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RESEARCH ARTICLE

MICROBIAL NECROMASS WITHIN AGGREGATES STABILIZES PHYSICALLY-PROTECTED C RESPONSE TO CROPLAND MANAGEMENT

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KEYWORDS

cropland management, microbial necromass, soil aggregates, soil carbon sequestration, soil organic matter

HIGHLIGHTS

- The contribution of fungal necromass C to SOC increased with aggregate sizes.
- Bacterial necromass had a higher proportion to SOC in silt and clay.
- Cropland management increased microbial necromass in macro- and microaggregates.
- Greater fungal necromass increases were found in macroaggregates under manure input and no or reduced tillage.
- Cover crops increased bacterial necromass in small macroaggregates.

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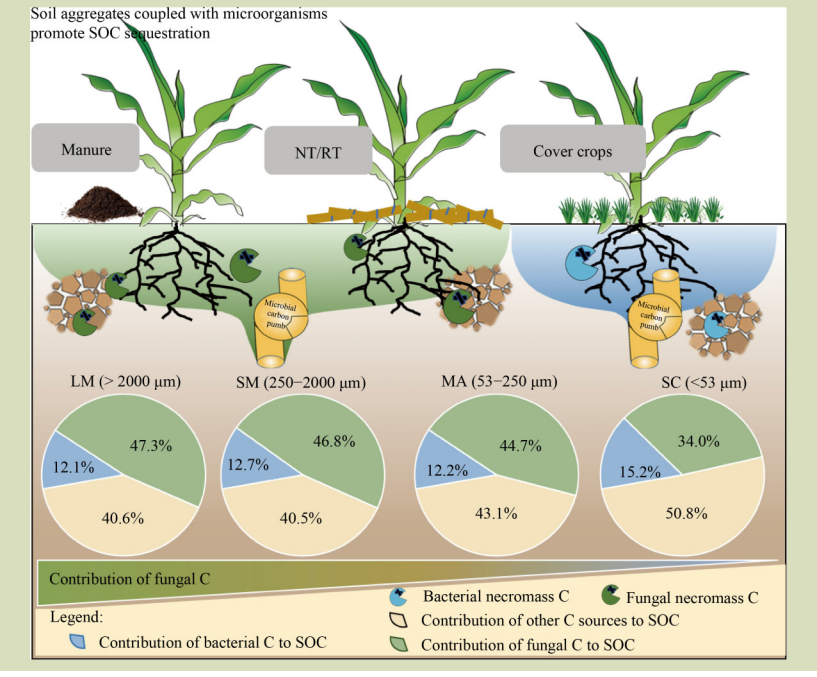
GRAPHICAL ABSTRACT



ABSTRACT

The interactions of soil microorganisms and structure regulate the degradation and stabilization processes of soil organic carbon (SOC). Microbial necromass is a persistent component of SOC, and its magnitude of accumulation dependent on management and aggregate sizes. A meta-analysis of 121 paired measurements was conducted to evaluate the management effects on contributions of microbial necromass to SOC depending on aggregate fractions. Results showed that the contribution of fungal necromass to SOC increased with aggregate sizes, while bacterial necromass had a higher proportion in silt and clay. Cropland management increased total and fungal necromass in large macroaggregates (47.1% and 45.6%), small macroaggregates (44.0% and 44.2%), and microaggregates (38.9% and 37.6%). Cropland management increased bacterial necromass independent of

aggregate fraction sizes. Greater fungal necromass was increased in macroaggregates in response to manure (26.6% to 28.5%) and no or reduced tillage (68.0% to 73.5%). Cover crops increased bacterial necromass by 25.1% in small macroaggregates. Stimulation of microbial necromass was proportional to the increases of SOC within soil aggregates, and the correlation was higher in macroaggregates. Increasing microbial necromass accumulation in macroaggregates can, therefore, be considered as a central component of management strategies that aim to accelerate C sequestration in agricultural soils.

1 INTRODUCTION

Soil organic carbon (SOC), as a key indicator of soil quality, has important functions such as nutrient supply, biodiversity maintenance and climate change mitigation[1,2]. Agricultural soils contain immense carbon pools but these are under considerable threat due to unsustainable cultivation practices[3]. At the global scale, agricultural soils have lost a half to two-thirds of total SOC compared with natural or uncultivated soils[4]. Increasing the potential for agricultural soils to sequester C, therefore, requires appropriate management practices, which are particularly important for agricultural sustainable development and climate change mitigation[5].

The SOC occlusion within aggregates is one of the most important physical preservation mechanisms because there are physical barriers between microorganisms, enzymes and their substrates[6]. Owing to different aggregate-size fractions provide spatially heterogeneous habitats, distribution of microorganisms and their activities among aggregates of different sizes are various[7,8]. Previous studies have shown that microbial products (e.g., residues or necromass) can enhance the stability of SOC through participating in soil aggregation, in turn, the degree to which microbial necromass accumulate in soil may depend on physical protection[9,10]. Generally, macroaggregates and microaggregates are hierarchically organized by organic matter from plant litters and microbial metabolites, which physically protect necromass from mineralizing within aggregates[11]. Microbial-derived C enrich in silt-clay fraction and was protected chemically via association with soil minerals[12]. These processes may further affect the distribution of microbial necromass in soil aggregates and would be regulated by management practices. Cropland management, i.e., nitrogen addition[13], manure[10], straw application[14], no or reduced tillage (NT/RT)[15], cover crops[16], influence microbial biomass and community structure composition, subsequently microbial necromass C associated with aggregates. Nonetheless, the magnitudes of change resulting from management effects varied among different assessments. Preferential accumulation of fungal-derived necromass in macroaggregates in response to no tillage was observed[17]. However, Li et al. showed bacterial necromass was highest in macroaggregates under conservation management[18]. Considering the significance of aggregates and microbial necromass to soil C pool, further research is needed to assess the proportions of microbial necromass C within soil aggregate fractions and the overall management effects. It is essential to enhance mechanistic comprehension of the vital roles of microbial byproducts and soil structure interaction drives physically C stabilization under cropland management and to formulate relevant management strategies.

Amino sugars have been widely used to study microbial necromass cycling and storage[10,19]. As many as 26 amino sugars have been identified in soil microorganisms, and various amino sugars are related to specific microbial populations[20]. Glucosamine, galactosamine, mannosamine and muramic acid, as four types of amino sugars, have been quantified in most studies[21,22]. Muramic acid occurs exclusively in the bacterial peptidoglycan. Glucosamine is a major source of fungal cell wall, and it is also found in bacterial peptidoglycan bonded to muramic acid[23]. Muramic acid and glucosamine have been employed to differentiate between fungal and bacterial necromass. In this study, we collected microbial necromass or muramic acid and glucosamine from soil aggregate fractions reported in experiments with paired management in cropland. We aimed to answer two questions: (1) How does microbial necromass distribute in different aggregates sizes in response to cropland management? (2) What are the key predictors for the accumulation of microbial necromass within soil aggregate fractions?

