Plastic film mulching maintains soil organic carbon by increasing fungal necromass carbon under manure application

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Highlights

- Manure increases microbial necromass C retention, especially bacteria-derived C.
- Mulching promotes fungal necromass C accumulation under manure application.
- Mulching decreases bacterial necromass C retention regardless of fertilization.
- The bacterial contribution to SOC is controlled by labile organic C fractions.

Abstract

Microbial necromass carbon (MNC) is a major contributor to soil organic carbon (SOC) sequestration. Fertilization combined with plastic film mulching, as an intensive agricultural practice to increase crop yields, affects soil microbial growth and metabolism. Nevertheless, how fertilization combined with mulching affects SOC sequestration by mediating MNC dynamics remains elusive. Here, the mulching and no-mulching sub-treatments were set under three fertilization treatments (no fertilization, NF; inorganic fertilization, IF; manure, MF), and a 900-day field incubation experiment using polyvinyl chloride containers was conducted in the buffer zone of NF treatment. We investigated the effects of fertilization combined with mulching on MNC composition (including fungal necromass carbon, FNC; and bacterial necromass carbon, BNC) and qualified their contributions to SOC sequestration. The MF treatment with/without mulching significantly increased the contents of MNC, FNC, BNC, and SOC by 97%–122%, 81%–94%, 152%–210%, and 60%–70% compared with the CK treatment without mulching over 900 days, respectively. The MNC content had a strongly positive correlation with particulate organic carbon (C) and microbial biomass C (P<0.01). During the incubation stage, the proportion of MNC in SOC was higher in the IF (37%–42%) and MF (40%–44%) treatments with/without mulching than that in the NF treatment with/without mulching (31%–35%). On day 900, mulching significantly decreased the MNC content by 8.7% and 7.8%, and decreased the proportion of MNC in SOC by 5.1% and 5.8% under the NF and IF treatments, respectively. In contrast, mulching did not

significantly (P>0.05) affect the MNC and SOC contents, but significantly increased the FNC content by 4.8% under the MF treatment on day 900. Mulching significantly decreased the proportion of BNC in SOC regardless of fertilization, and increased the proportion of FNC in SOC under the MF treatment on day 900. Overall, our findings suggest that mulching under manure application maintains SOC sequestration by promoting FNC retention.

Keywords: Fertilizer application; Microbial necromass carbon; Plastic film mulching; Soil organic carbon

1 Introduction

Climate change and unsustainable agricultural management cause a global issue of food security (Schmidhuber & Tubiello, 2007; van Dijk et al., 2021). Agricultural intensification (such as fertilization and mulching) is an important process to increase crop production without expanding agricultural land (Amare & Desta, 2021; Kopittke et al., 2019). Agricultural management practices alter soil physicochemical and biological properties under intensive agriculture (Jia et al., 2022; Skadell et al., 2023). However, unreasonable agricultural management has raised a series of environmental concerns, including soil organic carbon (SOC) depletion and greenhouse gases emissions (Burney et al., 2010). SOC is a key factor in improving soil productivity and maintaining soil quality (Lal, 2018). Soil is the largest reservoir of carbon (C) in terrestrial ecosystems, and a minor change in soil C pool can have considerable impacts on atmospheric CO_2 concentration (Bradford et al., 2016; Lal, 2004). Therefore, it is crucial to employ sustainable agricultural management practices for increasing the potential of SOC sequestration.

Soil microorganisms drive SOC cycling, they convert labile organic carbon C fractions (i.e., particulate organic C, POC; microbial biomass C, MBC; extractable organic C, EOC) into microbial necromass in iterative processes of microbial community turnover (Feng & Wang, 2023; Haynes, 2005; Sokol et al., 2022). Microbial necromass C (MNC) is stabilized in soil by association with soil minerals or by occlusion within soil aggregates (Buckeridge et al., 2022). More than 50% of SOC can be derived from MNC in croplands (Liang et al., 2019; Zhou et al., 2023).

However, microbial necromass is not always inert, and it is consumed by microorganisms as a substrate under soil nutrient-limited conditions (Kastner et al., 2021). Fungal and bacterial necromass have diverse biochemical composition, which might result in different decomposition rates (Fernandez et al., 2019; Hu et al., 2020). Bacterial cell walls are enriched in peptidoglycans, which are rapidly decomposed and utilized by microorganisms (Hu et al., 2020). Therefore, bacterial necromass actively regulates the stoichiometric balance of microbial C and nitrogen (N) (Hu et al., 2020; Shao et al., 2019). In contrast, fungal necromass plays a predominant role in SOC retention due to the recalcitrant structural compounds of fungal cell walls (Fernandez et al., 2019; Soares et al., 2017). Therefore, clarifying the dynamics of fungal necromasss C (FNC) and bacterial necromasss C (BNC) is of great significance for understanding the key processes of SOC sequestration.

Fertilization and plastic film mulching are intensive agricultural practices to increase crop productivity (Steinmetz et al., 2016). The application of inorganic fertilizers or manure increases the availability of soil nutrients and organic C substrate for microorganisms (Liu et al., 2020; van der Bom et al., 2019). Mulching regulates soil microbial activities due to the changes in soil temperature, moisture, and soluble organic substrates diffusion (Kader et al., 2017). Therefore, the above-mentioned practices change soil microbial biomass, activities, and community structure (Kracmarova et al., 2022; Li et al., 2021; Li et al., 2022), which likewise cascade to affect the production of FNC and BNC and the dynamics of total MNC. However, it remains unclear that how fertilization combined with mulching affects SOC

sequestration by altering the accumulation of fungal- and bacterial-derived organic C.

