Fungal necromass carbon contributes to organic carbon sequestration within soil macroaggregates under manure application combined with plastic film mulching

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Abstract

Purpose

Microbial necromass carbon (C) is a major contributor to soil organic C (SOC) sequestration. Soil aggregates are key functional units in regulating SOC dynamics. Fertilization and plastic film mulching, as common agricultural management practices, affect soil aggregation and microbial activities. However, how microbial necromass C is accumulated and contributes to organic C sequestration within soil aggregates remains poorly understood, especially under fertilization combined with plastic film mulching.

Methods

We set the mulching and no-mulching sub-treatments under three long-term (28 years) fertilization treatments (no fertilization, NF; inorganic fertilization, IF; manure application, MF) and conducted a 900-day field mesocosm experiment. We quantified the proportion of microbial necromass C in organic C within soil aggregates using amino sugar biomarkers, and investigated the effects of fertilization combined with mulching on the dynamics of microbial necromass C within soil aggregates.

Results

Microbial necromass C accounted for 28.2%–42.9% of organic C of macroaggregates (>0.25 mm) and 40.4%–55.8% of organic C of microaggregates (<0.25 mm) on day 900. The proportion of fungal necromass C in organic C of soil aggregates was nearly two times more than that of bacterial necromass C within 360–900 days. Regardless of mulching or no-mulching, the MF treatment increased the microbial necromass C

content within macroaggregates and microaggregates, on average, by 148.6% and 84.5% compared with the NF treatment during the entire incubation period, respectively. Mulching facilitated microbial necromass C accrual within macroaggregates under the NF and IF treatments only on day 360, but increased it under the MF treatment on both day 360 and day 900. Mulching increased the fungal necromass C content and its contribution to organic C within macroaggregates by an average of 17.4% and 11.2% under the MF treatment during the entire incubation period, respectively.

Conclusions

Manure application combined with mulching promoted organic C sequestration within macroaggregates via the accumulation of fungal necromass C.

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

1. Introduction

Soil organic carbon (SOC) plays a critical role in improving soil fertility and increasing soil productivity in agricultural ecosystems (Dheri and Nazir 2021; Stout et al. 2016). Recent studies have shown that microbial necromass carbon (C) is thought as an important contributor to SOC sequestration, and more than half of SOC in croplands derive from microbial necromass C (Buckeridge et al. 2022; Liang et al. 2019). Thus, clarifying the dynamics of microbial necromass C is of great importance to assess the key processes of SOC sequestration in agroecosystems.

Soil aggregates are key components of soil structure, and soil structural heterogeneity governs microbial activities (Gupta and Germida 2015; Rillig et al. 2017). Microbial biomass and activities within soil aggregates are increased with aggregate sizes due to higher available substrates and better microsites (Bhattacharyya et al. 2021; Fan et al. 2020). A large amount of living biomass contributes to microbial necromass production (Prommer et al. 2020). Moreover, soil aggregates provide physical and chemical protection for microbial necromass against enzymatic attack and microbial decomposition (Buckeridge et al. 2022; Dungait et al. 2012; Totsche et al. 2018). These evidences point out that microbial activities and the protection of soil aggregates drive the distribution of microbial necromass C within soil aggregates. Generally, fungi tend to colonize in large pores within macroaggregates (Schweigert et al. 2015). Bacteria predominantly proliferate in small pores within microaggregates (Six et al. 2006). Meanwhile, fungal and bacterial necromass have disparate turnover rates (Fernandez et al. 2019; Hu et al. 2020). Relative to fungal necromass, bacterial necromass is more

easily re-utilized to compensate microbial nutrient demand (He et al. 2011). Therefore, fungal and bacterial necromass C might play different roles in mediating organic C accumulation within soil aggregates. However, how much microbial necromass C derived from fungi and bacteria is sequestrated within soil aggregates needs to be further explored.

Fertilization, as a common agricultural management strategy, affects SOC dynamic within soil aggregates (Wu et al. 2023). Inorganic fertilization increases the availability of mineral nutrients and accelerates microbial metabolism, this process promotes aggregate-associated C mineralization (Lee et al. 2019; van der Bom et al. 2019; Wang et al. 2022). Manure application provides organic C substrates for microorganisms, and these C substrates are preferentially enriched in macroaggregates (Liu et al. 2020; Mustafa et al. 2020). Plastic film mulching is another common agricultural management practice (Steinmetz et al. 2016). Generally, the improved soil hydrothermal conditions by mulching accelerate microbial growth (Li et al. 2022). Given that fertilization-induced changes in soil resource availability within soil aggregates regulate the responses of soil microbial activities to mulching (Bo et al. 2024; Mo et al. 2021), these processes would further drive the redistribution of microbial-derived C between macroaggregates and microaggregates. However, the interactive effects between fertilizer application and film mulching on the dynamics of fungal and bacterial necromass C within soil aggregates remain poorly understood.

