Soybean inclusion reduces soil organic matter mineralization despite increasing its temperature sensitivity

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HIGHLIGHTS

- Soybean inclusion reduced CO_2 efflux by 17–24 % but increased its Q_{10} at topsoil
- Soybean inclusion decreased SOM mineralization in macroaggregates
- Soybean inclusion increased Q_{10} of SOM mineralization in meso- and micro-aggregates
- Soybean inclusion enhanced substrate supply for microbes thus reducing CO_2 efflux

GRAPHICAL ABSTRACT
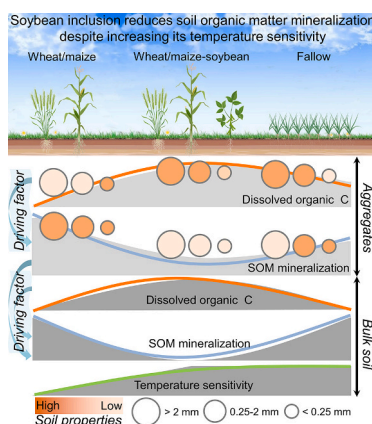
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ABSTRACT

Legume-based cropping increased the diversity of residues and rhizodeposition input into the soil, thus affecting soil organic matter (SOM) stabilization. Despite this, a comprehensive understanding of the mechanisms governing SOM mineralization and its temperature sensitivity across bulk soil and aggregate scales concerning legume inclusion remains incomplete. Here, a 6-year field experiment was conducted to investigate the effects of three cropping systems (i.e., winter wheat/summer maize, winter wheat/summer maize-soybean, and nature fallow) on SOM mineralization, its temperature sensitivity, and the main drivers in both topsoil (0–20 cm) and subsoil (20–40 cm). Soybean inclusion decreased the SOM mineralization by 17%–24%, while concurrently increasing the majority of soil biochemical properties, such as carbon (C) acquisition enzyme activities (5%–22%) and microbial biomass C (5%–9%), within the topsoil regardless of temperature. This is attributed to the

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increased substrate availability (e.g., dissolved organic C) facilitating microbial utilization, thus devoting less energy to mining nutrients under diversified cropping. In addition, SOM mineralization was lower within macroaggregates (~12%), largely driven by substrate availability irrespective of aggregate sizes. In contrast, diversified cropping amplified the Q_{10} of SOM mineralization in mesoaggregates (+6%) and microaggregates (+5%) rather than in macroaggregates. This underscores the pivotal role of mesoaggregates and microaggregates in dominating the Q_{10} of SOM mineralization under soybean-based cropping. In conclusion, legume-based cropping diminishes soil organic matter mineralization despite increasing its temperature sensitivity, which proposes a potential strategy for C-neutral agriculture and climate warming mitigation.

1. Introduction

Intensive agricultural practices accelerate soil organic matter (SOM) mineralization, approximately 1500 Gt of carbon dioxide (CO_2) have been released into the atmosphere from agroecosystem globally between 1751 and 2017 (Lal, 2011; Sanderman et al., 2017; Zhang et al., 2021). Given that SOM accumulation depends on the balance between carbon (C) input and output (Lal, 2004; Xie et al., 2017), limiting SOM mineralization holds a pivotal role in promoting C sequestration. Diversified cropping has been proposed to increase SOM accumulation (Yan et al., 2023). Proportionally, fallow reduced frequent soil disturbance by intensive tillage, which could increase aggregate stability and occlude microbial degradation for C sequestration physically (Jiang and Xie, 2009). However, the mechanistic comprehension of the driving factors behind SOM mineralization for promoting soil health and C-neutral under diversified cropping and fallow remains unclear.

