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Stoichiometry Influences on Microbial Necromass Carbon Contributions to Soil Organic Carbon in A Chinese Fir Plantation Under a 7-Year Litter Manipulation

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ABSTRACT

Microbial necromass is microbial assimilation metabolites that are important for soil organic carbon (SOC) accumulation. Stoichiometric ratios reflect the relative abundances of various elements in forest ecosystems, and the proportions of carbon (C), nitrogen (N), and phosphorus (P) in an organism affect its metabolic capacity, biomass, and final necromass yield. However, the mechanism by which soil stoichiometry regulates microbial necromass is unclear. The effect of soil stoichiometry on microbial necromass was investigated using a 7-year field manipulation experiment in a subtropical Chinese fir (*Cunninghamia lanceolata*) plantation with aboveground litter addition and removal. We measured microbial necromass C contributions to SOC (MNC/SOC) and the stoichiometric ratios of total and available nutrients, microbial biomass, and enzyme activities. MNC/SOC at the 0–10 cm soil layer was significantly increased by litter addition rather than litter removal. The stoichiometric ratios of available nutrients (31%) and enzymatic activities (15%) explained the variations in the MNC/SOC. The fungal MNC/SOC was positively correlated with the C/N ratios of microbial biomass and enzyme activities, whereas the bacterial MNC/SOC was only correlated with the C/P and N/P ratios of available nutrients and enzyme activities, which indicated that the relationship between fungal and bacterial MNC/SOC and stoichiometry ratios was not exactly the same. However, the MNC/SOC and stoichiometric ratios did not have significant correlations below the 20 cm soil layers, which indicated that the relationship was decoupled with increasing soil depth. Our results provide a holistic understanding of soil stoichiometry to elucidate the mechanisms of MNC accumulation and contribution along the soil profile in a subtropical forest, providing new insights and research directions for enhancing C sequestration and improving soil quality.

1 | Introduction

Microbial necromass carbon (MNC) is a crucial part of the soil organic carbon (SOC) in the form of necromass after the death of microorganisms, and the role in the soil carbon (C) cycle should not be neglected (Crowther et al. 2019). Previous research had found MNC contributions to SOC (MNC/SOC) can be as high as 30%–60% (Liang et al. 2019; Peng et al. 2024; Tian et al. 2024). Ecological stoichiometry (C: nitrogen (N): phosphorus (P) ratio) reveals the biogeochemical cycling of elements regarding ecosystem energy and elemental balance (Sterner and Elser 2002), which is the basis for the accumulation and decomposition of SOC in ecosystems (Delgado-Baquerizo et al. 2013). However, the effect of ecological stoichiometry on MNC accumulation and MNC/SOC remains unclear.

Nutrient and microbial stoichiometries may directly or indirectly alter the MNC/SOC. Research has found that the total nutrient stoichiometric ratios were the key mechanism to mediate soil C sequestration (Mou et al. 2023). However, relative to the total nutrient stoichiometry, the available nutrient stoichiometric ratios respond to the environment more sensitively and directly, reflecting the actual utilization of nutrients by microbes and plants (Gao et al. 2014). It seems that available nutrient stoichiometry may predict MNC/SOC better than total nutrient stoichiometry, which has not yet been tested. In addition, the role of microbial biomass and enzyme stoichiometric ratios could not be ignored when discussing the change of MNC/SOC (Liao et al. 2024). This is because an increase in microbial biomass generally means that more microorganisms are available for the formation of MNC, while high enzyme activity promotes the breakdown of organic matter or MNC, which in turn increases or decreases the turnover rate and accumulation of MNC (Liang et al. 2017; Wang et al. 2021a; Mou et al. 2023). Their stoichiometric ratios are regulated by nutrient stoichiometry (Wang et al. 2021b). However, how the ratios of nutrients and microbial stoichiometries affect the MNC/SOC remains argely unexplored. Therefore, we should differentiate from the functional perspective of stoichiometric ratios, such as total nutrients (C and nutrient pool), available nutrients (readily utilizable by microorganisms), microbial biomass (microbial growth), and enzyme activities (microbial decomposition capacity). Their comparative study is helpful for understanding the changes in MNC/SOC from a holistic perspective of soil stoichiometry.

Litter management is a common intensive management method that may disrupt the original soil balance by changing exogenous C and nutrient inputs, which can further change the soil nutrient and microbial stoichiometric balance, and the impacts may vary with soil depth (Wang et al. 2021b). For example, a change in exogenous C input considerably affects the microbial acquisition strategies and methods for nutrients through changing the soil C or other nutrient distributions (Wang et al. 2021b), which may consequently regulate the proportion of necromass generated during microbial turnover (Heuck et al. 2015). The responses of fungi and bacteria may not be identical (Liu et al. 2023). The positive effect of fungal biomass is greater on litter addition than that of bacterial biomass (Feng et al. 2022). Compared with bacterial cell walls, fungal cell walls contain chitin and melanin, and a higher proportion of melanin is relatively difficult to degrade (Li et al. 2022a). Thus, fungal cell walls may decompose more slowly and contribute more to MNC than bacterial MNC (Liang et al. 2019). Alterations in nutrients and microbes induced by changes in litter input occur mainly in the topsoil, with relatively weak effects on the subsoil (Liu et al. 2021). However, few studies have linked stoichiometry to microbial necromass driven by litter manipulation in forest ecosystems, and the internal coupling mechanisms along a profile remain unclear.