2 MATERIALS AND METHODS

2.1 Data compilation

The experiments to determine the concentrations of soil microbial necromass or amino sugars within soil aggregates in cropland were found in the Web of Science and China National Knowledge Infrastructure databases. The search terms were "microbial/bacterial/fungal necromass" or "microbial//bacterial/fungal residues" or "amino sugars" and "aggregates". All literature data were retrieved from peer-reviewed research articles published before October 2022. We focused on proportions of microbial necromass C in soil organic C within soil aggregates and the response of necromass C within aggregates to common cropland management. The following criteria were used to select suitable papers: (1) the experiments must include microbial necromass C or glucosamine and muramic acid within soil aggregates in cropland, excluding studies in grassland and forest ecosystems; (2) the experiment was implemented with a pairwise design, including cropland management and control (without respective amendment or practice); and (3) the means, standard deviation (SD), and sample sizes of the variables were available or could be obtained from the articles; if papers only included amino sugars, SD of microbial necromass was calculated by multiplying the mean by 0.1[24].

We extracted 121 independent observations from articles that met our criteria. These data covered four aggregates classifications[10]: large macroaggregates (LM; > 2000 μm), small macroaggregates (SM; 250–2000 μm), microaggregates (MA; 53–250 μm), silt and clay (SC; < 53 μm). The management practices with quantitative data, such as manure, straw, no or reduced tillage (NT/RT), and cover crops, were included. Most studies were concentrated in China and North America.

For each study in our data set, the mean values and standard deviations of the amino sugars (glucosamine and muramic acid) content under cropland management and control were extracted directly from the tables or were extracted by the free software GETDATA GRAPH DIGITIZER for data presented only in figures within the articles. The fungal and bacterial necromass C were calculated from glucosamine and muramic acid, respectively, using the following formula based on previously established stoichiometric conversion factors, as reviewed by Liang et al.[25].

$$\begin{aligned}&\text{Bacterial necromass C}\,(\text{mg}\cdot(\text{g soil})^{-1})\\&=\text{Muramic acid}\,(\text{mg}\cdot(\text{g soil})^{-1})\times 45\end{aligned}\tag{1}$$

$$\begin{aligned}\text{Fungal necromass C}=&[\text{Glucosamine as}\,(\text{mg}\cdot(\text{g soil})^{-1})\\&\div 179.17-2\times\text{Muramic acid}\,(\text{mg}\cdot(\text{g soil})^{-1})\\&\div 251.23]\times 179.17\times 9\end{aligned}\tag{2}$$

where, 45 is the conversion factor from muramic acid to bacterial necromass C, 9 is the conversion factor from glucosamine to fungal necromass C, 251.23 and 179.17 are the molecular weights of muramic acid and glucosamine, respectively.

The total microbial necromass C is the sum of fungal and bacterial necromass C. The ratios of fungal-to-bacterial necromass were used to evaluate the relative accumulation of fungal and bacterial necromass. The corresponding SOC contents within soil aggregates were extracted from the studies to evaluate the contributions of microbial-derived necromass to SOC.

Additionally, we recorded experiment location (e.g., longitude and latitude) and soil properties (initial soil pH, SOC, total N (TN), C/N and clay content). The mean annual temperature (MAT), mean annual precipitation (MAP), and aridity index were also extracted, or when not reported, extracted from the WorldClim database and the Global Aridity and PET database using latitude and longitude information.

2.2 Response metrics

A random-effect model was used to evaluate the effects of management on microbial necromass within soil aggregates and their contribution to SOC in cropland[26]. The natural log of the response ratio (RR) was calculated as the effect size, representing the management effects:

$$\text{RR}=\ln\left(\frac{X_\text{T}}{X_\text{C}}\right)=\ln(X_\text{T})-\ln(X_\text{C})\tag{3}$$

where, X_T and X_C are the mean of the management and control groups for variable X, respectively. The variance of RR was calculated as:

$$v=\frac{\text{SD}_\text{T}{}^2}{\text{N}_\text{T}X_\text{T}{}^2}+\frac{\text{SD}_\text{C}{}^2}{\text{N}_\text{C}X_\text{C}{}^2}\tag{4}$$

where, SD_T and SD_C are the standard deviations of the management and control groups, respectively, and N_T and N_C are the sample size of the management and control groups, respectively. The mean weighted response ratio (RR_{++}) was calculated from the individual pairwise comparison between management and control treatments:

$$\text{RR}_{++}=\frac{\Sigma(\text{w}_i\times\text{RR}_i)}{\Sigma\text{w}_i}\tag{5}$$

$$w_i = \frac{1}{v_i} \quad (6)$$

where, w_i is the weighting factor of the *i*th experiment in the group. To identify significant differences in the effect sizes, the 95% confidence intervals was calculated as $RR_{++} \pm 1.96 \times S(RR_{++})$, where, $S(RR_{++})$ is the standard error of RR_{++} calculated as $\sqrt{1/\Sigma w_i}$. If the 95% confidence intervals did not overlap with zero, the management effects significantly affected the target variables. To quickly account for the management effects, the percent change was calculated based on the weighted effect size as $(e^{RR_{++}} - 1) \times 100\%$ for all variables, including total, bacterial, and fungal necromass C; contributions of total, bacterial, and fungal necromass C to SOC and ratio of fungal-to-bacterial necromass C.

2.3 Statistical analysis

The effects size and 95% confidence intervals were calculated by using *rma.mv* function of the R package "metafor". Between-group heterogeneity and the probability described statistical differences of microbial necromass responses to management between different levels of the aggregate sizes. Linear regression was used to examine the relationship between the response ratios of microbial necromass C and the response ratios of SOC within soil aggregate fractions. Pearson correlation to assess the correlations between environmental variables and microbial necromass within soil aggregates was conducted using the R package "corrplot". These variables were collected at the treatment plots with cropland management at which the microbial necromass C data within soil aggregates were obtained. Egger's regression test and fail-safe analysis with Rosenberg method were used to test publication bias in the studies[27,28]. If *P* was > 0.05 in Egger's regression test or coefficients were > 5N + 10 in the fail-safe analysis (N is the sampling size in this study), then the effect sizes of variables are considered statistically significant, and the observed pattern indicated no sign of publication bias (Table S1).

3 RESULTS

3.1 Microbial necromass within soil aggregate fractions

The contributions of fungal necromass C to SOC were 47.3%, 46.8%, 44.7% and 34.0% in LM, SM, MA and SC fractions, respectively (Fig. 1). The contributions of bacterial necromass C to SOC were similar in LM, SM and MA, accounting for 12.1%, 12.7% and 12.2%, respectively. However, bacterial