In this study, we qualified the contributions of fungal and bacterial necromass C (using amino sugars as biomarkers) to organic C within soil aggregates, and evaluated

the effect of fertilization combined with mulching on the dynamics of fungal and bacterial necromass C within soil aggregates. We hypothesized that: manure application combined with mulching would promote microbial necromass C accumulation within macroaggregates, especially fungal necromass C.

2. Materials and Methods

2.1. Soil samples

The soil samples were collected from the 28-year fertilization experimental station (43.30°N, 124.48°E), in Gongzhuling County, Jilin Province, China. This area has a monsoon-influenced hot-summer humid continental climate. The mean annual precipitation was 560 mm and mean annual temperature was 4.5 °C in 2017–2019. At this region, more than 80% of total yearly precipitation is concentrated from June to August, and seasonal drought and low soil temperature frequently occur in Spring (March to May) (Wang et al. 2021; Zhang et al. 2023). The soil type at this station is classified as a Mollisol (USDA soil taxonomy), which is derived from the quaternary loess-like sediments. No inorganic C existed in the current soils. The soil texture consists of 39% clay, 30% silt, and 31% sand. Monoculture maize (*Zea mays* L.) was sown in late April or early May and harvested in October. After maize harvesting, all aboveground maize straw residues were completely removed, and maize roots were incorporated into soils using a rototiller. This long-term fertilization experimental station was arranged following a randomized block design, each treatment had three replicates.

The following three fertilization treatments were selected for this study: no

fertilization (NF), inorganic fertilization (IF), and manure application (MF). The application rates of fertilizer were shown in Table S1. Inorganic fertilizer was applied to soil surface as basal fertilizer before seed sowing, and pig manure was applied to the corresponding plots after maize harvesting. The contents of total N and organic C in pig manure were 5.0 $g\ kg^{-1}$ and 112.0 $g\ kg^{-1}$, respectively. Soil samples were randomly taken from two depths (0–20 and 20–30 cm) of each plot of the three corresponding fertilization treatments on May 5th, 2018 (before sowing). Then, the sub-samples were passed through a 7 mm sieve to ensure soil homogeneity and remove rocks and maize roots for subsequent field mesocosm experiment. The basic properties of topsoil (0–20 cm) were shown in Table S2.

2.2. Field mesocosm experiment and soil sampling

This experiment included three fertilization (NF, IF, and MF) treatments with/without mulching. To avoid the impact of mulching on field management of long-term fertilization station, we conducted this experiment in the buffer zone of NF treatment on May 5th, 2018. The soil pits (0.9×0.6×0.3 m) were dug before polyvinyl chloride (PVC) containers (0.9×0.6×0.3 m) were vertically put into them (Ge et al. 2021). Each PVC container was divided into nine equal sections (three fertilization treatments with three random replicates under the same mulching/no-mulching) with PVC sheets. The top of PVC container was 30 cm above the soil surface. The sub-samples from 0–20 cm and 20–30 cm depths were returned to corresponding depths in the section of PVC containers, and then flattened to a soil hardness similar to that in the original treatment. For the plastic film mulching sub-treatments, a transparent and

143 colorless polyethylene film (0.02 mm thickness) was mulched on the soil surface of all
144 sections in PVC container. The old polyethylene film was completely removed, and the
145 new film was covered again every April and after each soil sampling. The polyethylene
146 film remained intact throughout the entire incubation period. The edges of the
147 polyethylene films were compacted with soil. No plants were grown in any container
148 during the entire incubation period.

149 Soil samples were randomly collected on days of 360 (April 30th, 2019) and 900
150 (October 9th, 2020) after mulching. In each section of the PVC containers, three soil
151 core (3.5 cm diameter) were randomly collected from 0–20 cm depth and fully mixed
152 into one soil sample. All visible plant residues and stones were removed, and fresh soil
153 samples were then stored in rigid boxes (4 °C) and transported to the laboratory. After
154 soil clods were lightly broken along natural break points, soil samples (<5 mm) were
155 dried to 10% gravimetric water content under constant-temperature conditions (4 °C)
156 for soil aggregate isolation.

157 **2.3. Soil aggregate isolation and laboratory analysis**

158 The soil aggregates were separated by dry sieving method (Bach and Hofmockel
159 2014; Schutter and Dick 2002). Briefly, the 100 g cool-drying soil samples were placed
160 on a 0.25 sieve mm and vertically shaken for 2 min (30 times min^{-1}) using a sieve
161 shaker (Retsch, AS 200, Germany). Finally, macroaggregates and microaggregates
162 were weighed and then used for organic C and amino sugars analyses. The recovery
163 rate of soil aggregates after fractionation ranged from 98.4% to 99.9%.