Chinese fir (*Cunninghamia lanceolata*) is a unique artificial fast-growing forest species in China, widely distributed in southern China (Chen et al. 2015). The 9th national forest resources inventory showed that Chinese fir plantation covers an area of 9.8667 million hectares in China, with a stock volume of 755 million m^3, accounting for 1/4 and 1/3 of the total area and total stock volume of the country's artificial forest, ranking 1. Ecological stoichiometry is a key factor affecting necromass accumulation by regulating the growth and metabolism of microorganisms (Liang et al. 2020; Mou et al. 2023). However, few studies have established the intrinsic relationship between stoichiometry and MNC/SOC along a soil profile. Therefore, a 7-year aboveground litter manipulation experiment with doubled litter addition and total litter removal in a subtropical Chinese fir (*Cunninghamia lanceolata*) plantation was used to link the nutrient and microbial stoichiometry and MNC/SOC along a 60 cm soil profile. We hypothesized that: (1) the bacterial, fungal, and total MNC/SOC would be enhanced by double litter addition, while litter removal would decrease them; (2) the stoichiometry of available nutrients and enzyme activities, rather than the stoichiometry of total nutrients and microbial biomass, can reflect the change in MNC/SOC because available nutrients and enzymes are more responsive; and (3) the relationship between MNC/SOC and the stoichiometric ratios decouples as the soil profile deepens because nutrient and microbial availability control biomass turnover in surface soils, whereas aggregate protection mechanisms dominate the mineral stabilization of microbial residues in subsoils. The main objectives of this study were (a) to explore the influence of litter management on the MNC/SOC along the soil profile in subtropical Chinese fir plantations; and (b) what the intrinsic connection is between the stoichiometric ratio of nutrients and microorganisms and MNC/SOC.

2 | Materials and Methods

2.1 | Experimental Setup

This study was conducted at the Shixi forest farm in Ji'an City, Jiangxi Province, southern China (115.05° E, 26.73° N). The average annual temperature in the study area is 18°C, precipitation is 1616 mm, and average annual sunshine is 1408 h $year^{-1}$. The soil type is Ultisol, and the soil-forming matrix is mostly red sandstone, gravel, mudstone, or river alluvial (Liu et al. 2021).

In January 2013, the experiment was conducted in a completely randomized block design: each block consisted of three treatments, each treatment was replicated three times, with nine plots (15 m × 15 m). Each plot was separated by over 10 m. The three treatments were as follows: control, litter removal, and litter addition. The control treatment did not carry out any artificial operation, keeping it in a natural state. The

litter removal treatment: all aboveground litter was totally removed. The litter addition treatment: the litter from the litter removal treatment was transferred to this plot, which can form a double addition treatment. Because Chinese fir leaves fall at different times, and the amount of litter per month is small, maintenance is required every month to ensure the effectiveness of each treatment. In its natural state, the amount of aboveground litter in plantations is approximately 1×10^3 kg ha^{-1} year^{-1} (Liu et al. 2021).

2.2 | Soil Sampling and Processing

Soil samples from the 0–10, 10–20, 20–40, and 40–60 cm layers were collected after 7 years. Five random soil cores (5 cm in diameter) were collected using a soil auger at each plot and were mixed into one sample. A total of 36 soil samples were collected (three treatments × three replicates × four soil layers) and divided into two parts after pre-treatment. The part of the subsample was refrigerated for the determination of the microbiological index (microbial biomass and enzyme activities) within a week, and the other was used to measure the chemistry index (total and available nutrient) and amino sugars (ASs).

2.3 | Soil Chemistry Properties and Nutrient Stoichiometry

Soil pH and dissolved organic C (DOC) were determined using a pH meter and a total organic C (TOC) (multiN/C 2100, Analytik Jena, Germany) analyzer, respectively. SOC was determined using the H_2SO_4-$K_2Cr_2O_7$ heating method. Available N (NH_4^+ and NO_3^-) was extracted by KCl solution, available P was extracted by NH_4F, and total N and P were digested with H_2SO_4-H_2O_2; these extracted or digested solutions were measured using an automatic intermittent analyzer (Smartchem, AMS Alliance, Italy).

2.4 | Amino Sugars

The content of ASs was determined by gas chromatography (Shimadzu Gas Chromatograph GC-2014) equipped with a capillary column for meteorological chromatography (30 m × 0.25 mm ID × 0.25 μm) (Zhang and Amelung 1996). Briefly, 0.5 g of the sample was hydrolyzed with 6 M HCl, which was mixed with 100 μL of myo-inositol for a rotary evaporation and then filtered. After the pH was adjusted to 6.6–6.8, the solution was evaporated for a second time. The filtrate was dried using N_2 gas, and 100 μL *N*-methylglucamine was added. The mixture is freeze-dried and purified, and then subjected to cyanide and acetylation reactions. The resulting filtrate is extracted with dichloromethane to remove excess acetic acid, and the derivative is finally dissolved. Glucosamine (GluN), galactosamine (GalN), and muramic acid (MurN) were obtained.

MNC was calculated as (Appuhn et al. 2006; Liang et al. 2019):

$$\begin{aligned} &Fungal\ MNC\ \left(ug\ g^{-1}\ soil\right) \\ &= \left(GluN\ \left(ug\ g^{-1}\right) - 2 \times MurN\ \left(ug\ g^{-1}\right) \times \left(\frac{179.2}{251.2}\right)\right) \times 9 \end{aligned} \quad (1)$$

$$Bacterial\ MNC\ \left(ug\ g^{-1}\ soil\right) = MurN\left(ug\ g^{-1}\right) \times 45 \quad (2)$$

$$Total\ MNC\ \left(ug\ g^{-1}\ soil\right) = Fungal\ MNC + Bacterial\ MNC \quad (3)$$

where 179.2 and 251.2 are the molecular weights of GluN and MurN, respectively. The 9 and 45 are the conversion values of fungal MNC and bacterial MNC, respectively (Liang et al. 2019).

2.5 | Soil Microbial Biomass and Enzyme Activities

Soil microbial biomass C, N, and P contents were determined by chloroform fumigation and leaching (Vance et al. 1987). The conversion coefficients of the microbial biomass C, N, and P were 0.38, 0.54, and 0.40, respectively (Vance et al. 1987). The soil extracellular enzyme activities (β-1,4-glucosidase, BG; β-1,4-N-acetyl-glucosaminnidase, NAG; acid phosphatase, AP) were determined by the enzyme plate method (Saiya-Cork et al. 2002). The stoichiometric ratios of the microbial biomass and enzyme activities were calculated (Sinsabaugh et al. 2008).