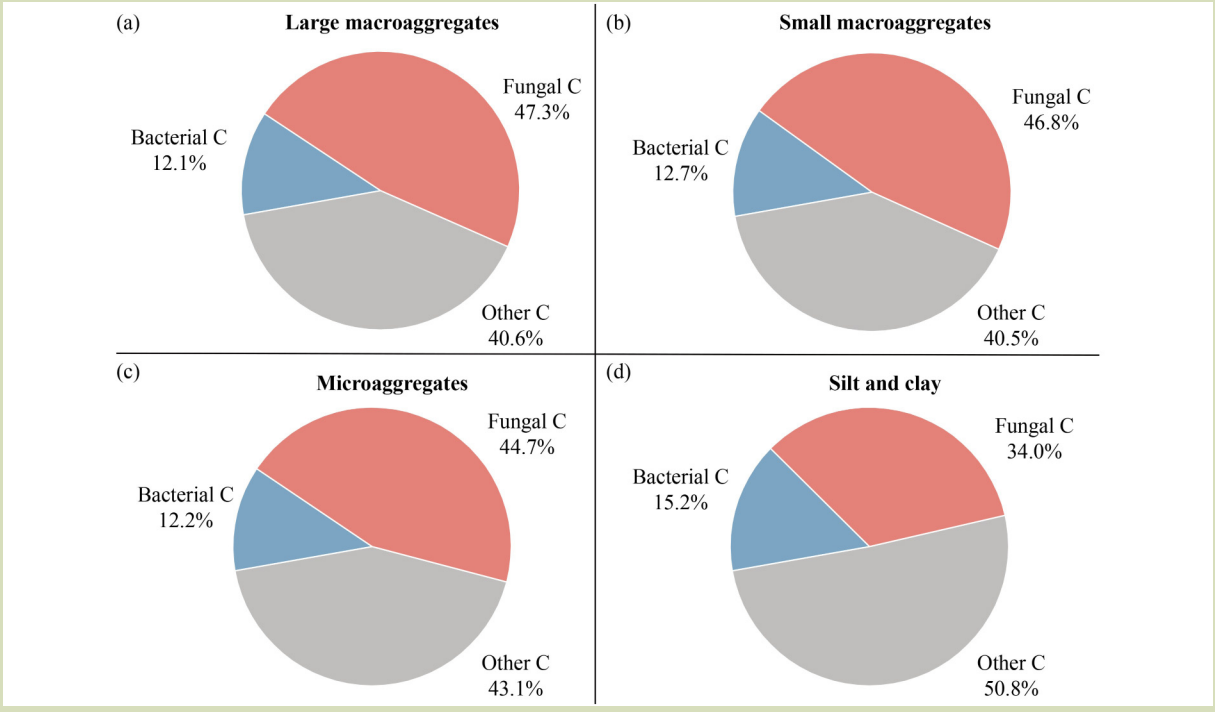


Fig. 1 Proportions of microbial necromass C in soil organic C within soil large macroaggregate (a), small macroaggregate (b), microaggregate (c), and silt and clay (d) in cropland.

necromass C had a higher proportion in SC than that in other fractions. Overall, total microbial necromass C contributed 59.4%, 59.5%, 56.9% and 49.2% of SOC of LM, SM, MA and SC, respectively.

3.2 Response of microbial necromass within soil aggregate fractions to cropland management

Across the data set, total microbial necromass C was not consistently affected by management across aggregate fraction sizes (Fig. 2(a)). The total microbial necromass C increased by 47.1%, 44.0% and 38.9% in LM, SM and MA, respectively, but the management effect was absent in SC. Bacterial and fungal necromass C within soil aggregate fractions responded differently to cropland management. Specifically, cropland management increased bacterial necromass C regardless of aggregate fraction sizes but the responses of fungal necromass C were contingent on soil aggregates (Table 1); cropland management significantly increased fungal necromass C in LM, SM, and MA, with some minor difference, but had no significant affect in SC (Fig. 2(b,c)), which is consistent with total necromass. The contributions of total and fungal necromass to SOC increased by 10.1% and 13.5% in SM fraction, respectively (Fig. 2(e,g)).

The microbial necromass C was significantly affected by management types, aggregate fractions and their interaction (Fig. 3). Especially, the responses of microbial necromass to manure application between different aggregate sizes was significant (Table 1). Manure application increased total microbial necromass C by 50.6% and 43.7% in LM and SM, respectively (Fig. 3(a)). Manure application increased fungal

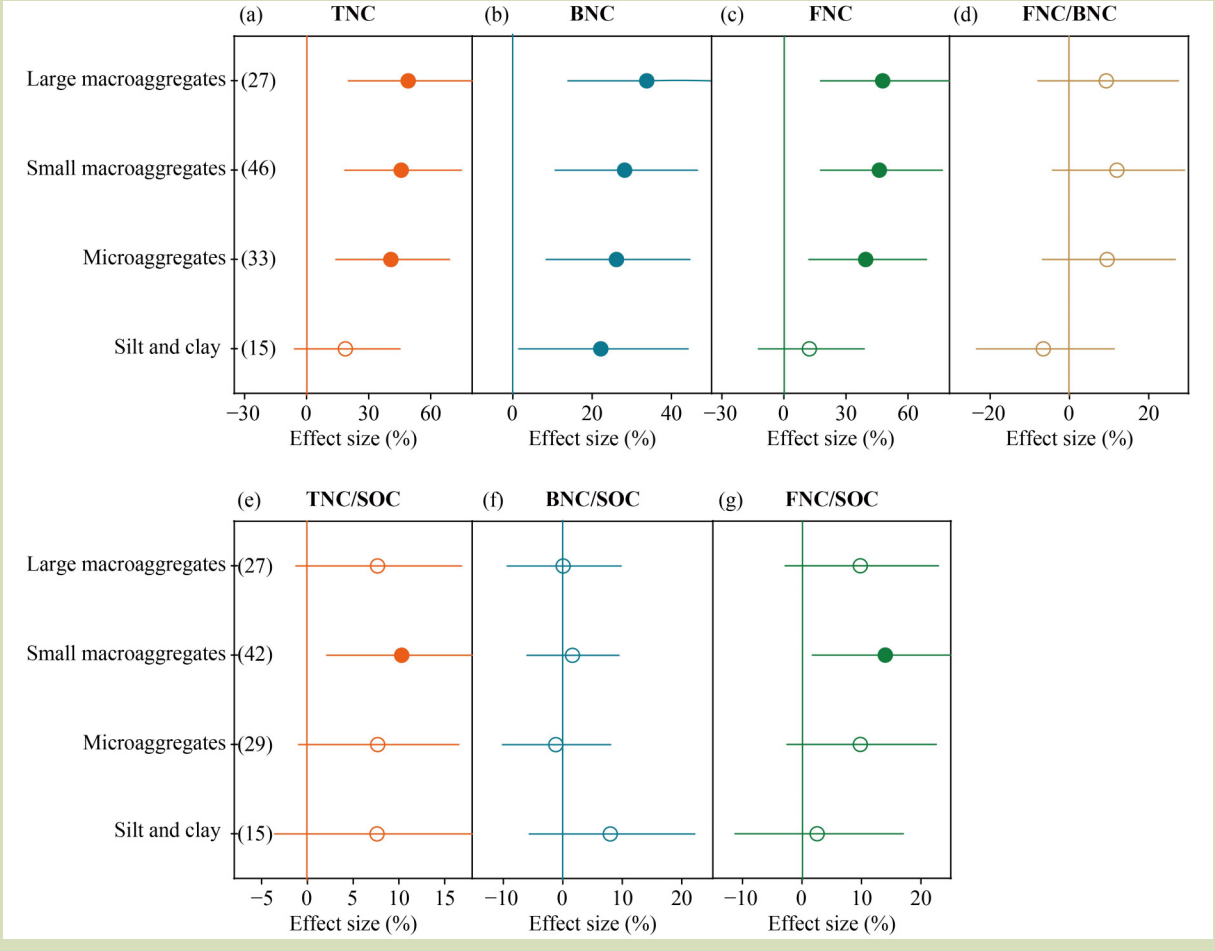


Fig. 2 The overall response of microbial necromass C (a–c), ratio of fungal-derived to bacterial-derived necromass (d) and necromass contribution to soil organic C (e–g) within soil aggregate fractions to management in cropland. TNC, total necromass C; BNC, bacterial necromass C; FNC, fungal necromass C; SOC, soil organic carbon; and FNC/BNC, the ratio of fungal-derived to bacterial-derived necromass C. The number of observations are shown in parentheses. Closed symbols indicate significant effects.