In this study, we evaluated the effects of mulching on MNC dynamics (using amino sugars as biomarkers) under different fertilization treatments, and assessed the fungal and bacterial contributions to SOC sequestration. We hypothesized that: (1) Mulching would accelerate microbial anabolism and intensify MNC accumulation under the manure application, because manure combined with mulching increases the input of labile organic substrates and improves soil hydrothermal conditions (Das et al., 2023; Kasirajan & Ngouajio, 2012); (2) Mulching would decrease the BNC accumulation in all treatments, because mulching enhances the SOC or N mineralization under soils with/without fertilization (Hai et al., 2015; Lee et al., 2019).

2 Materials and Methods

2.1 Study site and soil preparation

This experiment was carried out on the long-term fertilization experimental station (43°30′N, 124°48′E) at Gongzhuling County, Jilin Province, China. This station was initiated in 1990, and has a randomized block design with three replicates. The climate in this area is a warm-summer humid continental climate influenced by monsoon. The mean annual temperature was 4.5 °C and mean annual precipitation was 560 mm in 2017–2019. At this region, seasonal drought and low soil temperature frequently occur in March–May. More than 80% of precipitation was concentrated from June to August. Soils at this station develop from quaternary loess-like sediments and are classified as a Mollisol (USDA soil taxonomy), with 31% sand, 30% silt, and 39% clay. In this station, maize (*Zea mays* L.) is continuously sown in every

May, and maize residues are completely removed after harvesting in every October.

The soil samples were randomly taken from three fertilization treatments (no fertilizer, NF; inorganic fertilizer, IF; and manure, MF) at 0–20 cm and 20–30 cm depths before sowing on May 5th, 2018. All inorganic fertilizers (including urea, superphosphate, and potassium chloride) were applied to soils before seed sowing, and organic fertilizer (pig manure) were applied to soils after maize harvesting. The application rates of fertilizers in the IF and MF treatments were are showed in Table S1. The pig manure contained 5.0 g kg^{-1} total N and 112.0 g kg^{-1} organic C. The basic soil properties (0–20 cm) before the field incubation experiment were are shown in Table S2. Before the incubation experiment, the soil samples from 0–20 cm and 20–30 cm depths were separately sieved (<7 mm) to ensure soil homogeneity and remove rocks and maize roots.

2.2 Field incubation experiment design and soil sampling

We designed the sub-treatments with and without plastic film mulching under the NF, IF, and MF treatments. In order to avoid the impact of mulching on field management of long-term fertilization station, this experiment was conducted in the buffer zone of NF treatment. Two soil pits (0.9 × 0.6 × 0.3 m) were dug, and then two polyvinyl chloride (PVC) containers (0.9 × 0.6 × 0.6 m) were vertically inserted into soil pits on May 5th, 2018 (Ge et al., 2021). Each PVC container was divided into 9 equal sections with PVC sheets, 18 micro-plots (0.3 × 0.2 m) were randomly arranged in the field. The sub-samples from 0–20 cm and 20–30 cm depths were returned to corresponding depths of each micro-plot, and then compacted to a soil bulk density

similar to that in the original treatment. Plastic film (transparent and 0.01 mm thickness) was mulched on the soil surface in all mulching treatments. Plastic mulch we used in this experiment was primarily made from low-density polyethylene, with characteristics of good flexibility, strength, and barrier properties (Mooninta et al., 2020). The old plastic film was completely removed after each soil sampling and every April, and the soil was covered again in new plastic film. The plastic film remained intact throughout the entire incubation period. To exclude the effect of maize rhizodeposition on MNC dynamics, no plants were grown in any micro-plot.

Three soil core samples (5 cm diameter, 20 cm length) were randomly collected from 0–20 cm soil depth of each micro-plot on day 150 (October 2018), day 360 (April 2019), and day 900 (October 2020) after mulching, and then fully mixed into one soil sample. Soil samples were sieved (<2 mm) to remove all visible plant residues and stones. One part was stored at 4 °C for extractable organic C (EOC) and microbial biomass C (MBC) analyses, and the other part was air-dried for SOC, particulate organic C (POC), and amino sugars analyses.

2.3 Laboratory analysis

The content of SOC was determined using an elemental analyzer (Elementar Vario EL III, Germany). The MBC content was determined using the chloroform fumigation-extraction (with 0.5 mol L^{-1} K_2SO_4) method (Vance et al., 1987). The organic C content of the extracts was determined using a total organic C analyzer (Elementar High TOC II, Germany). The EOC content was determined from non-fumigated extracts (Schaeffer et al., 2013). The MBC content was calculated from the

difference between fumigated and non-fumigated extracts using a conversion factor of 0.45 (Joergensen, 2018). The POC was separated with 5 g L^{-1} sodium hexametaphosphate as described by Cambardella and Elliott (1992). The POC content was determined using an elemental analyzer (Elementar Vario EL III, Germany).