164 The contents of organic C and total N within soil aggregates were quantified using

an elemental analyzer (Elementar Vario EL III, Germany).

The contents of fungal and bacterial necromass C were calculated from the content of glucosamine and muramic acid based on the empirical conversion factors (Appuhn and Joergensen 2006; Liang et al. 2019). Glucosamine and muramic acid within soil aggregates were extracted following the method described by Zhang and Amelung (1996). The extraction procedures were given in detail in the Supplementary Materials. The calculations were as follows:

Fungal necromass C (g kg^{-1} soil) =

(glucosamine (g kg^{-1} soil)/179.2 - 2×muramic acid (g kg^{-1} soil)/251.2)×179.2×9 (1)

Bacterial necromass C (g kg^{-1} soil) = muramic acid (g kg^{-1} soil)×45 (2)

Microbial necromass C (g kg^{-1} soil) =

fungal necromass C (g kg^{-1} soil)+bacterial necromass C (g kg^{-1} soil) (3)

Where 179.2 and 251.2 are the molecular weight of glucosamine and muramic acid, respectively. The conversion coefficients of glucosamine to fungal necromass C and muramic acid to bacterial necromass C are 9 and 45, respectively (Joergensen 2018).

The proportions of microbial, fungal, and bacterial necromass C in organic C of soil aggregates represent the contributions of microbial, fungal, and bacterial necromass C to organic C of soil aggregates, respectively (Liang et al. 2019).

2.4. Statistical analysis

Two-way analysis of variance was used to analyze the effects of fertilization, mulching, and their interactive effects on the dependent variables. One-way analysis of variance with Tukey's HSD post-hoc test was conducted to examine differences in the

187 dependent variables among the treatments. Independent sample T-test was used to
188 compare the dependent variables between macroaggregates and microaggregates. For
189 all data, the homogeneity of variance was verified using Levene's test, and the
190 normality of residuals was checked using Shapiro-Wilk's test (P>0.05), histograms,
191 and normal Q-Q plots. Statistical analyses were conducted using SPSS 19.0 (IBM
192 Corporation, USA).

193 **3. Results**

194 **3.1. Contents of organic carbon and total nitrogen within soil aggregates**

195 Fertilization and mulching had interactive effects on the content of organic C of
196 soil aggregates (P<0.01, Fig. 1). Regardless of mulching or no-mulching, the MF
197 treatment increased the content of organic C within macroaggregates and
198 microaggregates, on average, by 71.4% and 44.9% compared with the NF treatment
199 during the entire incubation period. On day 360, mulching increased the organic C
200 content of macroaggregates by 5.4% and 5.8% under the IF and MF treatments,
201 respectively. On day 900, mulching increased the organic C content of macroaggregates
202 by 5.3% under the MF treatment, but decreased it by 6.8% under the NF treatment.
203 Mulching decreased the organic C content of microaggregates, on average, by 28.4%,
204 34.4%, and 38.8% under the NF, IF, and MF treatments during the entire incubation
205 period, respectively.

206 Fertilization and mulching had significant effects on the total N content within
207 macroaggregates (P<0.05, Fig. 1). During the entire incubation period, the MF
208 treatment increased the content of total N within macroaggregates, on average, by 52.6%

209 compared with the NF treatment under the same mulching and no-mulching. Mulching
210 decreased the content of total N within microaggregates under the NF, IF, and MF
211 treatments on day 360 and day 900 (P<0.05). On day 900, mulching decreased the ratio
212 of organic C to total N within macroaggregates under the NF and IF treatments, but
213 increased this ratio under the MF treatment (Fig. 1).

214 **3.2. Contents of microbial, fungal, and bacterial necromass carbon within soil**
215 **aggregates**

216 Fertilization and mulching had interactive effects on the microbial necromass C
217 content within soil aggregates (P<0.01, Fig. 2). Regardless of mulching or no-mulching,
218 the MF treatment increased the content of microbial necromass C within
219 macroaggregates and microaggregates, on average, by 148.6% and 84.5% compared
220 with NF treatment during the entire incubation period. On day 360, mulching increased
221 the microbial necromass C content within macroaggregates by 10.1%, 21.5%, and 23.5%
222 under the NF, IF, and MF treatments, respectively. On day 900, mulching increased the
223 microbial necromass C content within macroaggregates by 8.4% under the MF
224 treatment, but decreased it by 12.4% and 17.3% under the NF and IF treatments,
225 respectively. Mulching decreased the microbial necromass C content within
226 microaggregates, on average, by 23.1%, 36.5%, and 43.9% under the NF, IF, and MF
227 treatments during the entire incubation period, respectively.