2.6 | Statistical Analysis

Data were statistically analyzed by using 19.0 software of the IBM SPSS Statistics. Differences in soil chemical properties, microbial biomass, enzyme activities, MNC, MNC/SOC, and stoichiometric ratios of nutrients, microbial biomass, and enzyme activities among litter manipulation treatments and different soil layers were analyzed by two-way ANOVA and post hoc multiple comparisons (Tukey). In addition, Pearson's correlation and the Mantel test were used to examine the potential relationships between MNC/SOC and the stoichiometric ratios of nutrients and microorganisms. By using redundancy analysis (RDA) and variance partitioning analysis (VPA), the key drivers influencing the changes in MNC/SOC and their relative contributions can be identified and quantified. The bar chart was produced using the Origin 2021 software package (Origin Lab, Hampton, UAS, USA), the RDA plot was drawn using Canoco 5, and the varpart ("vegan" package) and mutate ("ggcor" package) functions were performed using VPA and Mantel tests analysis in R software (version 4.3.1).

3 | Results

3.1 | The Effect of Litter Treatments on Soil Nutrients and Microbial Stoichiometric Ratios

The stoichiometric ratios of all the nutrients and microbial parameters were affected by litter manipulation and soil depth (Table S1). Litter removal significantly reduced the ratios of total and available C/P in the 10–20 cm layer, the ratio of available N/P above the 40 cm soil layers, and the ratio of enzymatic N/P in the 40–60 cm layer, while it significantly increased the ratios of microbial biomass C/P, microbial biomass N/P,

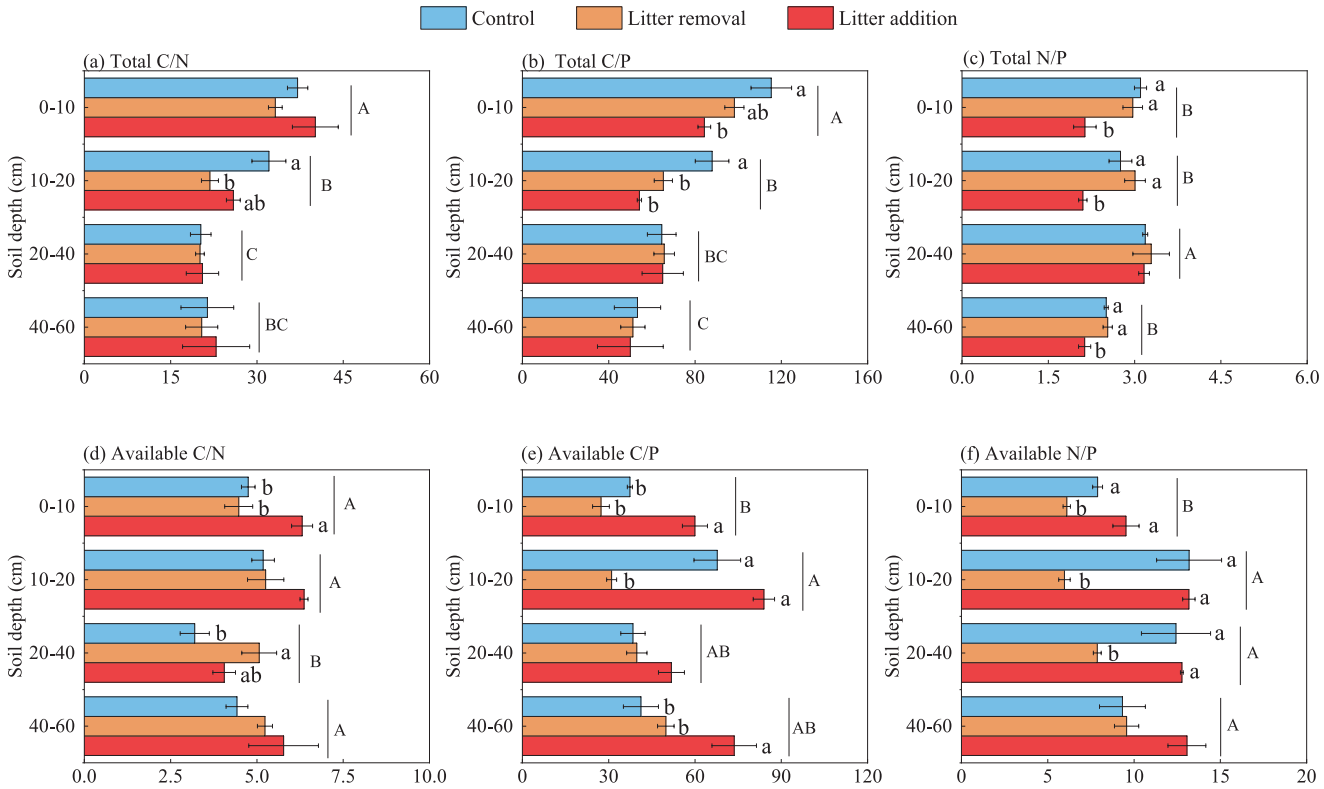

FIGURE 1 | Effects of litter addition and removal on stoichiometric ratios of total and available nutrients along a 60-cm soil profile in a Chinese fir plantation. Values are means ± standard errors ($n = 3$). Different lowercase letters indicate significant differences among three litter manipulation treatments within the same soil depth and different uppercase letters indicate significant differences among four soil depths for the averages of three litter manipulation treatments ($p < 0.05$). [Colour figure can be viewed at wileyonlinelibrary.com]

and enzymatic C/N in the 0–10 or 40–60 cm layer ($p < 0.05$; Figures 1 and 2). Litter addition significantly decreased the ratios of total C/P and total N/P in the 10–20 cm soil layer, while it significantly increased the ratio of available C/N in the 0–10 cm soil layer, the ratio of available C/P in the 0–10 and 40–60 cm layers, and the ratio of microbial biomass C/N, enzymatic C/N, C/P, and N/P in the upper soil layer ($p < 0.05$; Figures 1 and 2).