The contents of FNC (g kg^{-1} soil) and BNC (g kg^{-1} soil) were calculated from the contents of glucosamine (g kg^{-1} soil) and muramic acid (g kg^{-1} soil) according to the empirical conversion factors (Appuhn & Joergensen, 2006; Liang et al., 2019). The analysis of glucosamine and muramic acid were conducted following the method described by Zhang and Amelung (1996). The details of the extraction procedures were given in the Supplementary Materials. Glucosamine occurred in both fungal and bacterial cell walls, FNC was calculated by subtracting bacterial-derived glucosamine from total glucosamine, assuming that muramic acid and glucosamine occurred at the molar ratio of 1 to 2 in the cell walls of bacteria (Engelking et al., 2007). The contents of FNC, BNC, and MNC were calculated as follows:

$$FNC=(\text{glucosamine}/179.2-(2\times\text{muramic acid}/251.2))\times179.2\times9 \quad (1)$$

$$BNC=\text{muramic acid}\times45 \quad (2)$$

$$MNC = FNC + BNC \quad (3)$$

Where 179.2 is the molecular weight of glucosamine and 251.2 is that of muramic acid. 9 is the conversion coefficient of glucosamine to FNC and 45 is that of muramic acid to BNC (Joergensen, 2018).

The ratio of MNC to MBC indicates the amount of microbial necromass accumulated per unit of MBC (Zhang et al., 2021a).

2.4 Statistical analysis

Statistical analyses were performed using SPSS 19.0 (IBM Corporation, USA). Prior to statistical analysis, the homogeneity of variance was verified using Levene's test, and the normality of variance was checked using Shapiro-Wilk's test (P>0.05), histograms, and normal Q-Q plots. If the data were not normally distributed, a natural logarithm transformation was performed. Two-way analysis of variance was conducted on all variables to examine the effects of fertilization and mulching. All variables between different treatments were evaluated using one-way analysis of variance with Tukey's HSD post-hoc test. Spearman's correlation analysis was performed to compare the correlations between FNC, BNC, and MNC and SOC fractions.

3 Results

3.1 Soil organic carbon fractions

During the entire incubation period, the MF treatment with/without mulching increased (P<0.05) the contents of SOC, MBC, and POC by 60%–70%, 50%–104%, and 79%–178% relative to the NF treatment without mulching, respectively (Fig. 1). Mulching increased the MBC content under the treatment with/without fertilization within 150–360 days. On day 900, mulching decreased the SOC content by 3.7% and 2.2% under the NF and IF treatments, respectively. On day 900, mulching decreased the MBC and POC contents by 10% and 17% under the IF treatment, respectively. During the entire incubation period, mulching decreased the EOC content under the NF, IF, and MF treatments (Fig. 1).

3.2 Microbial, fungal, and bacterial necromass carbon

Within 150–900 days, the MF treatment with/without mulching increased (P<0.05) the MNC, FNC, and BNC contents by 97%–122%, 81%–94%, and 152%–210% compared with the NF treatment without mulching, respectively (P<0.05, Fig. 2). Mulching increased the MNC content, on average, by 12% and 6.3% under the IF and MF treatments within 150–360 days, respectively. Specifically, mulching increased the FNC content by 16% under the IF treatment, and increased the BNC content by 15% under the MF treatment within 150–360 days. On day 900, mulching decreased the MNC, FNC, and BNC contents by 8.7%, 5.8%, and 19% under the NF treatment, and decreased those contents by 7.8%, 6.1%, and 13% under the IF treatment, respectively. On day 900, mulching increased the FNC content by 4.8% and decreased the BNC content by 14% under the MF treatment, but there was no significant difference (P>0.05) in the MNC content between mulching and no-mulching under the MF treatment.

During the entire incubation period, the proportion of MNC in SOC was 37%–44% in the IF and MF treatments with/without mulching, and was 31%–35% in the NF treatment with/without mulching (Table 1). Within 150–900 days, the MF treatment with/without mulching had the highest proportion of BNC in SOC among all the six treatments (Table 1). Within 150–360 days, mulching increased the proportions of MNC and FNC in SOC under the IF treatment, and increased the proportions of MNC and BNC in SOC under the MF treatment (Table 1). On day 900, mulching decreased the proportions of MNC and BNC in SOC by 5.1% and 15.0% under the NF treatment,

and decreased the proportions of MNC, FNC, and BNC in SOC by 5.8%, 4.0% and 11% under the IF treatment, respectively. On day 900, mulching increased the proportion of FNC in SOC by 4.5% and decreased that of BNC in SOC by 14% under the MF treatment, but there was no significant difference (P>0.05) in the proportion of MNC in SOC between mulching and no-mulching under the MF treatment. Mulching increased the ratio of MNC to MBC under the NF, IF, and MF treatments within 150–360 days (Table 1).

3.3 Correlation between microbial necromass carbon and soil organic carbon fractions

The SOC content was strongly correlated with the contents of MNC (R^2=0.98, P<0.01), FNC (R^2=0.98, P<0.01), and BNC (R^2=0.95, P<0.01) (Fig. 3). The MNC content had the strongest correlation with the POC content (R^2=0.95, P<0.01), followed by the contents of MBC (R^2=0.83, P<0.01) and EOC (R^2=0.53, P<0.01). The proportion of BNC in SOC was positively correlated with the contents of POC (R^2=0.76, P<0.01), MBC (R^2=0.81, P<0.01), and EOC (R^2=0.53, P<0.01).