Table 1 Between-group heterogeneity (Q_M) and the probability (P) showing statistical differences of microbial necromass responses to management between different levels of the aggregate sizes

	Overall		Manure		Straw		NT/RT		Cover crops	
	Q_M	P	Q_M	P	Q_M	P	Q_M	P	Q_M	P
TNC	7.26	0.064	14.6	**0.002**	1.55	0.670	6.54	0.088	0.81	0.667
BNC	1.17	0.759	8.59	**0.035**	2.34	0.506	6.21	0.102	1.40	0.497
FNC	8.79	**0.032**	10.5	**0.014**	1.78	0.620	6.58	0.086	0.34	0.844
FNC/BNC	3.95	0.267	6.36	0.096	5.55	0.136	6.53	0.089	0.14	0.934
TNC/SOC	0.43	0.933	1.85	0.604	0.76	0.859	2.97	0.397	1.28	0.528
BNC/SOC	1.21	0.750	0.97	0.809	2.32	0.509	5.16	0.160	1.74	0.418
FNC/SOC	2.14	0.545	2.53	0.471	0.83	0.843	3.85	0.278	0.63	0.731

Note: Q_M is the heterogeneity of the weighted effect size associated with different aggregate sizes, and $P < 0.05$ is bold and indicates significant differences among different aggregate sizes. TNC, total necromass C; BNC, bacterial necromass C; FNC, fungal necromass C; SOC, soil organic carbon; FNC/BNC, the ratio of fungal-derived to bacterial-derived necromass C; and NT/RT, no or reduced tillage.

necromass C by 28.6% and 26.6% in LM and SM, which was higher than those in MA at 15.0% and SC at 14.5% (Fig. 3(c)). Greater accumulation of bacterial necromass C in response to manure was in LM at 60.2% (Fig. 2(b)). Straw application did not significantly increase microbial necromass C in any aggregate size (Fig. 3(a–c)). Straw application significantly increased the ratio of fungal-derived to bacterial-derived necromass in LM (Fig. 3(d)). NT/RT had a faster accumulation of total and fungal necromass C than bacterial necromass; increased total necromass C by 64.2% and 61.6%, and increased fungal necromass C by 68.0% and 73.5% in LM and SM, respectively (Fig. 3(a,c)). NT/RT led to greater accumulation of fungal necromass than bacterial necromass with the ratio of fungal-derived to bacterial-derived necromass being significantly greater in SM and MA (Fig. 3(d)). In SM, cover crops significantly increased total and bacterial necromass C by 22.9% and 25.1%, respectively, but not fungal necromass (Fig. 3(a–c)). For the contribution of necromass to SOC, the proportions of total necromass C in SOC increased 17.8% and 23.0% in LM and SM under NT/RT, respectively (Fig. 3(e)). NT/RT also increased the contribution of fungal necromass C to SOC in SM (Fig. 3(g)).

3.3 Relationships between environmental variables and microbial necromass within soil aggregate fractions

Correlation analyses indicated that both climatic conditions and soil properties are important factors associated with microbial necromass C and necromass contribution to SOC in LM, SM and MA (Fig. 4). MAT, MAP, SOC, TN and soil clay content were most strongly associated with microbial necromass C in SC, but had no significant correlation with both necromass contribution to SOC and ratio of fungal-derived to bacterial-derived necromass. The SOC, TN and soil clay content had positive relationships with microbial necromass C. MAT, MAP were negatively associated with microbial necromass in SC. Specially, microbial necromass C increased with SOC, TN, C/N and clay content in LM and MA whereas ratio of fungal-derived to bacterial-derived necromass decreased. In addition, microbial necromass within soil aggregate fractions increased with soil clay content increased.

3.4 Contribution of microbial necromass to physically stabilized C

The response ratios of total microbial necromass C were positively correlated with the response ratio of SOC within soil aggregate fractions (Fig. 5). The response ratios of bacterial necromass C were positively correlated with the response ratios of SOC in LM, SM and SC whereas there was no significant correction in MA. The response ratios of fungal necromass C were positively correlated with the response ratios of SOC in all aggregate fractions, especially in macroaggregates (Fig. 5), in which soil aggregates coupled with microorganisms (microbial necromass) physically stabilized SOC sequestration (Fig. 5 and Fig. 6).

4 DISCUSSION

Soil microbial necromass substantially contributes to SOM (15% to 80%)[9,25], which include intact or burst cells or hyphae, fragments of cell walls and monomers or polymers that were in the cytoplasm, biofilm or hyphal mucilage[9]. As binding agents, necromass help form or stabilize soil