3.2 | The Effect of Litter Treatments on Microbial Necromass Carbon

Litter removal significantly decreased the total ASs, GluN, GalN, total and fungal MNC in the upper layer ($p < 0.05$; Table S3 and Figure S2), while litter addition significantly increased the total ASs, GluN, GalN, MurN, total MNC, fungal MNC, and bacterial MNC in the 0–10 cm layer, and GluN, total MNC, and fungal MNC in the 10–20 cm layer ($p < 0.05$; Table S3 and Figure S2). The ASs and MNC generally decreased with increasing depth (Tables S1 and S3 and Figure S2).

The total, fungal, and bacterial MNC/SOC were significantly altered by litter addition rather than by litter removal, specifically showing significant enhancement in the 0–10 cm soil layer due to litter addition ($p < 0.05$; Figure 3). Total and fungal MNC/SOC generally increased with increasing depth (Figure 3).

3.3 | The Effect of Litter Treatments on Microbial and Abiotic Factors

Soil chemical and microbial properties were generally and significantly affected by litter treatments ($p < 0.05$; Tables S1 and S2 and Figure S1). Litter removal significantly increased soil pH and Olsen-P content in the 0–10 and 10–20 cm soil layers but significantly decreased DOC and available N in the upper three layers ($p < 0.05$; Table S2). Litter addition significantly increased the total nutrients, microbial biomass, and enzyme activities in the upper soil layers and the contents of DOC and available N in the 40–60 cm soil layer ($p < 0.05$; Table S2 and Figure S1). Soil chemical properties, microbial biomass, and enzyme activities decreased with increasing depth (Tables S1 and S2 and Figure S1).

3.4 | Correlation Between Microbial Necromass Carbon to Soil Organic Carbon and Soil C: N: P Stoichiometry

The stoichiometric ratios of nutrients and microorganisms and the MNC/SOC were closely related. The RDA1 axis showed that the stoichiometry explained the various between 81.69% and 90.52% (Figure 4). The major variations in MNC/SOC were explained by the available and enzymatic stoichiometry ratios in the top soil layer, while they were predominantly determined by the microbial biomass N/P and total

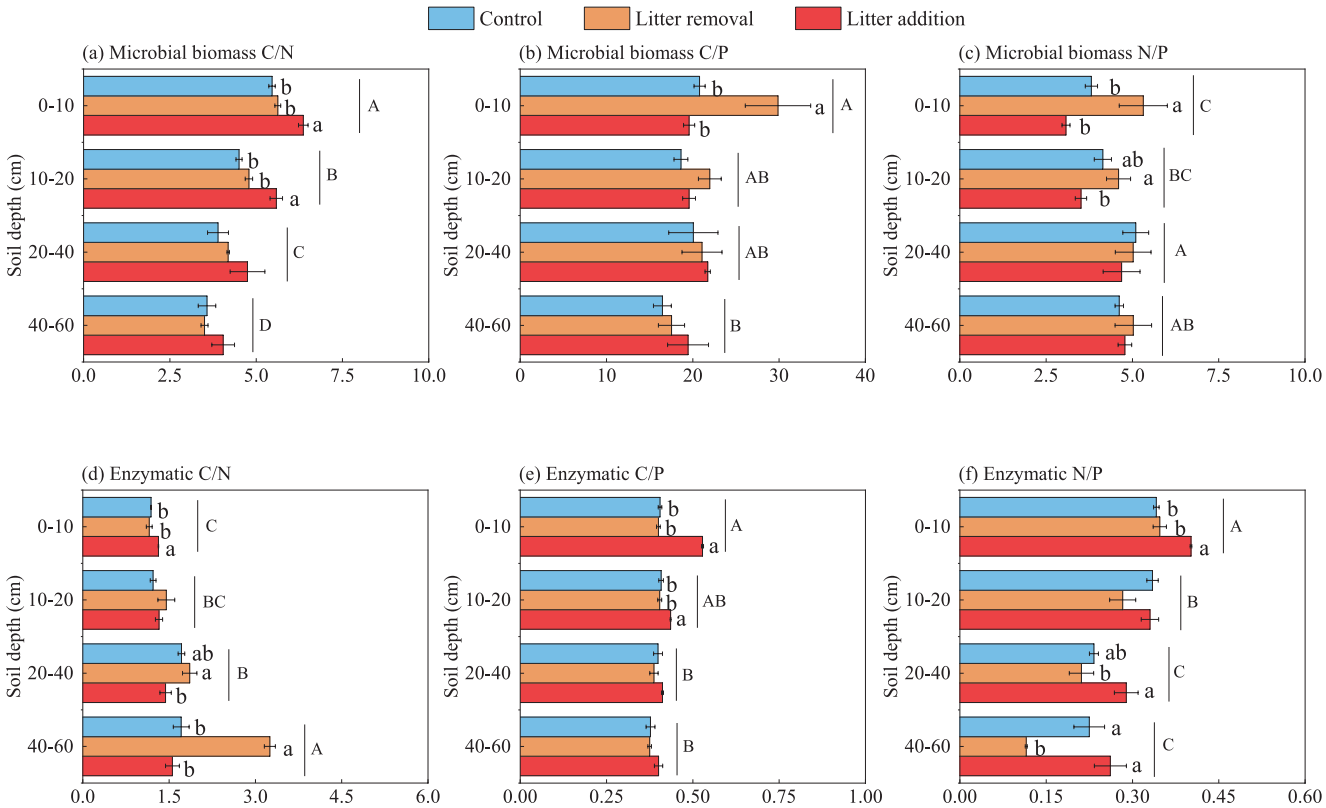

FIGURE 2 | Effects of litter addition and removal on stoichiometric ratios of microbial biomass and enzyme activity along a 60-cm soil profile in a Chinese fir plantation. Values are means ± standard errors ($n = 3$). Different lowercase letters indicate significant differences among three litter manipulation treatments within the same soil depth and different uppercase letters indicate significant differences among four soil depths for the averages of three litter manipulation treatments ($p < 0.05$). BG: β-1,4-glucosidase, NAG: β-1,4-N-acetyl-glucosaminnidase, AP: Acid phosphatase. Enzymatic C/N: In (BG)/In (NAG); Enzymatic C/P: In (BG)/In (AP); Enzymatic N/P: In (NAG)/In (AP). [Colour figure can be viewed at wileyonlinelibrary.com]