4 Discussion

4.1 Soil microbial necromass carbon accumulation and its contribution to soil organic carbon sequestration under the fertilization combined with mulching

The MF treatment with/without mulching increased the SOC content compared with the other four treatments (Fig. 1). And the SOC content was positively correlated with the MNC content (Fig. 3), which highlights the importance of microbial anabolism in SOC accumulation (Liang et al., 2017). On the one hand, particulate

organic matter in pre-se manure might be loosely bound in large soil aggregates or located between soil aggregates (Bartuška et al., 2015). As a readily accessible SOC fraction, POC is utilized by microorganisms and favors microbial growth (Lavallee et al., 2020; Rocci et al., 2021). High microbial biomass contributes to MNC production and accumulation (Luan et al., 2022). On the other hand, manure harbors diverse microorganisms (Semenov et al., 2021). Manure application could introduce a large number of exogenous microorganisms into soils, thereby directly increasing MNC accumulation. Notably, MF treatment with/without mulching had the highest MNC content among all treatments. But the proportion of MNC in SOC was not consistently higher in the MF treatment with/without mulching (37%–42%) than that in the IF treatment with/without mulching (40%–44%) over 900 days (P>0.05, Fig. 2 and Table 1). Manure application can directly increase SOC content by adding organic matter to the soil (Mustafa et al., 2020). Moreover, plant lignin phenol accumulates more readily than other SOC components (i.e., microbial necromass) under manure application (Li et al., 2020). These processes might relatively weaken microbial contribution to SOC accumulation in the MF treatment with/without mulching. In this study, we calculated MNC content from soil amino sugar content using conversion factors (Appuhn & Joergensen, 2006; Engelking et al., 2007), and then estimated the contribution of MNC to SOC. However, these assessments were based on the assumption that the proportions of gram-positive bacteria and gram-negative bacteria account for 65% and 35% of total bacteria, respectively (Appuhn & Joergensen, 2006). When the microbial community composition in this study differs from that previously

observed, the content of MNC might be overestimated or underestimated. In further study, the ratio of soil gram-positive to gram-negative bacteria should be measured to generate a correct conversion coefficient in each soil, which will reduce existing uncertainties (Joergensen, 2018; Salas et al., 2023).

The MF treatment with/without mulching increased (P<0.05) the FNC and BNC contents by 81%–94% and 152%–210% relative to the CK treatment without mulching, respectively (Fig. 2). Application of manure provides a large amount of energy and diverse resources for microbial growth and reproduction (Das et al., 2023). It has been reported that manure application promotes bacterial growth and induces a significant decrease in the ratio of fungal biomass to bacterial biomass, because bacteria are more sensitive to changes in available substrates in farmland systems (Marschner et al., 2011; Shen et al., 2023; Wei et al., 2017). Therefore, the competitive advantage of bacteria might lead to the highest contribution of BNC to SOC (12%–15%) under the MF treatment with/without mulching (Table 1).

4.2 Impact of mulching on microbial necromass carbon accumulation under the same fertilization treatment

Within 150–360 days, mulching increased the MNC content by promoting fungal necromass formation under the IF treatment, and it enhanced MNC accumulation by increasing the BNC content under the MF treatment. The accumulation of FNC and BNC not only was associated with the increase in MBC (Fig. 3), but also correspond to the dominant microbial community in soils (Wang et al., 2021). Mulching decreases urea-N loss, and high N availability favors microbial growth (Hu et al.,

2022; Ma et al., 2018). Long-term application of inorganic fertilizer reduces soil pH (Table S2), and low soil pH is favorable for fungal growth (Grosso et al., 2016). Therefore, mulching might stimulate the proliferation of fungi, leading to intensified FNC production under the IF treatment within 150–360 days (Fig. 2). In contrast, mulching promotes labile organic substrates diffusion in the manure-treated soil (Manzoni et al., 2014). Labile organic substrates are preferentially utilized by bacteria (Marschner et al., 2003; Marschner et al., 2011). Moreover, long-term application of manure adjusts the soil pH to a neutral range (Table S2). Neutral soil pH is conducive to the growth of bacteria (Liu et al., 2020; Zhang et al., 2021b). Therefore, mulching might promote bacterial colonization and further increased BNC retention under the MF treatment within 150–360 days (Fig. 2). Interestingly, mulching under the MF treatment promoted MNC accumulation on day 150 and day 360 (Fig. 2). While mulching had no significant effect on the SOC content ($P>0.05$) on day 150, and decreased ($P<0.05$) the SOC content on day 360 (Fig. 1). Previous studies have observed that mulching greatly enhanced SOC decomposition under green manure application (Hwang et al., 2020; Lee et al., 2021). A lower ratio of MNC to MBC indicated that MNC could be less efficiently stored in the MF treatment with mulching than that in the MF treatment without mulching within 150–360 days (Table 1). Together, these results suggested that mulching might highly increase mineralized C loss. This process could counteract microbial necromass-derived C accumulation under the MF treatment.

On day 900, mulching decreased MNC accumulation and SOC sequestration

317 under the NF and IF treatments (Fig. 1, Fig. 2 and Table 1). Low contents of POC and
318 MBC and low C/N ratio indicated that mulching aggravated microbial C limitation
319 under the NF and IF treatments on day 900 (Fig. 1 and Table S3). Poor organic C
320 substrate limits microbial growth and its necromass C formation (Schmidt et al.,
321 2017). Moreover, soil microorganisms can directly decompose microbial necromass
322 to meet microbial C demand (Coonan et al., 2020; Kastner et al., 2021). The recycling
323 of microbial necromass is enhanced under soil nutrient-limited condition (Meier et al.,
324 2017). Therefore, the rate of microbial necromass production was insufficient to offset
325 its degradation rate under the NF and IF treatments with mulching on day 900.
326 Notably, the presence of living roots could be also a key factor affecting experimental
327 conclusions (Maillard et al., 2021). Living roots regulate the turnover of microbial
328 necromass by both releasing labile organic C substrate (root exudates) and competing
329 N with microorganisms (Canarini et al., 2019; Sokol et al., 2019). Root exudates also
330 selectively promote the growth of fungi and bacteria by changing their chemical
331 composition (Canarini et al., 2019; Hartmann et al., 2009), thereby affecting the
332 source of microbial necromass. Considering that MNC exhibits complex responses to
333 living roots, simultaneous changes in the rhizosphere under mulching and fertilization
334 might confound the effects of mulching and fertilization on MNC dynamics.
335 Therefore, we excluded the effect factor of living roots and focused on the effect
336 factors of mulching and fertilization on MNC dynamics in the no plant root system.
337 Future research should also deeply evaluate these effects under the actual field
338 systems.