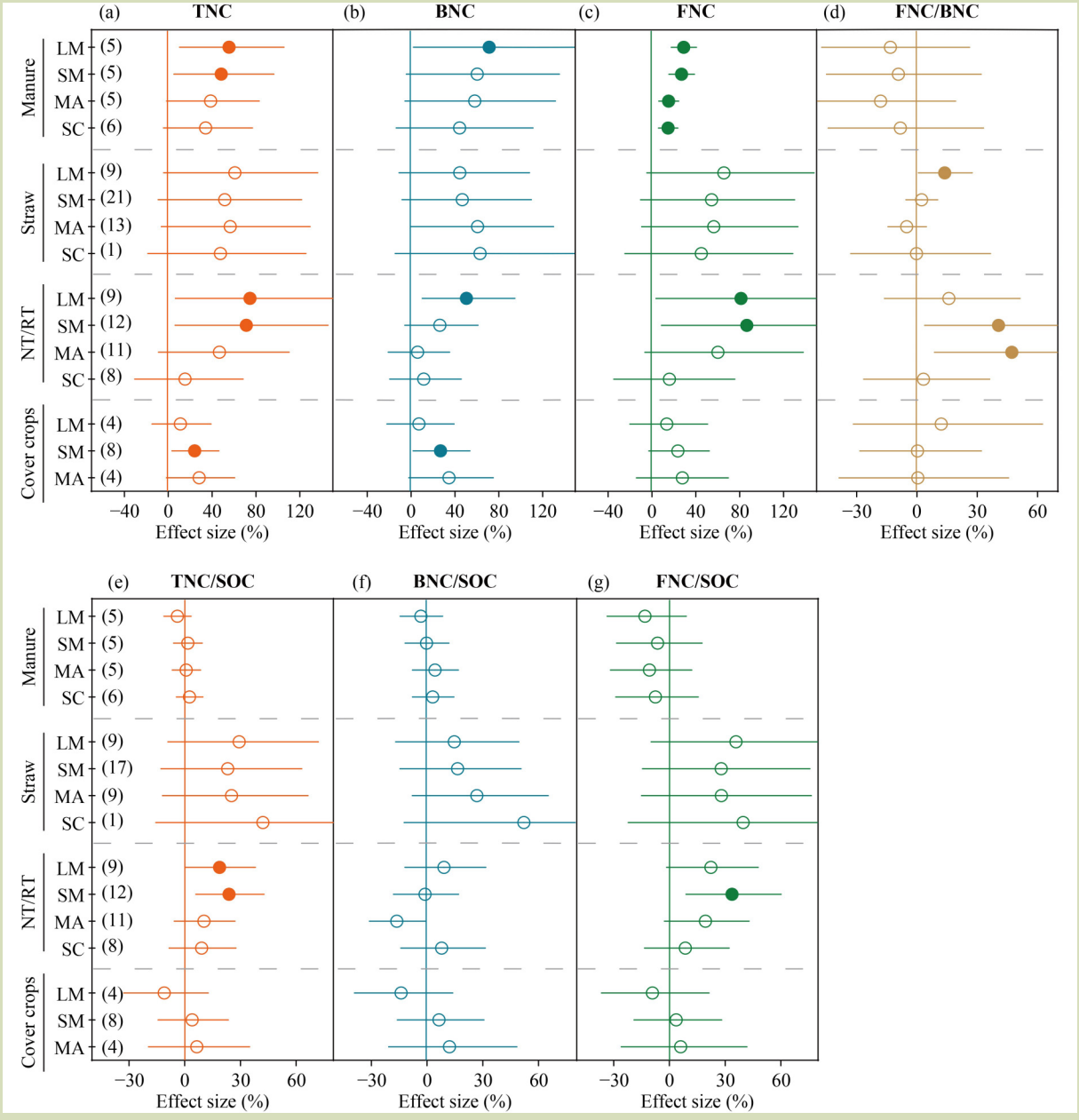


Fig. 3 Percent changes in microbial necromass C (a–c), ratio of fungal-derived to bacterial-derived necromass (d) and necromass contribution to soil organic C (e–g) within soil aggregate fractions dependent on cropland management. TNC, total necromass C; BNC, bacterial necromass C; FNC, fungal necromass C; SOC, soil organic carbon; FNC/BNC, the ratio of fungal-derived to bacterial-derived necromass C; NT/RT, no or reduced tillage; LM, large macroaggregates; SM, small macroaggregates; MA, microaggregates; and SC, silt and clay. The number of observations are shown in parentheses. Closed symbols indicate significant effects.

aggregates[29]. In turn, aggregates physically protect microbial necromass from degradation, which promotes more necromass accumulation[30]. Various aggregates fraction have different potential to influence the contribution microbial necromass to SOC. Our results illustrated total microbial necromass C can contribute 59.4%, 59.5%, 56.9%, and 49.2% of SOC in LM, SM, MA, and SC (Fig. 1). These proportions were higher than previous studies reporting that microbial necromass account for 47.2%, 49.7%, and 38.6% of stabilized SOM for macroaggregates, microaggregates, and silt and clay fraction, respectively[12]. In the present study, we only included data from managed cropland, while data from forest and

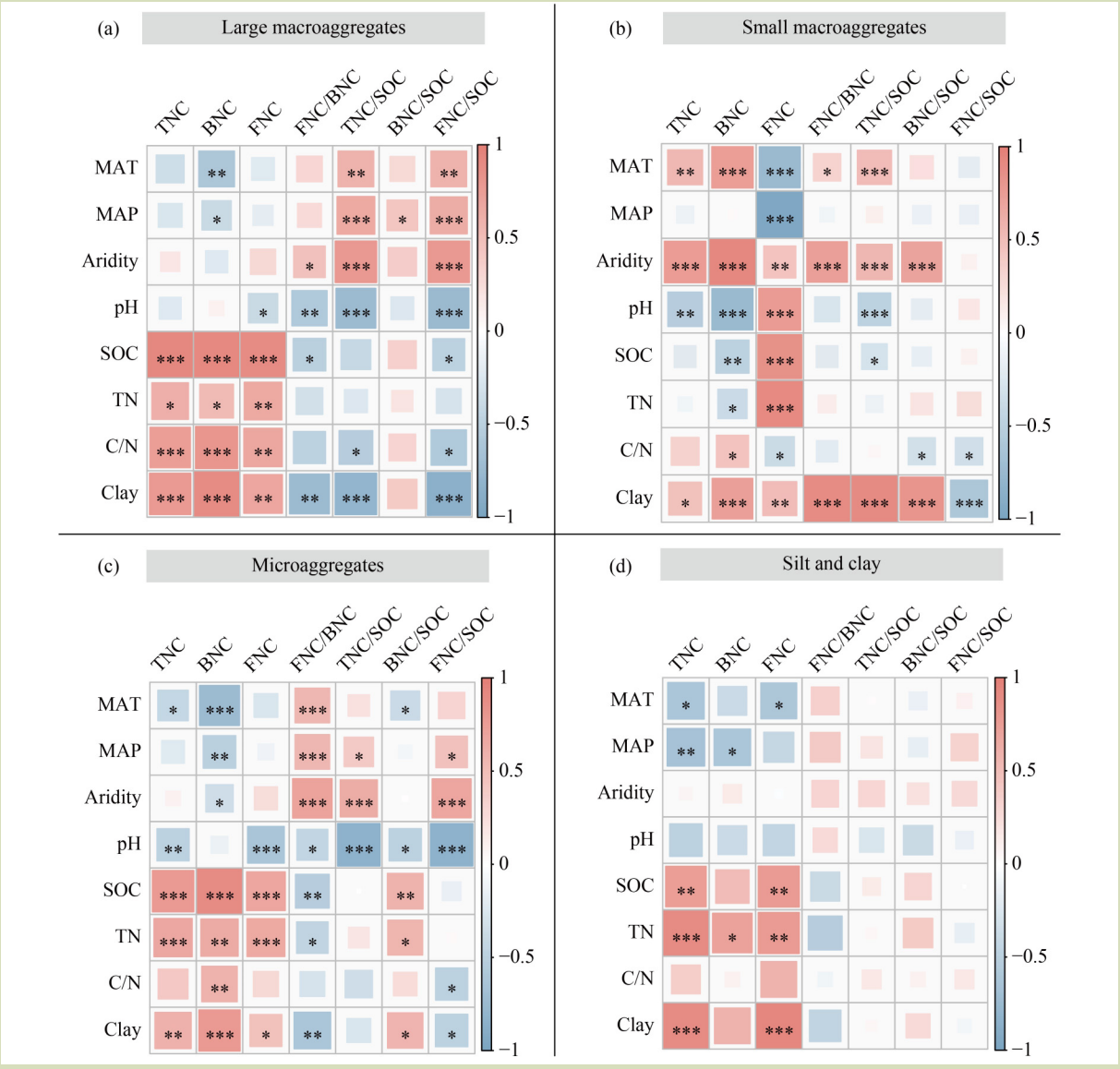


Fig. 4 Correlations between environmental variables and microbial necromass within soil large macroaggregate (a), small macroaggregate (b), microaggregate (c), and silt and clay (d). All microbial necromass C data within soil aggregate fractions were obtained from samples collected after the application of management practice. TNC, total necromass C; BNC, bacterial necromass C; FNC, fungal necromass C; MAT, mean annual temperature; MAP, mean annual precipitation; pH, soil pH; SOC, soil organic carbon; TN, total nitrogen; C/N, ratio of soil carbon to nitrogen; and Clay, soil clay content. *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.5$.