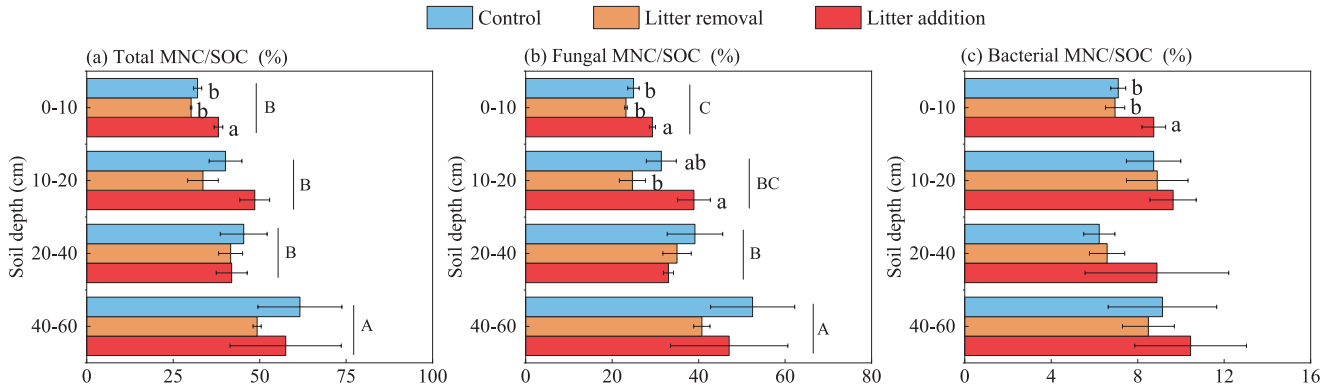

FIGURE 3 | Effects of litter addition and removal on microbial necromass carbon (MNC) percentages to soil organic carbon (SOC) along a 60-cm soil profile in a Chinese fir plantation. Values are means ± standard errors ($n = 3$). Different lowercase letters indicate significant differences among three litter manipulation treatments within the same soil depth and different uppercase letters indicate significant differences among four soil depths for the averages of three litter manipulation treatments ($p < 0.05$). [Colour figure can be viewed at wileyonlinelibrary.com]

C/P ratios below 20 cm soil layers, respectively (Figure 4). The VPA results showed that the available nutrient stoichiometry had the largest explanation degrees (31%) in the 0–10 cm soil layer, while the microbial biomass stoichiometry had the largest explanation degrees in the 10–20 and 20–40 cm soil layers (Figure 5). In the 40–60 cm soil layer, total nutrient stoichiometry explained the MNC/SOC variable (Figure 5). The mantel test showed that the ratios of available nutrient C/P and N/P and the ratios of enzymatic C/P and N/P significantly affected total, fungal, and bacterial MNC/SOC in the 0–10 cm soil layer (Figure 6). The ratios of microbial C/N and enzymatic C/N significantly affected fungal but not bacterial MNC/SOC in the 0–10 cm layer (Figure 6 and Table S4).