Mulching decreased the contribution of BNC to SOC on day 900 regardless of fertilization (Table 1), indicating that bacterial necromass had a strong sensitivity to plastic film mulching. This result was consistent with our second hypothesis. The decrease in the BNC accumulation could be attributed to substrate limitation under long-term mulching condition, because bacterial necromass actively regulates the stoichiometric balance of microbial C and N (Hai et al., 2015; Hu et al., 2020; Lee et al., 2019; Shao et al., 2019). This was also supported by our result that the proportion of BNC in SOC was positively correlated with the contents of MBC, EOC, and POC (Fig. 3). Although mulching decreased BNC accumulation, it increased the FNC content and its proportion in SOC under the MF treatment on day 900 (Fig. 2). The results of our study showed that the contribution of FNC to SOC (25%–31%) was nearly two times more than that of BNC to SOC (6.1%–15%) (Table 1), indicating that FNC was the major contributor to SOC sequestration over BNC. Therefore, mulching maintained SOC content through FNC accumulation under the MF treatment on day 900 (Fig.1, Fig. 2, and Table 1).

5 Conclusions

Fertilization and mulching had interactive effects on microbial necromass carbon accumulation. Manure application with/without mulching increased soil organic carbon sequestration compared with the other four treatments, this was attributed that the high contents of particulate organic carbon and microbial biomass carbon led to microbial necromass carbon accumulation, especially as bacterial necromass carbon. Mulching decreased microbial necromass carbon accumulation under the no fertilizer

and the application of inorganic fertilizer treatments on day 900. Although mulching had no significant effect on the microbial necromass carbon content and its contribution to soil organic carbon under the manure application, it promoted fungal necromass carbon accumulation, contributing to maintaining soil organic carbon content on day 900. Moreover, mulching decreased bacterial contribution to soil organic carbon regardless of fertilization on day 900. The proportion of bacterial necromass carbon in soil organic carbon was regulated by labile organic carbon substrates. Therefore, manure application combined with mulching maintained soil organic carbon through fungal necromass carbon accumulation in soils with crop removal. The dynamics of microbial necromass carbon under fertilization combined with plastic film mulching should be further explored in the actual field systems.

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Table 1. The MNC/SOC, FNC/SOC, and BNC/SOC proportions and the MNC/MBC ratio under the fertilization treatments with/without plastic film mulching

Fertilization treatment	No-mulching/ mulching	MNC/SOC (%)			FNC/SOC (%)			BNC/SOC (%)			MNC/MBC		
		Day 150	Day 360	Day 900	Day 150	Day 360	Day 900	Day 150	Day 360	Day 900	Day 150	Day 360	Day 900
NF	No-mulching	33.7±0.3e	33.4±0.6d	32.4±0.1d	25.6±0.4b	25.6±0.3d	25.1±0.2c	8.1±0.1d	7.8±0.3d	7.3±0.1e	20.9±1.8b	19±0.1c	21.3±0.4b
	Mulching	34.9±0.7d	34.2±1.1d	30.7±0.4e	26.2±0.6b	26.2±1.0cd	24.6±0.3c	8.7±0.2d	8.0±0.4d	6.1±0.1f	14.8±0.3c	16.2±0.4e	21.5±0.3b
IF	No-mulching	37.0±0.2c	38.7±0.9c	40.6±0.5b	25.7±0.3b	27.4±1.1bcd	30.0±0.6a	11.3±0.1c	11.3±0.3bc	10.6±0.1c	23.5±0.3ab	18.8±0.3c	20±0.9b
	Mulching	41.6±0.4b	41.5±0.2ab	38.2±0.4c	29.7±0.6a	30.8±0.2a	28.8±0.5b	12.0±0.2c	10.7±0.1c	9.4±0.1d	17.2±0.5c	17.3±0.2d	20.5±1b
MF	No-mulching	42.0±0.5b	39.7±0.3bc	42.4±0.6a	28.6±0.1a	27.8±0.8bc	28.6±0.5b	13.3±0.6b	11.8±0.6b	13.7±0.1a	24.5±1.2a	24.8±0.2a	29±0.6a
	Mulching	43.9±0.3a	43.2±0.6a	41.8±0.5ab	29.2±0.4a	29.0±0.7ab	29.9±0.3a	14.7±0.3a	14.3±0.0a	11.8±0.3b	22.6±0.4ab	23.6±0.2b	29.3±0.5a
Two-way analysis of variance													
Fertilization (F)		**	**	**	**	**	**	**	**	**	**	**	**
Mulching (M)		**	**	**	**	**	ns	**	**	**	**	**	ns
F×M		**	*	*	**	*	**	ns	**	**	**	**	ns

MNC/SOC: the proportion of microbial necromass carbon (MNC) in soil organic carbon (SOC); FNC/SOC: the proportion of fungal necromass carbon in SOC; BNC/SOC: the proportion of bacterial necromass carbon in SOC; MNC/SOC: the ratio of MNC to microbial biomass carbon. "**", "*", and "ns" indicate significant levels at $P<0.01$, $P<0.05$, and $P>0.05$, respectively. NF, IF, and MF denote treatments of no fertilization, inorganic fertilization, and manure, respectively. Values are mean ± standard errors (n=3). Different lowercase letters show significant differences ($P < 0.05$) among the treatments.