228 The contents of fungal and bacterial necromass C within soil aggregates were
229 significantly affected by the interaction between fertilization and mulching (P<0.01,
230 Fig. 2). Relative to the NF treatment, the MF treatment increased the contents of fungal

231 and bacterial necromass C within soil aggregates and decreased the ratio of fungal to
232 bacterial necromass C within soil aggregates under the same mulching and no-mulching
233 during the entire incubation period. On day 360, mulching increased the contents of
234 fungal and bacterial necromass C within macroaggregates under the NF, IF, and MF
235 treatments. On day 900, mulching increased the content of fungal necromass C within
236 macroaggregates by 15.4% under the MF treatment. On day 900, the NF, IF, and MF
237 treatments with mulching decreased the bacterial necromass C content within
238 macroaggregates by 29.8%, 34.7%, and 7.9% relative to the corresponding treatments
239 without mulching, respectively. During the entire incubation period, mulching
240 decreased the contents of fungal and bacterial necromass C within microaggregates
241 under the NF, IF, and MF treatments, and decreased the ratio of fungal to bacterial
242 necromass C within microaggregates under the IF and MF treatments.

243 **3.3. Proportions of microbial, fungal, and bacterial necromass carbon in organic**
244 **carbon of soil aggregates**

245 On day 900, the microbial necromass C occupied 28.2%–42.9% of organic C
246 within macroaggregates, and 40.4%–55.8% of organic C within microaggregates,
247 respectively (Fig. 3). Fertilization and mulching had interactive effects on the
248 proportion of microbial necromass C in organic C of soil aggregates ($P<0.01$). During
249 the entire incubation period, the MF treatment increased the proportion of microbial
250 necromass C in organic C of soil aggregates compared with the NF treatment under the
251 same mulching and no-mulching. On day 360, mulching increased the proportion of
252 microbial necromass C in organic C of macroaggregates under the NF, IF, and MF

253 treatments. On day 900, mulching decreased the proportion of microbial necromass C
254 in organic C of macroaggregates by 6.0% and 13.9% under the NF and IF treatments,
255 respectively.

256 During the entire incubation period, the fungal and bacterial necromass C occupied
257 21.2%–32.0% and 6.1%–12.4% of organic C within macroaggregates, and the fungal
258 and bacterial necromass C occupied 29.1%–38.3% and 11.3%–18.5% of organic C
259 within microaggregates, respectively (Fig. 3). The proportion of fungal necromass C in
260 organic C of soil aggregates was significantly affected by the interaction between
261 fertilization and mulching (P<0.01). Mulching increased the proportion of fungal
262 necromass C in organic C of macroaggregates under the MF treatment during the entire
263 incubation period. The proportion of bacterial necromass C in organic C of
264 macroaggregates was affected by the interaction between fertilization and mulching
265 (P<0.05). During the entire incubation period, the MF treatment increased the
266 proportion of bacterial necromass C in organic C of soil aggregates compared with the
267 NF treatment regardless of mulching or no-mulching. On day 900, mulching decreased
268 the proportion of bacterial necromass C in organic C of macroaggregates by 24.7%,
269 32.0%, and 12.6% under the NF, IF, and MF treatments, respectively.

270 **4. Discussions**

271 **4.1. Impact of fertilization combined with mulching on microbial necromass**
272 **carbon accumulation within soil aggregates**

273 The contribution of microbial necromass C to organic C sequestration of
274 microaggregates was larger than that of macroaggregates on day 900 (Fig. 3). On the

one hand, microbial necromass binds with clay particles and metal oxides to form the core/building unit of microaggregates (Miltner et al. 2012; Totsche et al. 2018). In this case, microbial necromass C became a predominant SOC fraction of microaggregates. On the other hand, high proportions of plant-derived organic matter and polysaccharides in organic C weaken the importance of microbial necromass C in organic C sequestration within macroaggregates (Angst et al. 2021; Golchin et al. 1994; Tisdall 1994).

Regardless of mulching or no-mulching, the MF treatment promoted microbial necromass C accumulation and increased its contribution to organic C within soil aggregates compared with the NF treatment, especially within macroaggregates (Figs 2 and 3). Our previous study showed that manure application increased particulate organic C content (Liu et al. 2023). As labile SOC fractions, particulate organic C is readily utilized by microorganisms, thus accelerating microbial growth and turnover (Lavallee et al. 2020). Given that particulate organic matter is preferentially sequestrated in macroaggregates (Mustafa et al. 2020; Wang et al. 2017), manure application greatly enhanced microbial anabolism products accumulation within macroaggregates.

