important role in driving the SOC turnover (Treseder, 2008; Zhang et al., 2018). Soil pH is an important limiting factor for the growth of actinomycetes (Shahbaz et al., 2023). The acid resistance of actinomycetes is weak and the addition of N may lead to a decrease in soil pH, which may inhibit the growth and reproduction of actinomycetes. This may have an important impact on key ecosystem C cycle processes, such as litter decomposition and SOC mineralization. To date, most studies have focused only on the overall response of bacterial communities to environmental changes, neglecting the important role of actinomycetes in driving SOC turnover. Exploring the response of soil actinomycetes to N addition is important for understanding SOC dynamics.

4.2 The effect of N addition on soil microbial residues

Microbial residues are important components of SOC pools. Our research shows that microbial residue C accounted for 64.4%–68.7% of SOC, of which fungal source C accounted for 41.32%–45.35% of SOC (Fig. 2 A, B), which is similar to the research results of Ma et al. (2018). Soil microorganisms convert organic matter in plant residue into biomass through assimilation and produce soil organic matter as microbial residues (Klink et al., 2022; Bao et al., 2023). However, our results showed that N addition did not significantly change the contribution of microbial residue C to SOC (Fig. 2).The insignificant changes in the biomass of living microorganisms may be an important reason for the non-significant changes in the contribution of microbial residue C (Table 1). It is also possibe that the degradation and synthesis of microbial residues reach a balance.

This study showed that N addition had no significant effect on soil TAS and bacterial residues, but significantly increased soil fungal and fungal/bacterial residues (Table 2). An analysis of the impact of N deposition on microbial residues at seven forest sites in eastern China corroborates this finding, indicating that the response of fungal and bacterial residue C to N addition is inconsistent (Ma et al., 2021). This may be the key driving factors are different for fungal and bacterial residues C. Our results showed that bacterial residues were mainly affected by bacterial biomass (Fig. 4 A), therefore, the response of bacterial biomass to N addition was not significant, resulting in no significant changes in microbial residues. However, fungal residues were more affected by chemical factors (Figs. 3 and 4 B), and SEM results showed that, under N addition, the accumulation of fungal residues was mainly affected by reducing pH (Fig. 5). Numerous studies have shown that N addition has a significant and negative impact on microbial biomass and microbial biomass carbon, and the acidification effect induced by N input plays a dominant role (Zhang et al., 2018; Wang et al., 2022b; Das et al., 2023). The decrease in living microbial biomass caused by pH decrease may promote the accumulation of microbial residues. Generally speaking, there is a positive correlation between soil microbial biomass and the accumulation of microbial residues (Li et al., 2019, 2021a; Yang et al., 2022b). However, no similar pattern was observed in this study. There are two possible reasons for this finding. The accumulation of microbial residues depends on the balance between synthetic microbial metabolites and microbial degradation. A significant decrease in fungal and actinomycete biomass may lead to a decrease in microbial decomposition ability. Studies have shown that N addition reduces microbial biomass and, extracellular enzyme activity, thus reducing the decomposition rate of litter and SOC (Manning et al., 2008; Yang et al., 2022c; Zheng et al., 2023). Although the addition of N leads to a decrease in the overall fungal biomass, the changes in different populations within the fungal community can also affect the accumulation and degradation of microbial residue C. Long-term N addition may reduce the fungal population associated with organic matter decomposition, increasing the growth of N tolerant and copiotrophic species, which typically have a lower C decomposition ability, which

may promote the accumulation of microbial residual carbon in the soil (van Diepen et al., 2013; Han et al., 2020; Moore et al., 2021). Secondly, soil enzyme activity is crucial for the decomposition and formation of soil microbial residues (Burns et al., 2013). Studies have shown that microbial demand for N in N-limited ecosystems promotes enzyme activity to decompose microbial residues and obtain sufficient N nutrients (Wang et al., 2022a). When N availability increases, the cost of microbial N acquisition decreases, thereby weakening the acquisition of N from microbial residues and soil organic matter (Chen et al., 2018; Li et al., 2021c), which is beneficial for accumulating microbial residues. Therefore, in the future, it will be necessary to further clarify the microbial-mediated processes of SOC changes under N addition.

5 Conclusion

Soil microbial residues in the meadow grassland accounted for more than 50% of SOC. Long-term N addition significantly affects the accumulation of soil microbial residues by changing soil chemical properties and microbial community structure. Our study found neither bacterial biomass nor bacterial residues responded significantly to N addition. In contrast, N addition significantly increased the accumulation of fungal residues in the soil and reduced F/B. The decrease in soil pH was the main factor driving the accumulation of fungal residues under N addition conditions. Our research indicated a differential response of fungal and bacterial residue accumulation in meadow grassland soil to N addition. Our study provides an important theoretical supplement for predicting the C dynamics of meadow grassland soils with an increase of N input.

Acknowledgments

This study was funded by the High-End Foreign Expert Introduction Program of the Natural Science Foundation of the Inner Mongolia Autonomous Region.

Conflict of interest

The authors declare that they have no competing interest.

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