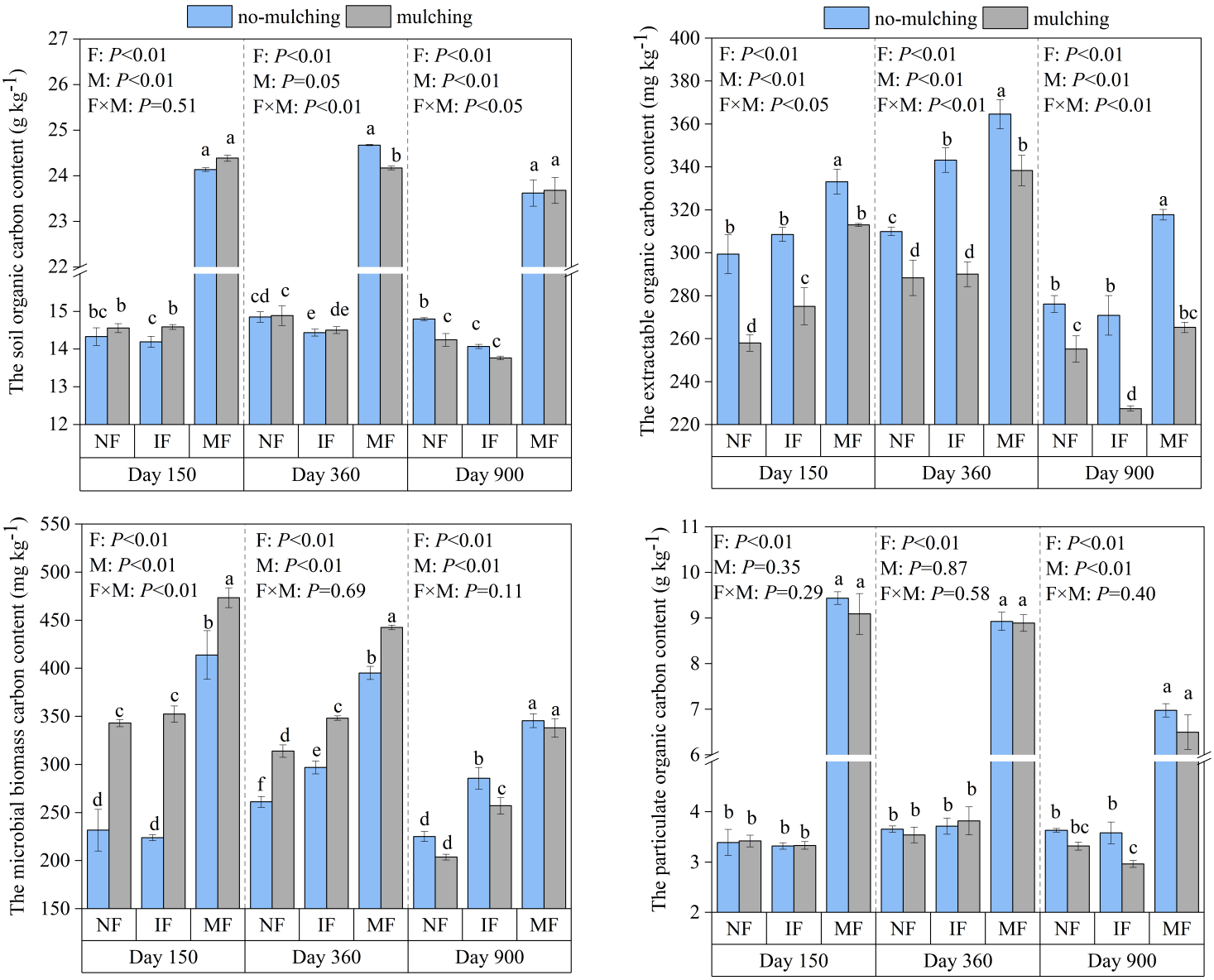

Fig. 1. The contents of soil organic carbon, extractable organic carbon, microbial biomass carbon, and particulate organic carbon under the fertilization treatments with/without plastic film mulching. NF, IF, and MF denote treatments of no fertilization, inorganic fertilization, and manure, respectively. Error bars indicate standard errors (n=3). Different lowercase letters show significant differences (P<0.05) among the treatments. The P values behind 'fertilization (F)', 'mulching (M)', and 'F× M' show the effects of fertilization, mulching, and their interactions on the contents of soil organic carbon, extractable organic carbon, microbial biomass carbon, and particulate organic carbon.