Mulching further stimulated microbial necromass C accumulation within macroaggregates under the MF treatment (Fig. 2). Large amounts of available organic C and nutrients are preferentially accumulated within macroaggregates after manure is applied to the soil (Mustafa et al. 2021). Meanwhile, mulching increases microbial biomass and activities due to the improvements of soil moisture and temperature (Wang

297 et al. 2015). Thus, the MF treatment with mulching might lead to the intensified
298 production of microbial-derived C within macroaggregates. In contrast, mulching
299 decreased the microbial necromass C content within soil aggregates under the NF and
300 IF treatments on day 900 (Fig. 2). There are two possible explanations for this
301 phenomenon. First, mulching in soils with limited organic C accelerates SOC
302 mineralization (Lee et al. 2019). This is evidenced by our previous result that mulching
303 decreased the contents of particulate organic C and extractable organic C under the NF
304 and IF treatments (Liu et al. 2023). In this case, microorganisms might invest less
305 energy for their growth, but more energy for decomposing persistent SOC (Burns et al.
306 2013; Henneron et al. 2022), which limits microbial necromass C accumulation.
307 Secondly, living soil microorganisms also directly decompose microbial necromass to
308 alleviate microbial constraints on nutrients stoichiometry (Kastner et al. 2021). The
309 recycling of microbial necromass can be enhanced under soil nutrients-limited
310 condition (Meier et al. 2017).

311 **4.2. Impact of fertilization combined with mulching on fungal and bacterial**
312 **necromass carbon accumulation within soil aggregates**

313 Regardless of soil macroaggregates or microaggregates, the contribution of fungal
314 necromass C to organic C was nearly two times more than that of bacterial necromass
315 C to organic C (Fig 3). Fungal necromass is more resistant to be decomposed relative
316 to bacterial necromass due to the relatively recalcitrant component of fungal cell wall
317 (Fernandez et al. 2019; Maillard et al. 2020). Moreover, fungal biomass is nearly two
318 times higher than that of bacteria in topsoil (He et al. 2020; Khan et al. 2016). During

319 the iterative process of microbial cell growth, lysis, and fragmentation, high fungal
320 biomass results in a large amount of fungal-derived C in organic C pool (Tian et al.
321 2022).

322 Manure application led to a decrease in the ratio of fungal to bacterial necromass
323 C within soil aggregates compared with the NF treatment under the same mulching and
324 no-mulching (Fig 2), indicating that bacterial necromass accumulation within soil
325 aggregates was more intense than that of fungal necromass following manure
326 application. Although manure particles are preferentially distributed within
327 macroaggregates, the turnover of soil aggregates drives the migration and redistribution
328 of manure particles between macroaggregates and microaggregates (Ding et al. 2015;
329 Mustafa et al. 2020). The locations of manure particles can be acted as hotspots for
330 microbial activities (Kuzyakov and Blagodatskaya 2015). Bacteria are more sensitive
331 to changes in available substrates than fungi (Marschner et al. 2011). Therefore, the
332 competitive advantage of bacteria might lead to a high contribution of bacterial
333 necromass to organic C after biomass turnover under the MF treatment.

334 Our study showed that mulching decreased the bacterial necromass C content
335 within macroaggregates among all treatments on day 900 (Fig. 3), which might be
336 related to their decomposition triggered by changes in soil nutrients availability.
337 Mulching changed the ratio of organic C to total N within macroaggregates (Fig. 1).
338 Bacterial necromass can actively regulate the stoichiometric balance of microbial C and
339 N (He et al. 2011; Hu et al. 2020). Thus, mulching would result in differential
340 accumulation patterns of fungal and bacterial necromass C within macroaggregates,

and exhibit strong bacterial responses. Moreover, it was also noted that the fungal necromass C content within macroaggregates was similar or lower in the NF and IF treatments with mulching than the corresponding treatments without mulching on day 900, respectively, but mulching stimulated fungal necromass C accumulation within macroaggregates under the MF treatment within 360–900 days (Fig. 2). Mulching increased the proportion of macroaggregates (Table S3). The increased proportion of macroaggregates could enhance fungal biomass and promote fungal necromass C production, because fungi predominantly proliferate in large pores (Huang et al. 2023; Murugan et al. 2019; Ye et al. 2019). Given that fungi are involved in the process of macroaggregates formation (Huang et al. 2023; Ye et al. 2019), we speculated that under C-rich MF treatment with mulching, macroaggregates might effectively protect fungal necromass from decomposition. Whereas under the NF and IF treatments, mulching might be not conducive to fungal necromass C accumulation within macroaggregates due to C substrates and energy.