agricultural soil were included in a previous study[12]. Due to increased proportions of mineral-associated C and more rapid microbial transformation of litter in croplands, the microbial necromass contribution to SOC has been found to be larger than that in forests[25,31]. It is generally believed that microbial necromass may favorably accumulate in mineral fractions (silt and clay) due to reducing the distances between necromass and sorption sites[32]. However, the contribution of total necromass to SOC in SC fraction was lower than those in LM, SM, and MA (Fig. 1). This may be due to microbial necromass in macro- and microaggregates, apart from being attached to mineral surfaces, can also be protected in aggregates associated small pores[33]. The contribution of fungal necromass to SOC increased with aggregate sizes (Fig. 6), which highlights the role of fungi in aggregate formation and stabilization[30]. Meanwhile, high correlations between the response ratios of fungal necromass and SOC associated with aggregates were observed (Fig. 5). Bacterial necromass can directly, but non-

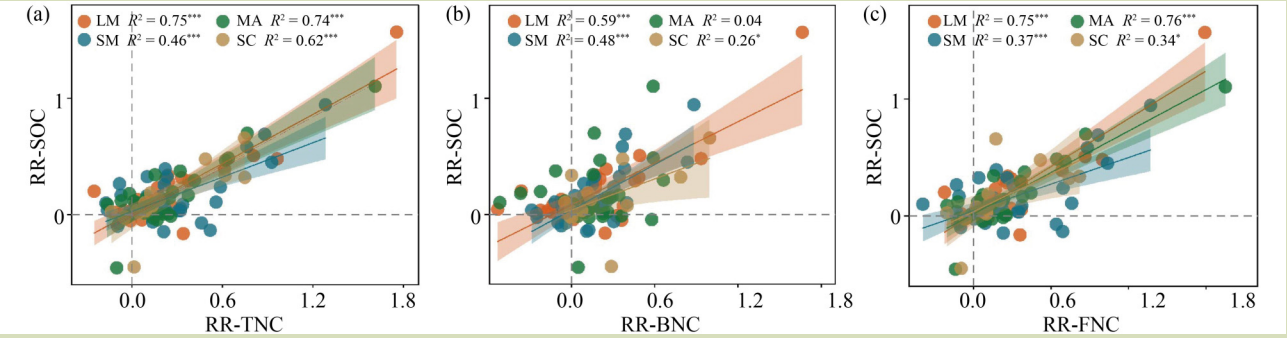


Fig. 5 Relationship between the response ratios (RRs) of total microbial necromass C (a), bacterial necromass C (b), fungal necromass C (c) and the response ratios of SOC within soil aggregate fractions. TNC, total necromass C; BNC, bacterial necromass C; FNC, fungal necromass C; SOC, soil organic carbon; LM, large macroaggregates; SM, small macroaggregates; MA, microaggregates; and SC, silt and clay. Shaded areas represent the 95% confidence band of the regression models. *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.5$.

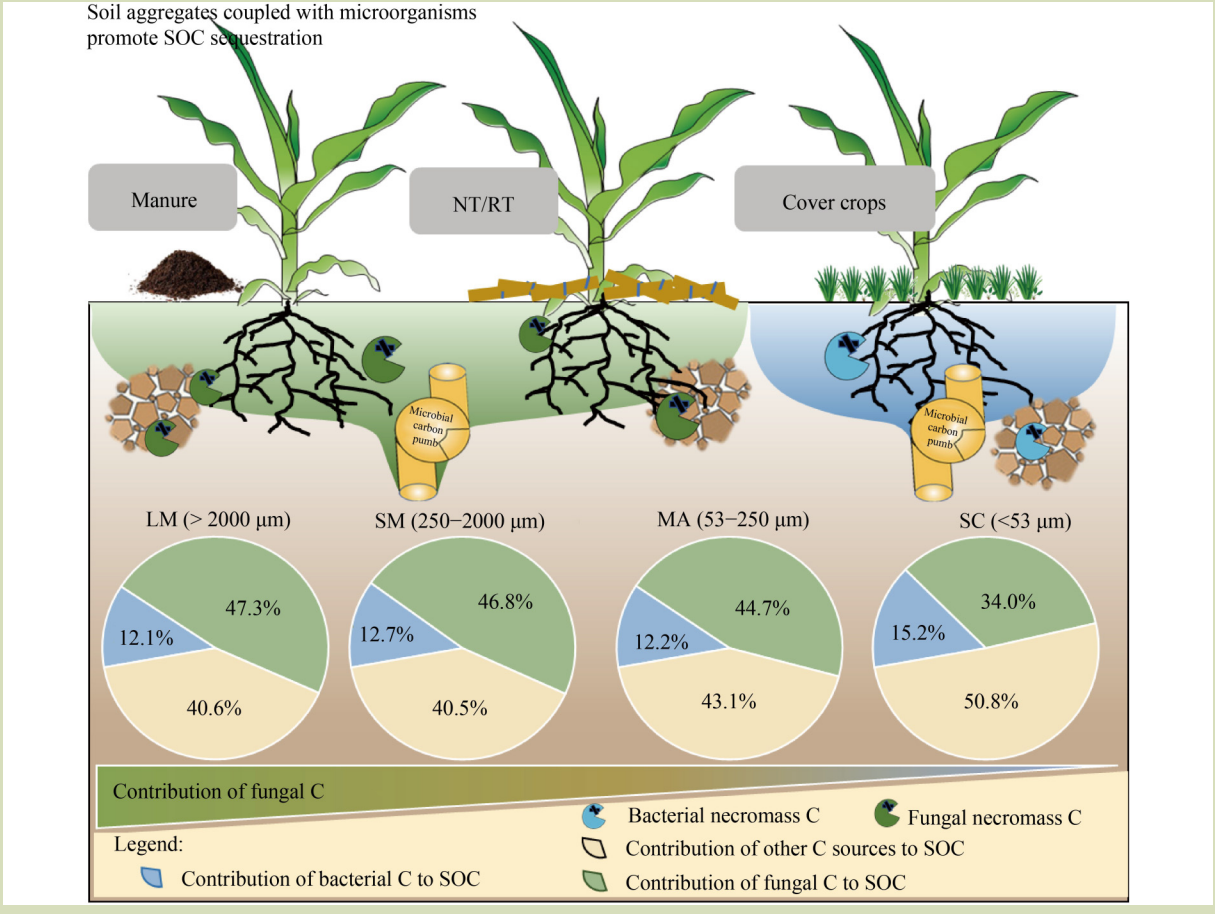


Fig. 6 Concept and meta-analysis results of the responses of necromass C within soil aggregate fractions to cropland management. NT/RT, no or reduced tillage; LM, large macroaggregates; SM, small macroaggregates; MA, microaggregates; and SC, silt and clay.

specifically, attach to clay surfaces[34], thus have a higher proportion in SC than that in other fractions (Fig. 1). Therefore, the contribution of fungal and bacterial necromass to SOC largely is dependent of aggregates fraction. It will be

necessary to understand the regulation of soil aggregates coupled with microorganisms on the organic matter upgrading.