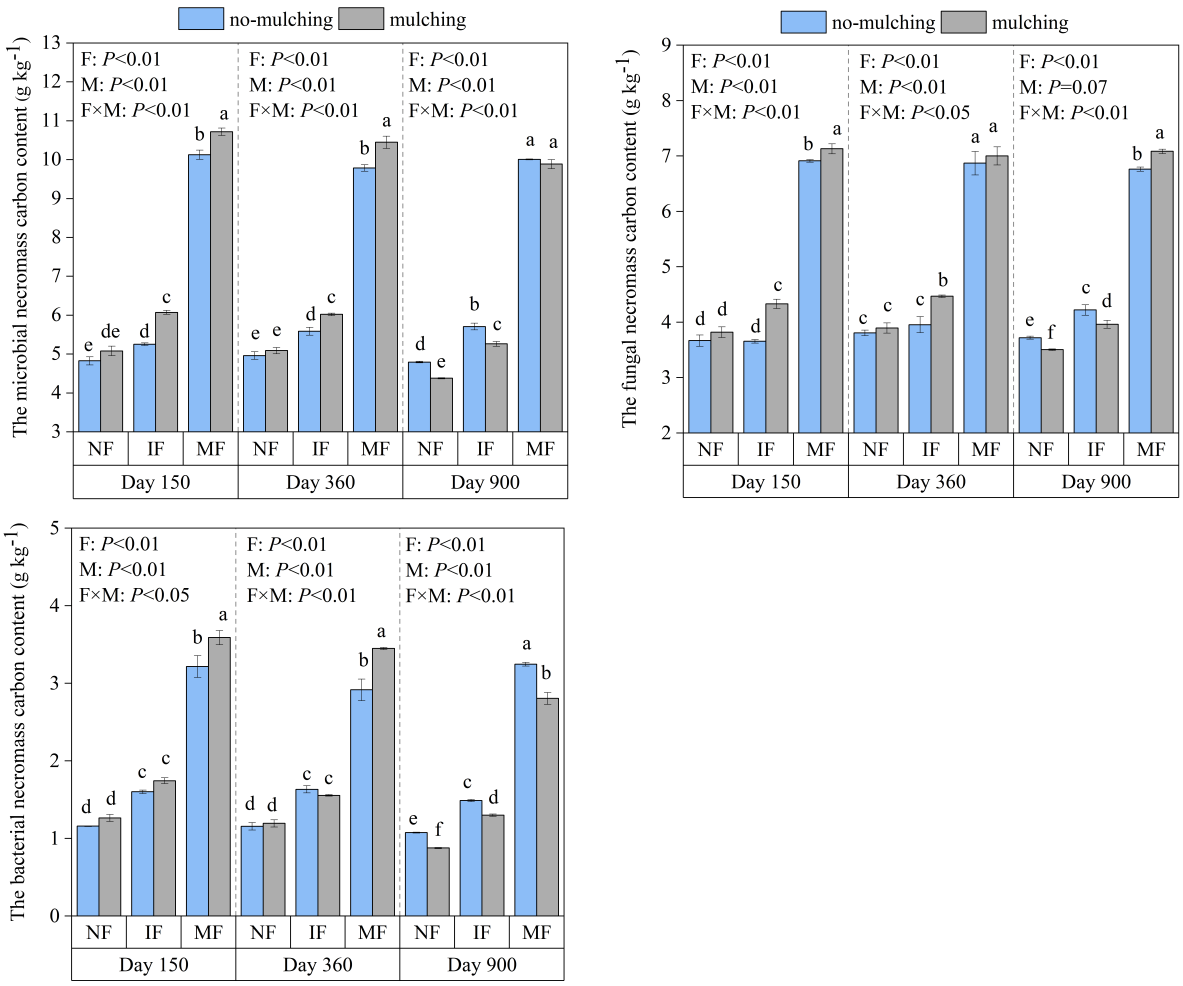

Fig. 2. The contents of microbial, fungal, and bacterial necromass carbon under the fertilization treatments with/without plastic film mulching. NF, IF, and MF denote treatments of no fertilization, inorganic fertilization, and manure, respectively. Error bars indicate standard errors (n=3). Different lowercase letters show significant differences (P<0.05) among the treatments. The P values behind 'fertilization (F)', 'mulching (M)', and 'F× M' show the effects of fertilization, mulching, and their interactions on the contents of microbial, fungal, and bacterial necromass carbon.

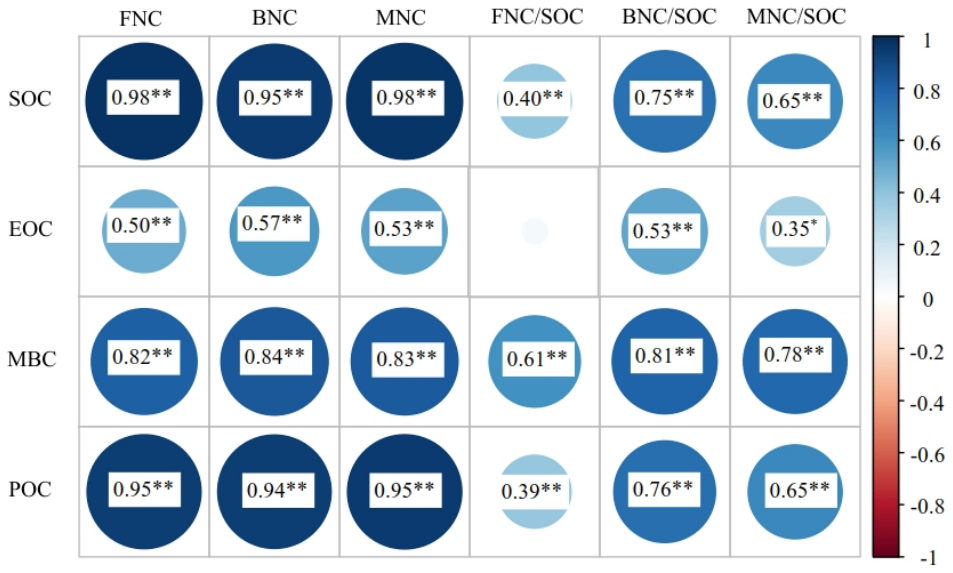

Fig. 3. The correlations between microbial necromass carbon (MNC) and soil organic carbon (SOC) fractions. FNC: fungal necromass carbon (C), BNC: bacterial necromass C, MNC/SOC: the proportion of MNC in SOC; FNC/SOC: the proportion of FNC in SOC; BNC/SOC: the proportion of BNC in SOC; EOC: extractable organic C, MBC: microbial biomass C, POC: particulate organic C. The size of the circles represents the magnitude of correlations. "**", "*", and "ns" indicate significant levels at $P<0.01$, $P<0.05$, and $P>0.05$, respectively.