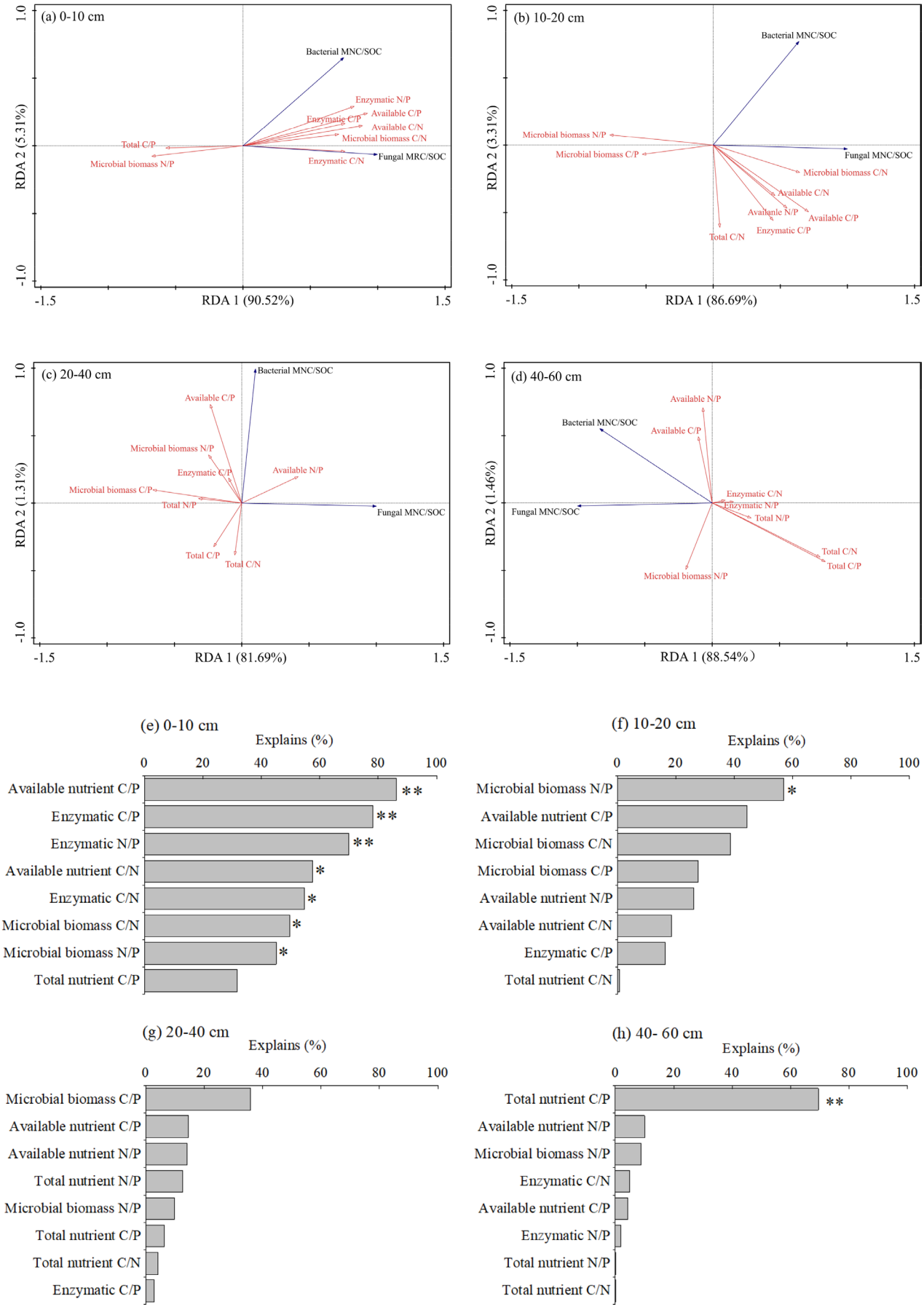

FIGURE 4 | Legend on next page.

FIGURE 4 | Redundancy analysis of microbial necromass carbon contribution to soil organic carbon (MNC/SOC), stoichiometric ratios of nutrients, microbial biomass and enzymatic activities along a 60-cm soil profile including the 0–10 cm depth (a and e), 10–20 cm depth (b and f), 20–40 cm depth (c and g), and 40–60 cm depth (d and h) in a Chinese fir plantation. [Colour figure can be viewed at wileyonlinelibrary.com]

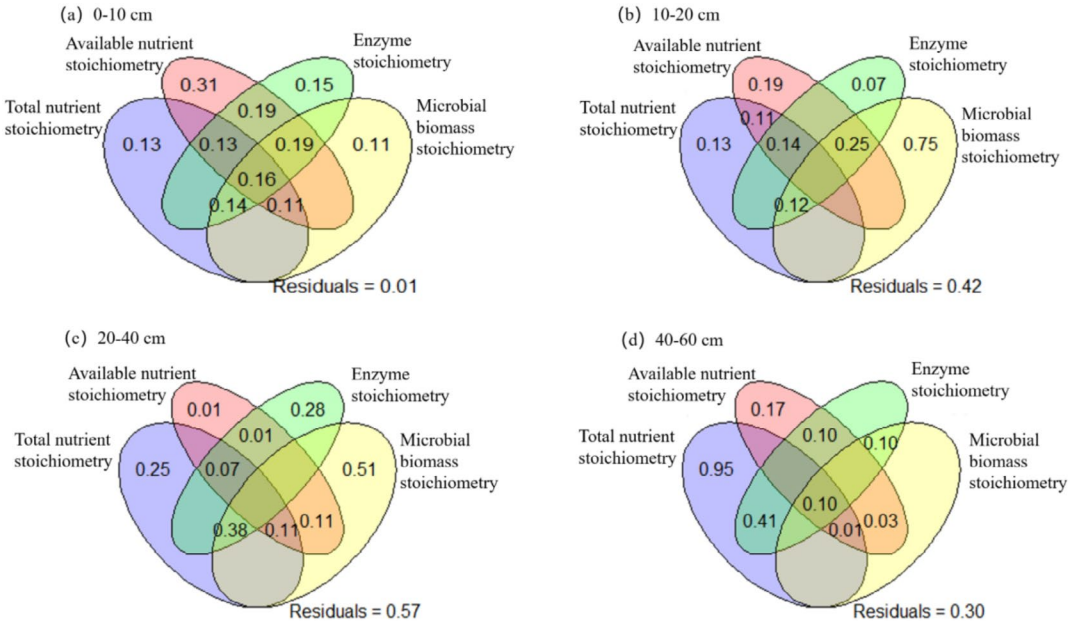

FIGURE 5 | The variance partitioning analysis (VPA) of the stoichiometry of total nutrients, available nutrients, microbial biomass and enzymes on microbial necromass carbon contributions to soil organic carbon along a 60-cm soil profile in a Chinese fir plantation. [Colour figure can be viewed at wileyonlinelibrary.com]

4 | Discussion

4.1 | Asymmetric Responses of Microbial Necromass Carbon to Litter Addition and Removal

As we hypothesized, the total, fungal and bacterial MNC/SOC was all increased due to litter addition in the upper soil layer (Figure 3). Previous studies have also shown that the input of exogenous C is conducive to the increase of MNC/SOC (Jia et al. 2022). The input of aboveground litter disrupts the initial balance between the availability of soil resources and the requirements of microorganisms (Shao et al. 2021). Microbial physiological metabolism is an important mechanism to adapt to imbalances in stoichiometric ratios (Mou et al. 2023). The "resource allocation theory" shows that the increased soil C availability induced by litter addition could reduce the secretion of C-enzyme by microorganisms (Zechmeister-Boltenstern et al. 2015). Instead, we observed the ratios of enzyme C/N and C/P increased under the exogenous C input (Figure 2). Litter addition increased the C input, resulting microbes secrete more C-related enzymes (Liu et al. 2021). These results indicated enzyme activities did not always follow the "resource allocation theory". "Economic theory" shows that high soil C availability compensates for the energy consumption required to produce microbial enzyme secretions, which is conducive to MNC accumulation (Allison et al. 2011; Mou et al. 2023). In addition, microorganisms can adjust their community composition to adapt to stoichiometric imbalances (Wang and Kuzyakov 2023). However, previous study found litter addition did not change the fungi/bacteria ratio in any of the soil layers in this study area, indicating that litter addition did not adjust the community composition to change the MNC/SOC (Liu et al. 2021). Therefore, the increase in MNC/SOC under litter addition may be mainly related to changes in the stoichiometric ratios.