5. Conclusions

Our results indicated that microbial necromass carbon made higher contribution to organic carbon of microaggregates than that of macroaggregates on day 900. Regardless of macroaggregates or microaggregates, the content of fungal necromass carbon was nearly two times higher than that of bacterial necromass carbon. Manure application, especially combined with mulching, promoted microbial-derived C preservation and microbial contribution to organic carbon sequestration within macroaggregates. In contrast, mulching resulted in the loss of microbial necromass

363 carbon and the decline of organic carbon within soil aggregates under no fertilization
364 and inorganic fertilization on day 900. Moreover, mulching enhanced fungal necromass
365 carbon production and increased its contribution to organic carbon with
366 macroaggregates under manure application. The accumulation of bacterial necromass
367 carbon within soil macroaggregates was weakened after plastic film was mulched for
368 900 days. Our result highlighted that manure application with mulching was a
369 recommended agricultural practice to increase organic carbon sequestration within
370 macroaggregates by enhancing fungal-derived carbon accumulation.

371 **Conflict of interest**

372 The authors declare no competing interests.

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Figure

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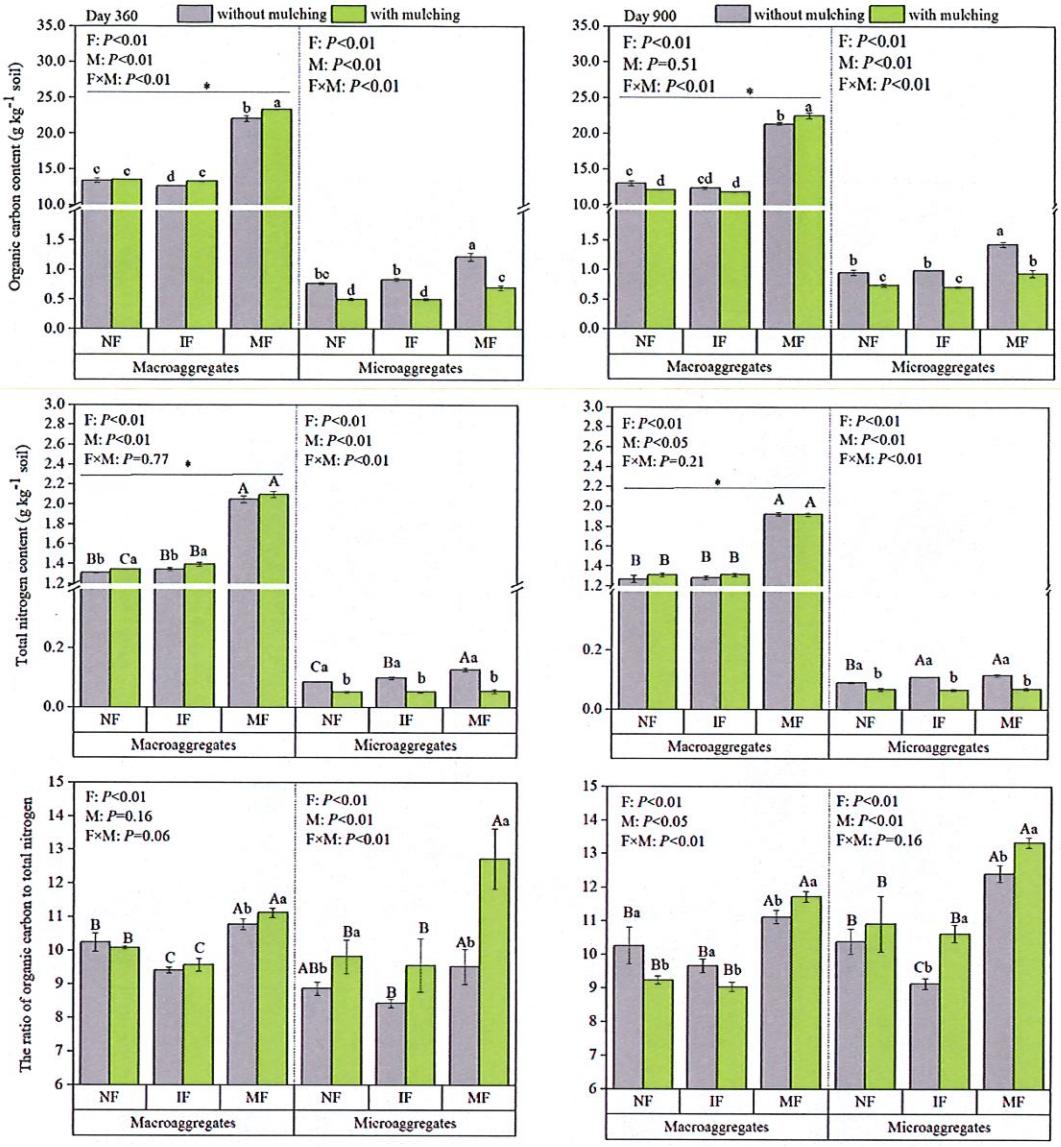