Management practices can increase microbial necromass accumulation in cropland soil[35–37], which depended on soil aggregates. An increase in the total microbial necromass was observed in LM, SM and MA but this was not evident in SC (Fig. 2(a)). This result answered our first question that cropland management affected total microbial necromass dependent on aggregate fractions. It is possible that saturation of microbial necromass in silt-clay occurred earlier than in greater aggregates leading to additional C only accumulating in larger aggregates[10]. However, cropland management increased bacterial necromass C in all aggregate fractions. Especially, bacterial necromass also increased in the SC fraction (Fig. 2(b)). Studies have reported a dominance of bacterial, rather than fungal amino sugars in the SC fraction[38]. Meanwhile, bacterial necromass held a higher proportion in SC than other fractions (Fig. 1 and Fig. 6). These results indicated that bacterial necromass may be an important variable influenced by management in SC fraction[17].

The management effects were strongly depended on different practices (Fig. 3). Greater accumulation of total microbial necromass in LM and SM in respond to manure and NT/RT were observed (Fig. 3(a)). The statistical difference was absent due to the small number of studies under NT/RT (Table 1). Manure and NT/RT increased more fungal necromass in LM and SM than those in MA and SC (Fig. 3(c)), which indicates that fungi in macroaggregates was most influenced by cropland management[39]. NT/RT reduced soil disturbance, promoted aggregates formation and protected fungal hyphae, thus a preferential accumulation of fungal necromass in macroaggregates[40]. Strengthen of stable aggregates under manure can be spatially protected from microbial decomposition when microbial necromass occlude in soil aggregates[36]. Under cover crops, supply of diverse microbial substrates through litter, root exudates and rhizodeposits increase soil bacterial activity and/or growth[18], leading to intensified production of bacterial, but not fungal, necromass (Fig. 3(b,c)). Management promoted only microbial contribution to SOC in SM, especially under NT/RT (Fig. 2(e) and Fig. 3(e)). NT/RT increased greater contribution of fungal necromass to SOC rather than bacterial necromass (Fig. 2(g) and Fig. 3(g)), which indicated that fungal-derived C is predominantly contributes to stable SOC accrual. The increased contribution of microbial necromass in SM may be because SM have more capacity for necromass accumulation, compared to microaggregates and silt-clay. Meanwhile, SM has a more stable necromass pool as SM stability is higher than LM[34]. Management influence microbial necromass by the growth of plant roots and microorganism, thus microbial necromass in macro- and microaggregates was easily controlled by management whereas those in silt-clay depend on sorption sites, independent of management[41]. Increasing microbial necromass accumulation within aggregates could be considered important management strategies that aim to accelerate C sequestration in agricultural soils.

Soil nutrients exert significant control over microbial necromass accumulation. As observed, SOC and TN had positive correlations with microbial necromass C within aggregates (Fig. 4(a,c,d)). Microbial necromass sequestration will be more efficient in nutrient-rich soil due to high C use efficiency[42]. The ratio of fungal-derived to bacterial-derived necromass decreased with nutrients increased because higher nutrients condition favor bacterial growth and subsequent increases in bacterial necromass[10]. Soil texture is also one of the key factors regulating SOC stability[43]. In general, high clay content resulted in the ability to stabilize microbial necromass C by physicochemical protection, thus increased necromass accumulation in all aggregates fraction. Climactic conditions had no obvious consistent impact on microbial necromass within aggregates (Fig. 4), therefore further study the climatic conditions favoring microbial necromass accumulation within soil aggregates is needed.

5 CONCLUSIONS

Cropland management practices increased microbial necromass associated with aggregates with an increase of bacterial necromass in all aggregate fraction sizes and an increase of fungal necromass, except for in SC. Manure and NT/RT increased fungal necromass in macroaggregates, and cover crops increased bacterial necromass in SM. The response ratios of fungal necromass positively correlated with SOC associated with aggregates, especially in macroaggregates. Consequently, it is necessary to consider the accumulation of microbial necromass associated with aggregates under cropland management, which could favor stable soil C formation and accrual in croplands soil.

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2023498 contains supplementary material (Table S1)

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Compliance with ethics guidelines

Ranran Zhou, Jing Tian, Zhengling Cui, and Fusuo Zhang declare that they have no conflict of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

REFERENCES

1. Lehmann J, Bossio D A, Kögel-Knabner I, Rillig M C. The concept and future prospects of soil health. *Nature Reviews. Earth & Environment*, 2020, **1**(10): 544–553
2. Hoffland E, Kuyper T W, Comans R N J, Creamer R E. Eco-functionality of organic matter in soils. *Plant and Soil*, 2020, **455**(1–2): 1–22
3. Eswaran H, Vandenberg E, Reich P. Organic-carbon in soils of the world. *Soil Science Society of America Journal*, 1993, **57**(1): 192–194
4. Lal R. Soil carbon sequestration to mitigate climate change. *Geoderma*, 2004, **123**(1–2): 1–22
5. Lessmann M, Ros G H, Young M D, de Vries W. Global variation in soil carbon sequestration potential through improved cropland management. *Global Change Biology*, 2022, **28**(3): 1162–1177
6. Six J, Callewaert P, Lenders S, De Gryze S, Morris S J, Gregorich E G, Paul E A, Paustian K. Measuring and understanding carbon storage in afforested soils by physical fractionation. *Soil Science Society of America Journal*, 2002, **66**(6): 1981–1987
7. Schutter M E, Dick R P. Microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil. *Soil Science Society of America Journal*, 2002, **66**(1): 142–153
8. Rillig M C, Muller L A, Lehmann A. Soil aggregates as massively concurrent evolutionary incubators. *ISME Journal*, 2017, **11**(9): 1943–1948
9. Buckeridge K M, Creamer C, Whitaker J. Deconstructing the microbial necromass continuum to inform soil carbon sequestration. *Functional Ecology*, 2022, **36**(6): 1396–1410
10. Ding X L, Liang C, Zhang B, Yuan Y R, Han X Z. Higher rates of manure application lead to greater accumulation of both fungal and bacterial residues in macroaggregates of a clay soil. *Soil Biology & Biochemistry*, 2015, **84**: 137–146
11. Liang C, Schimel J P, Jastrow J D. The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, 2017, **2**(8): 17105
12. Angst G, Mueller K E, Nierop K G J, Simpson M J. Plant- or microbial-derived? A review on the molecular composition of stabilized soil organic matter. *Soil Biology & Biochemistry*, 2021, **156**: 108189
13. Lu X, Hou E, Guo J, Gilliam F S, Li J, Tang S, Kuang Y. Nitrogen addition stimulates soil aggregation and enhances carbon storage in terrestrial ecosystems of China: a meta-analysis. *Global Change Biology*, 2021, **27**(12): 2780–2792
14. Huang R, Lan M, Liu J, Gao M. Soil aggregate and organic carbon distribution at dry land soil and paddy soil: the role of different straws returning. *Environmental Science and Pollution Research International*, 2017, **24**(36): 27942–27952
15. Zheng H, Liu W, Zheng J, Luo Y, Li R, Wang H, Qi H. Effect of long-term tillage on soil aggregates and aggregate-associated carbon in black soil of Northeast China. *PLoS One*, 2018, **13**(6): e0199523
16. Topps D, Khabir M I U, Abdelmagid H, Jackson T, Iqbal J, Robertson B K, Pervaiz Z H, Saleem M. Impact of cover crop monocultures and mixtures on organic carbon contents of soil aggregates. *Soil Systems*, 2021, **5**(3): 43
17. Simpson R T, Frey S D, Six J, Thiet R K. Preferential accumulation of microbial carbon in aggregate structures of no-tillage soils. *Soil Science Society of America Journal*, 2004, **68**(4): 1249–1255
18. Li L D, Wilson C B, He H B, Zhang X D, Zhou F, Schaeffer S M. Physical, biochemical, and microbial controls on amino sugar accumulation in soils under long-term cover cropping and no-tillage farming. *Soil Biology & Biochemistry*, 2019, **135**: 369–378
19. Tian J, Zong N, Hartley I P, He N, Zhang J, Powlson D, Zhou J, Kuzyakov Y, Zhang F, Yu G, Dungait J A J. Microbial