However, we found that surface litter removal did not change the total, fungal, or bacterial MNC/SOC (Figure 3), which is inconsistent with our first hypothesis. Generally, MNC accumulation is limited by C and other nutrients (Mou et al. 2023). Sufficient C and nutrients are essential for microbial growth and biomass, while when these nutrients are lacking, microbial growth and activity are inhibited, which in turn limits the formation and stabilization of MNC (Mou et al. 2023). Therefore, surface litter removal may lead to C limitation owing to low C content and the ratios of C/nutrient, thus inhibiting microbial growth and decreasing MNC/SOC (Liu et al. 2021). Interestingly, we did not find that litter removal changed the MNC/SOC (Figure 3). One plausible explanation is that the supply of underground C may offset the decrease in the input of aboveground C and maintain the nutrient balance (Pausch and Kuzyakov 2018; Feng et al. 2022). Underground C input is mainly derived from an efficient and simple C source released by the roots (Varik et al. 2013; Pausch and Kuzyakov 2018). The frequent "drip" of this unstable C source is more likely to be preferentially utilized by microorganisms (Li et al. 2020). Research had shown that surface litter exclusion had no significant effect on MNC/SOC in the forest ecosystem because the input of underground root C and nutrients maintained a balance between soil nutrients and microorganisms (Xu

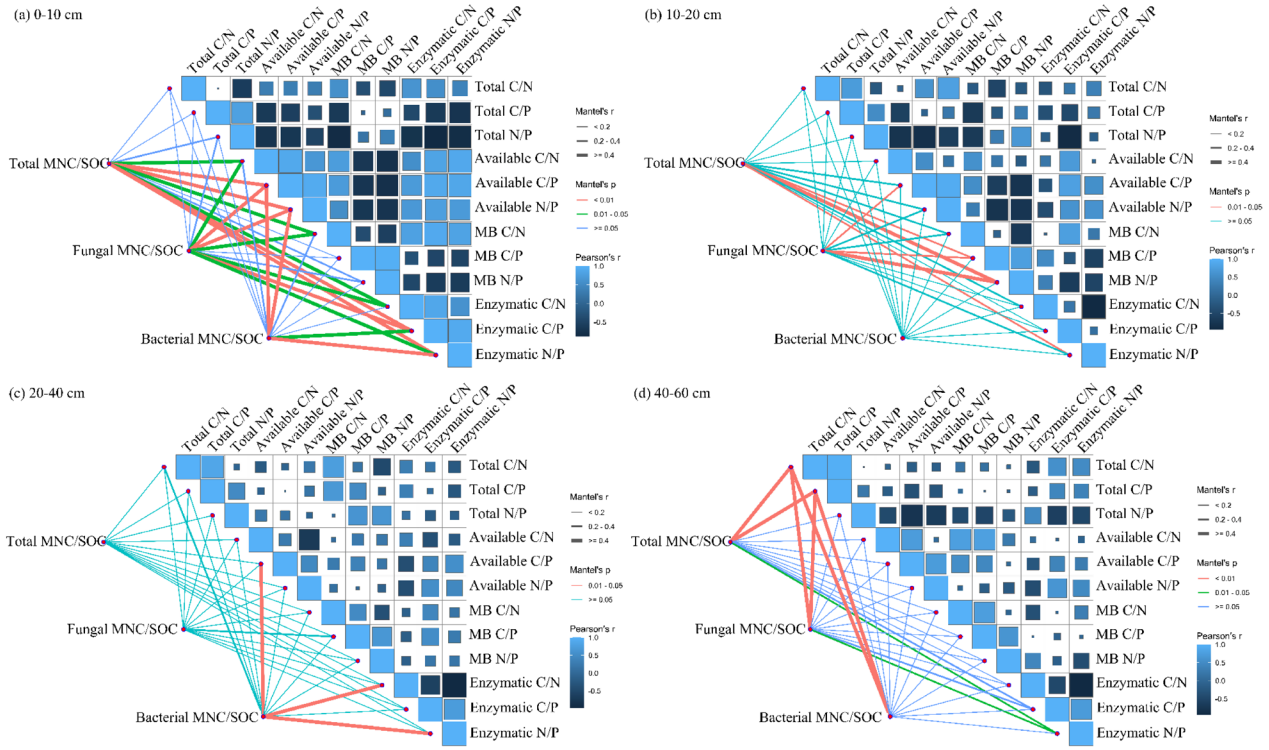

FIGURE 6 | Mantel test on correlations of total microbial necromass carbon contribution to soil organic carbon (MNC/SOC), fungal MNC/SOC, bacterial MNC/SOC with the stoichiometric ratios of nutrients, microbial biomass and enzyme activities along a 60-cm soil profile including the 0–10 cm depth (a), 10–20 cm depth (b), 20–40 cm depth (c), and 40–60 cm depth (d) in a Chinese fir plantation. The size of the squares indicates the size about the Pearson correlation coefficient and the color of the squares indicates the positive or negative correlation. The color and width of the line indicates *R* value and *P* value in mantel test, respectively. MB C/N: Microbial biomass carbon/mitrogen; MB C/P: Microbial biomass carbon/phosphorus; MB N/P: Microbial biomass nitrogen/phosphorus. [Colour figure can be viewed at wileyonlinelibrary.com]

et al. 2022). Clearly, although surface litter removal is expected to reduce the accumulation of MNC, the rapid supplementation of root C can compensate for the deficiency of this aboveground C source input and maintain the soil C balance. Collectively, our results indicated that the responses of MNC/SOC to litter addition and removal were asymmetric.