Fig. 1 Contents of organic carbon and total nitrogen and their ratio within soil aggregates under the fertilization treatments with/without plastic film mulching. NF, IF, and MF denote treatments of no fertilization, inorganic fertilization, and manure, respectively. Error bars indicate standard errors (n = 3). Different lowercase letters show significant differences (P<0.05) among the treatments. Effects of fertilization (F), mulching (M), and their interaction (F× M) on the organic carbon content were determined by two-way analysis of variance. Asterisks (*) indicate significant differences (P < 0.05) between soil macroaggregates and microaggregates

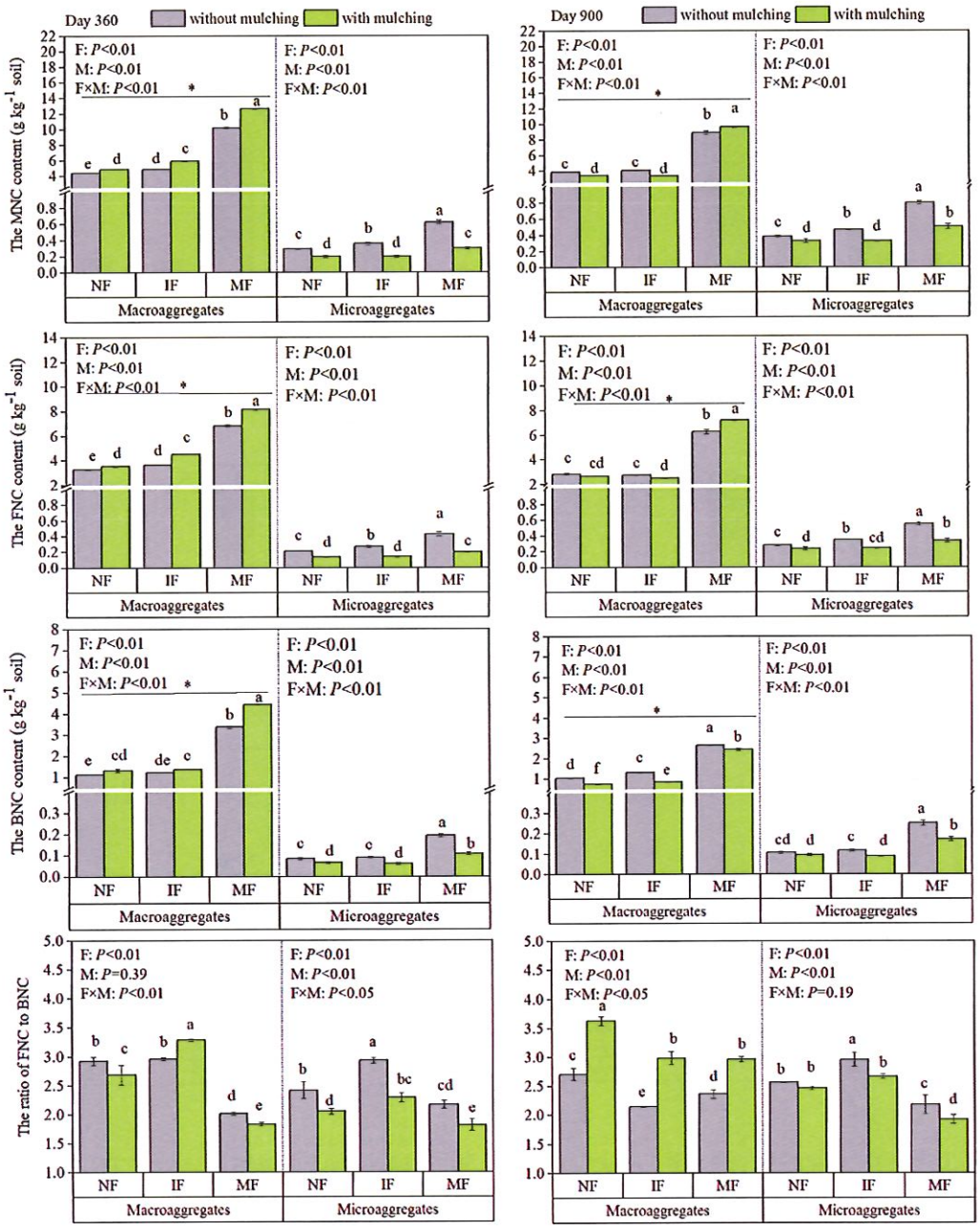

Fig. 2 Contents of microbial necromass carbon (MNC), fungal necromass carbon (FNC), and bacterial necromass carbon (BNC), and the ratio of FNC to BNC within soil aggregates under the fertilization treatments with/without plastic film mulching. NF, IF, and MF denote treatments of no fertilization, inorganic fertilization, and manure, respectively. Error bars indicate standard errors (n = 3). Different lowercase letters show significant differences (P<0.05) among the treatments. Effects of fertilization (F), mulching (M), and their interaction (F× M) on the contents of microbial, fungal, and bacterial necromass carbon were determined by two-way analysis of variance.