metabolic response to winter warming stabilizes soil carbon. *Global Change Biology*, 2021, **27**(10): 2011–2028

20. Joergensen R G. Amino sugars as specific indices for fungal and bacterial residues in soil. *Biology and Fertility of Soils*, 2018, **54**(5): 559–568
21. Chen X B, Xia Y H, Rui Y C, Ning Z, Hu Y J, Tang H M, He H B, Li H X, Kuzyakov Y, Ge T D, Wu J S, Su Y R. Microbial carbon use efficiency, biomass turnover, and necromass accumulation in paddy soil depending on fertilization. *Agriculture, Ecosystems & Environment*, 2020, **292**: 106816
22. Luo Y, Xiao M L, Yuan H Z, Liang C, Zhu Z K, Xu J M, Kuzyakov Y, Wu J S, Ge T D, Tang C X. Rice rhizodeposition promotes the build-up of organic carbon in soil via fungal necromass. *Soil Biology & Biochemistry*, 2021, **160**: 108345
23. Hu Y T, Zheng Q, Noll L, Zhang S S, Wanek W. Direct measurement of the in situ decomposition of microbial-derived soil organic matter. *Soil Biology & Biochemistry*, 2020, **141**: 107660
24. Du Y D, Cui B J, Zhang Q, Wang Z, Sun J, Niu W Q. Effects of manure fertilizer on crop yield and soil properties in China: a meta-analysis. *Catena*, 2020, **193**: 104617
25. Liang C, Amelung W, Lehmann J, Kästner M. Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology*, 2019, **25**(11): 3578–3590
26. Hedges L V, Gurevitch J, Curtis P S. The meta-analysis of response ratios in experimental ecology. *Ecology*, 1999, **80**(4): 1150–1156
27. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* (*Clinical Research Edition*), 1997, **315**(7109): 629–634
28. Rosenberg M S. The file-drawer problem revisited: a general weighted method for calculating fail-safe numbers in meta-analysis. *Evolution*, 2005, **59**(2): 464–468
29. Chantigny M H, Angers D A, Prévost D, Vézina L P, Chalifour F P. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. *Soil Science Society of America Journal*, 1997, **61**(1): 262–267
30. Six J, Frey S D, Thiet R K, Batten K M. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal*, 2006, **70**(2): 555–569
31. Throckmorton H M, Bird J A, Dane L, Firestone M K, Horwath W R. The source of microbial C has little impact on soil organic matter stabilisation in forest ecosystems. *Ecology Letters*, 2012, **15**(11): 1257–1265
32. Wei H, Guenet B, Vicca S, Nunan N, Asard H, Abdelgawad H, Shen W, Janssens I A. High clay content accelerates the decomposition of fresh organic matter in artificial soils. *Soil Biology & Biochemistry*, 2014, **77**: 100–108
33. Totsche K U, Amelung W, Gerzabek M H, Guggenberger G, Klumpp E, Knief C, Lehndorff E, Mikutta R, Peth S, Prechtel A, Ray N, Kögel-Knabner I. Microaggregates in soils. *Journal of Plant Nutrition and Soil Science*, 2018, **181**(1): 104–136
34. Six J, Elliott E T, Paustian K. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology & Biochemistry*, 2000, **32**(14): 2099–2103
35. Samson M E, Chantigny M H, Vanasse A, Menasseri-Aubry S, Royer I, Angers D A. Management practices differently affect particulate and mineral-associated organic matter and their precursors in arable soils. *Soil Biology & Biochemistry*, 2020, **148**: 107867
36. Ye G P, Lin Y X, Kuzyakov Y, Liu D Y, Luo J F, Lindsey S, Wang W J, Fan J B, Ding W X. Manure over crop residues increases soil organic matter but decreases microbial necromass relative contribution in upland Ultisols: results of a 27-year field experiment. *Soil Biology & Biochemistry*, 2019, **134**: 15–24
37. Zhou R, Liu Y, Dungait J A J, Kumar A, Wang J, Tiemann L K, Zhang F, Kuzyakov Y, Tian J. Microbial necromass in cropland soils: a global meta-analysis of management effects. *Global Change Biology*, 2023, **29**(7): 1998–2014
38. Griepentrog M, Bodé S, Boeckx P, Hagedorn F, Heim A, Schmidt M W I. Nitrogen deposition promotes the production of new fungal residues but retards the decomposition of old residues in forest soil fractions. *Global Change Biology*, 2014, **20**(1): 327–340
39. Hannula S E, Morriën E. Will fungi solve the carbon dilemma. *Geoderma*, 2022, **413**: 115767
40. Yang Y, Xie H, Mao Z, Bao X, He H, Zhang X, Liang C. Fungi determine increased soil organic carbon more than bacteria through their necromass inputs in conservation tillage croplands. *Soil Biology & Biochemistry*, 2022, **167**: 108587
41. Tisdall J M, Oades J M. Organic matter and water-stable aggregates in soils. *Journal of Soil Science*, 1982, **33**(2): 141–163
42. Clayton J, Lemanski K, Bonkowski M. Shifts in soil microbial stoichiometry and metabolic quotient provide evidence for a critical tipping point at 1% soil organic carbon in an agricultural post-mining chronosequence. *Biology and Fertility of Soils*, 2021, **57**(3): 435–446
43. Wan X, Xiao L, Vadeboncoeur M A, Johnson C E, Huang Z. Response of mineral soil carbon storage to harvest residue retention depends on soil texture: a meta-analysis. *Forest Ecology and Management*, 2018, **408**: 9–15