4.2 | Key Factors to Regulate Microbial Necromass Carbon Contribution to Soil Organic Carbon

We observed that the stoichiometric ratios of the available nutrients and enzyme activities were the primary factors driving the total, fungal, and bacterial MNC/SOC rather than those of the total nutrients and microbial biomass (Figures 4 and 5 and Table S4), which support our second hypothesis. This could be related to the functional specificity of the stoichiometric ratio (Mou et al. 2023; Liao et al. 2024). The available nutrient stoichiometric ratio usually refers to the proportion of elements that plants and microorganisms can use directly (Schleuss et al. 2021). The enzyme stoichiometric ratio represents the characteristics of the metabolism and biochemical reactions in microorganisms (Tapia-Torres et al. 2015). Actually, the stoichiometric ratios of available nutrients and enzyme activities reveal a balance between nutrient supply and microbial demand, which are highly sensitive to environmental changes (Tapia-Torres et al. 2015). In contrast, the stoichiometric ratios of the total nutrients and microbial biomass were relatively stable, which was consistent with the "homeostasis hypothesis" of ecological stoichiometric ratios (Schleuss et al. 2021). Microbial communities can alleviate the microbial stoichiometric imbalance by increasing extracellular enzyme secretion or changing element utilization efficiency in response to the excess or short nutrient element in the soil (Mou et al. 2023). Furthermore, the available nutrients and enzymes act as substrates and metabolites of microorganisms, and their stoichiometric ratios can more directly and quickly reflect the supply and demand relationship between microorganisms and nutrients, and ultimately determine MNC/SOC (Tapia-Torres et al. 2015; Liang et al. 2017). According to the "nutrient mining" strategy and metabolic tradeoff of microorganisms, microorganisms need to secrete more degrading enzymes to obtain restricted nutrients when the ratios of available C/N or C/P are high (Chen et al. 2022). Microbial growth efficiency is reduced and cell turnover is accelerated, thus increasing the MNC/SOC (Liang et al. 2019). On the contrary, when nutrients are balanced, microbial metabolism is more inclined to assimilate and reduce residue accumulation (Liang et al. 2019). Thus, a more active stoichiometric ratio (available nutrients and enzyme activities) can better characterize changes in MNC/SOC than a more stable stoichiometric ratio (total nutrients and microbial biomass).

4.3 | Divergent Mechanisms of Fungi and Bacterial Microbial Necromass Carbon Regulated by Soil C: N: P Stoichiometry

We observed that the ratios of microbial biomass and enzymatic C/N were significantly positively correlated with fungal MNC/

SOC rather than bacterial MNC/SOC in the top soil (Figure 6 and Table S4). The results indicated that fungal MNC/SOC was more sensitive to stoichiometric changes than bacterial MNC/SOC in the topsoil and that increases in microbial C/N ratio promoted the accumulation and contribution of fungal MNC. The increase of microbial biomass C/N ratio would be related to C utilization efficiency (CUE) (Tian et al. 2024). The higher CUE of microorganisms is conducive to microbial fixation of C rather than the release of C by CO_2 (Li et al. 2022a; Tian et al. 2024). This is conducive to fungal growth rather than bacterial growth and turnover because fungi have a higher C/N ratio (Hu et al. 2023). Instead, the decomposition activities of bacteria are restricted when the ratio of C/N changes because they require a relatively balanced environment for growth (Li et al. 2022b). Moreover, fungi, due to their faster turnover rate of biomass, can rapidly decompose organic matter into MNC, thereby making a larger contribution to MNC in soil (Tian et al. 2024). Thus, changes in the fungal MNC/SOC rather than bacterial MNC/SOC are regulated by microbial C/N ratio.

Additionally, we observed that only the ratios of available nutrients and enzyme C/P and N/P showed a positive relationship with bacterial MNC/SOC in the upper soil layer (Figure 6 and Table S4). In our study, the AP activity was notably higher than the C and N acquired enzyme activities, and the ratios of enzyme C/P and N/P were significantly lower than the global average (0.62 and 0.44, respectively) (Figure 3) (Sinsabaugh et al. 2008). These results indicate that there may be an insufficient P supply in subtropical regions (Liu et al. 2021; Chen et al. 2022). P deficiency may change the bacterial community structure because bacteria are the dominant communities in subtropical areas and are easily affected by the surrounding environment (Li et al. 2023; Ding et al. 2024; Wang et al. 2024). A study found that oligotrophic bacteria in microbial communities gradually play a dominant role when P content is insufficient (Li et al. 2023). Correspondingly, the change in bacterial MNC/SOC may also be affected by the P content (Liang et al. 2017; Wang et al. 2022). Thus, the ratios of C/P and N/P of available nutrients and enzyme activities regulate the accumulation and contribution of bacterial MNC.

4.4 | Changing Relationship Between Microbial Necromass Carbon and Soil C: N: P Ratios Along a Soil Profile

We found that the internal coupling relationship between nutrient and microbial stoichiometric ratios and MNC/SOC gradually declined with increasing soil depth (Figures 4–6; Table S4), which is consistent with our third hypothesis. This may be due to reduced nutrients and enzyme activities along the profile (Liu et al. 2021). Compared with upper soil, which contains more labile organic matter, deep soil is dominated by more stable organic matter. Because unstable and labile organic C and nutrients are absorbed and utilized by plants and microorganisms before, they reach the deep soil (He et al. 2022). Microbes invest more energy in decomposing the remaining stable organic matter (Huang et al. 2023). Therefore, microorganisms in deep soil may reduce their investment in extracellular enzymes because the energy required for extracellular enzymatic reactions is much higher than that required for intracellular compound reutilization (Huang et al. 2023). In contrast, physical protection of aggregates and chemical protection of minerals and iron/aluminum oxides strengthen with increasing soil depth, which is conducive to an increase in MNC/SOC (He et al. 2022). Because microbial necromass is usually attached to clay minerals, it requires more energy for destruction (Ni et al. 2020). In summary, the internal relationships between MNC/SOC and nutrient and microbial stoichiometries were decoupled as the soil profile deepens.

5 | Conclusion

In this study, the contribution of MNC to SOC (MNC/SOC) showed a positive response to litter addition but did not respond to litter removal. The stoichiometric ratios of available nutrients and enzyme activities were more strongly coupled with MNC/SOC than the stoichiometric ratio of total nutrients and microbial biomass. Microbial biomass and enzyme C/N ratios were significantly correlated with fungal MNC but not with bacterial MNC, indicating that the effect of the microbial stoichiometric ratio on MNC depended on microbial functional group specificity. The relationship between MNC/SOC and the stoichiometric ratios decoupled with increasing soil depth. Our results provide a new theoretical mechanism for the changes in MNC regulated by soil stoichiometry.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.