Asterisks (*) indicate significant differences ($P < 0.05$) between soil macroaggregates and microaggregates

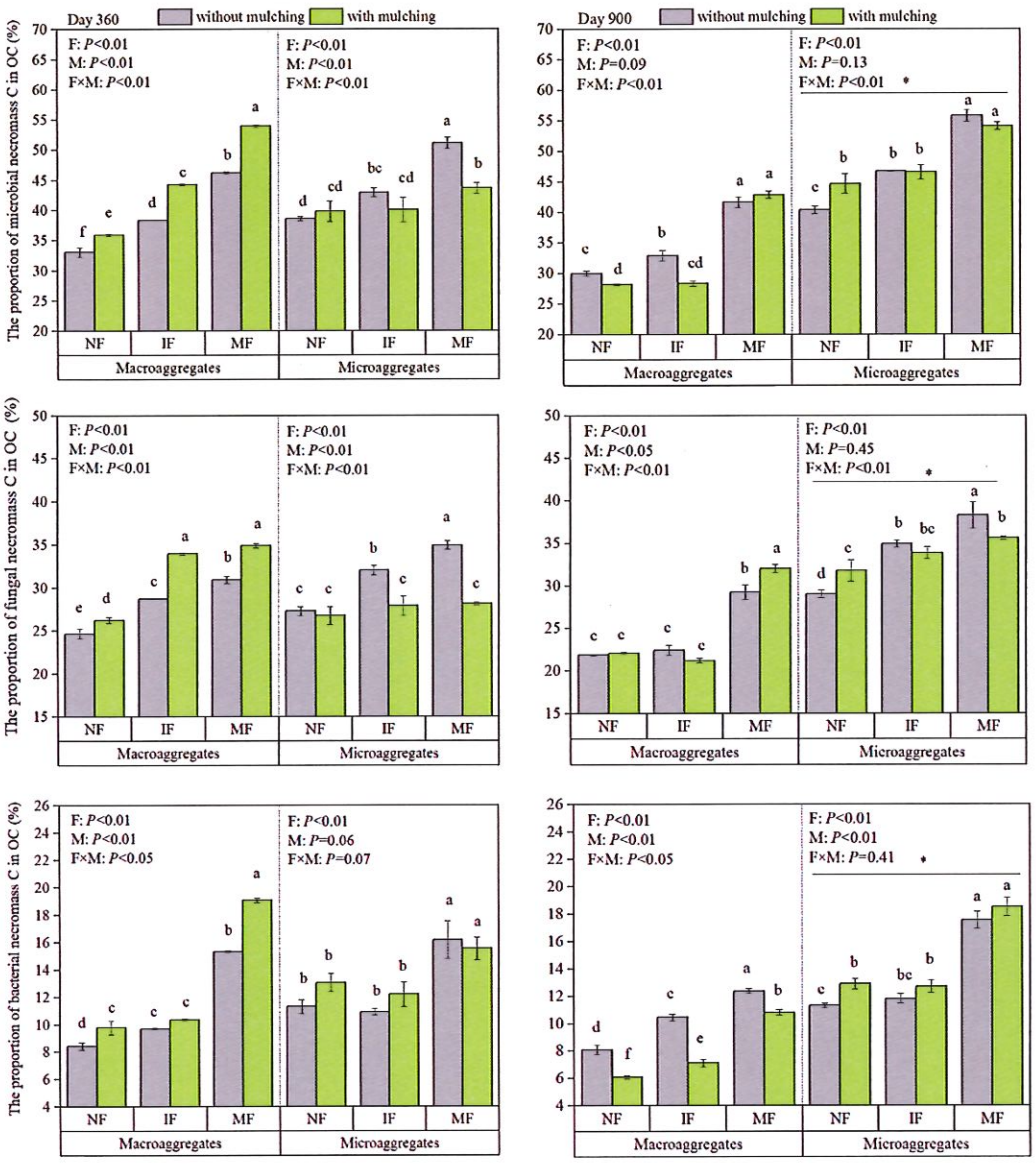

Fig. 3 Proportions of microbial, fungal, and bacterial necromass carbon in organic C (OC) of soil aggregates under the fertilization treatments with/without plastic film mulching. NF, IF, and MF denote treatments of no fertilization, inorganic fertilization, and manure, respectively. Error bars indicate standard errors (n = 3). Different lowercase letters show significant differences (P<0.05) among the treatments. Effects of fertilization (F), mulching (M), and their interaction (F× M) on the proportions of microbial, fungal, and bacterial necromass carbon in organic carbon were determined by two-way analysis of variance. Asterisks (*) indicate significant differences (P < 0.05) between soil macroaggregates and microaggregates