Table S1 The application rates of fertilizer in different treatments

Fertilization treatment	Urea ($N\ ha^{-1}\ yr^{-1}$)	Superphosphate ($P_2O_5\ ha^{-1}\ yr^{-1}$)	Potassium chloride ($K_2O\ ha^{-1}\ yr^{-1}$)	Pig manure ($N\ ha^{-1}\ yr^{-1}$)
NF	0.0	0.0	0.0	0.0
IF	165.0	82.5	82.5	0.0
MF	50.0	82.5	82.5	115.0

NF, IF, and MF denote treatments of no fertilization, inorganic fertilization, and manure, respectively.

Table S2 Basic soil properties of the tested soil in 2018

Fertilization treatment	Soil organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	C/N	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})	pH (H_2O)
NF	15.0±0.2 b	1.42±0.05 c	10.6±0.3 a	15.2±0.6 c	65.1±2.8 c	7.8±0.1 a
IF	14.9±0.2 b	1.55±0.01 b	9.7±0.1 b	20.5±0.8 b	82.4±2.5 b	5.8±0.1 c
MF	25.2±0.3 a	2.59±0.04 a	9.7±0.1 b	80.1±2.0 a	138.3±3.3 a	7.2±0.1 b

C/N: the ratio of soil organic carbon to total nitrogen. NF, IF, and MF denote treatments of no fertilization, inorganic fertilization, and manure, respectively. Different lowercase letters show significant differences ($P < 0.05$) among the treatments. Values are mean ± standard errors (n=3).

Table S3. Effect of fertilization combined with plastic film mulching on the total nitrogen content, the C/N ratio and soil water content

Fertilization treatment	No-mulching/ mulching	Total nitrogen (g kg^{-1})			C/N			Soil water content (%)		
		Day 150	Day 360	Day 900	Day 150	Day 360	Day 900	Day 150	Day 360	Day 900
NF	No-mulching	1.43±0.01d	1.41±0.01e	1.42±0.02c	10±0.1a	10.5±0.0a	10.4±0.1a	24.7±0.5d	20.0±0.4d	25.6±0.6b
	Mulching	1.45±0.01d	1.51±0.01c	1.44±0.03c	10±0.1a	9.8±0.1c	9.9±0.1b	25.7±0.7c	23.6±0.5b	25.1±0.0b
IF	No-mulching	1.53±0.03c	1.49±0.02cd	1.47±0.00c	9.3±0.1b	9.7±0.1c	9.6±0.0b	24.5±0.1d	20.7±0.1d	25.0±0.1b
	Mulching	1.54±0.01c	1.48±0.01d	1.43±0.02c	9.5±0.0b	9.8±0.1c	9.6±0.1b	25.9±0.2c	22.1±0.7c	25.8±0.3b
MF	No-mulching	2.39±0.00b	2.39±0.00b	2.41±0.01a	10.1±0.0a	10.3±0.0b	9.8±0.1b	29.3±0.1b	24.4±0.0b	27.9±0.9a
	Mulching	2.48±0.06a	2.44±0.01a	2.26±0.06b	9.9±0.2a	9.9±0.0c	10.5±0.2a	30.8±0.3a	28.1±0.0a	28.9±0.8a
Two-way analysis of variance										
Fertilization (F)		**	**	**	**	**	**	**	**	**
Mulching (M)		*	**	**	ns	**	ns	**	**	ns
F×M		ns	**	**	*	**	**	ns	**	ns

C/N: the ratio of soil organic carbon to total nitrogen. NF, IF, and MF denote treatments of no fertilization, inorganic fertilization, and manure, respectively. Values are mean ± standard errors (n=3). Different lowercase letters show significant differences ($P < 0.05$) among the treatments. “**”, “*”, and “ns” indicate significant levels at $P<0.01$, $P<0.05$, and $P>0.05$, respectively.

Amino sugars analysis

The weighed soil aggregate samples (<0.15 mm) were hydrolyzed with 6 mol L^{-1} HCl solution. The hydrolysates were filtered, dried with N_2 gas, and re-dissolved with deionized water. After adjusting the pH to 6.6–6.8, the samples were centrifuged and freeze-dried. Amino sugars were extracted from freeze-dried supernatants using methanol. The recovered amino sugars were then transformed into aldononitrile derivatives by reaction with hydroxylamine hydrochloride, 4-dimethylamino-pyridine, and acetic anhydride. Dichloromethane and HCl were sequentially added to the mixture. Finally, excess anhydride was removed using 1 mol L^{-1} HCl and deionized water. After drying the extracts using an N_2 stream, the amino sugar derivatives were re-dissolved with hexane-ethyl acetate (1:1) and quantified using an Agilent 7890B gas chromatograph (Agilent Technologies, California, USA) equipped with an HP-5 column (30 m length, 0.25 mm diameter, and 0.25 μm thickness) and a flame ionization detector.