Diversified cropping alters soil aggregation, and nutrient cycling, as well as microbial diversity and activity, thus governing SOM mineralization (Zhang et al., 2021). Previous studies have provided paramount insights on SOM mineralization under different cropping systems, albeit with inconsistent results. For instance, diversified cropping had the potential to decrease SOM mineralization, which was attributed to the physical occlusion via increased soil aggregation (Yan et al., 2022; Yan et al., 2023). The SOM within macroaggregates could be physically protected from microbial decomposition, resulting in lower SOM mineralization (Bachmann et al., 2008). Additionally, the increased substrate availability (e.g., low-molecular mucilage and root exudates) from diversified cropping is readily utilized by microorganisms, reducing the additional energy invested in secreting extracellular enzymes for nutrition mining from SOM (Meyer et al., 2017; Singh et al., 2017). Conversely, the negative response advocated that diversified cropping provided abundant labile C sources, which changed soil microbial activities and stimulated soil priming, thus resulting in the acceleration of SOM mineralization (King and Blesh, 2018; Kumar et al., 2018). Since aggregates offer a habitat for microbial-mediated biochemical reactions, the microbial-derived by-products (e.g., metabolites and enzymes), and substrate availability in them subsequently regulate the SOM mineralization (Trivedi et al., 2015; Walker et al., 2018). Improved fallow could also maintain higher microbial biomass C and aggregate-protected C, thus leading to reduced SOM mineralization (Nyamadzawo et al., 2009). However, some studies indicated that fallow practice contributes to an increase in SOM mineralization (Karbozova-Saljnikov et al., 2004; Ryan et al., 2008). This is primarily attributed to the fact that nitrogen (N)-deficient soils under fallow stimulate microbial decomposition of recalcitrant SOM for N (Bottner et al., 2006; Meyer et al., 2017). Despite these, little knowledge of the responses of SOM mineralization, particularly in spatially heterogeneous aggregates under diversified cropping and fallow is available.

Soil C stability and accumulation are pronounced responses to global warming due to the temperature-dependency of SOM mineralization (Davidson and Janssens, 2006). Temperature sensitivity (Q_{10}) often describes the response of SOM mineralization to warming, which represents a vital parameter for predicting the fates of SOM under climate change (Zang et al., 2020). The Q_{10} of SOM mineralization is mediated dominantly by the sensitivities of substrate availability and microbial

metabolism to temperature (Thiessen et al., 2013; Nottingham et al., 2019; Walker et al., 2018). Typically, SOM mineralization is positively correlated with higher temperature, which is responsible for the accelerated C loss under global warming (Trumbore et al., 1996; Conant et al., 2011; Qin et al., 2019). Diversified cropping increased the temporal diversity in plants and functional traits, which stimulated soil biodiversity and agroecosystem functions, thus determining the direction and magnitude of SOM mineralization in response to increased temperatures (Lefèvre et al., 2014; Ghimire et al., 2019; Wen et al., 2019). Previous studies have demonstrated that legume-based cropping minimized the Q_{10} of SOC mineralization at 0–20 cm soil depth (Ghimire et al., 2019). Such a result might be attributed to the increased substrate availability under diversified cropping less sensitive to temperature changes requiring low activation energy (Qin et al., 2019; Lai et al., 2021). Despite a few studies on decreasing Q_{10} of SOM mineralization by legume-based cropping (Ghimire et al., 2019) or by nature fallow (Zhang et al., 2022), large knowledge gaps remained on the fate and driving factors of SOM mineralization in response to increasing temperature.

To fill this knowledge gap, a 90-day incubation experiment was set up to investigate the effects of three 6-year cropping systems (i.e., winter wheat/summer maize, winter wheat/summer maize-soybean, and natural fallow) on SOM mineralization and its temperature sensitivity, as well as their main drivers. We hypothesized that (1) diversified cropping decreases SOM mineralization due to the increased substrate availability being easily utilized by microbes without investing more energy to decompose the recalcitrant SOM and the occlusion physically within macroaggregates; (2) the temperature-sensitive response of SOM mineralization decreases under diversified cropping.

2. Materials and methods

2.1. Site description

This long-term field experiment was established in 2015 at the Wuqiao Experimental Station of the China Agricultural University, Hebei Province, China (37°36′N, 116°21′E). The mean annual temperature was 12.9°C, and annual precipitation was 500 mm over the past 30 years, classified as a temperate continental condition. The initial (2015) soil properties at 0–20 cm depth were: SOM 15.52 g kg^{-1}, total nitrogen (TN) 1.30 g kg^{-1}, total phosphorus (P) 1.72 g kg^{-1}, available P 89.80 mg kg^{-1}, and soil pH 7.74.

2.2. Experimental design and management

The experiment consisted of three cropping systems as follows: (1) winter wheat/summer maize (WM) as control treatment; (2) optimized winter wheat/summer maize-soybean (WM-soybean) as the diversified cropping to achieve sustainable development of agroecosystem; and (3) nature fallow. The plots were completely randomized design and each plot size was 72 m^2. Before sowing, the N: P: K fertilizers as urea, diammonium phosphate, and potassium sulfate respectively were applied annually for crops as follows: 160: 60: 93 kg ha^{-1} for winter wheat, 180: 45: 93 kg ha^{-1} for summer maize, and 60: 48: 113 kg ha^{-1} for soybean. The natural fallow received no fertilizer. Winter wheat was sown in mid-

October and harvested in early June of the following year, respectively; summer maize was sown in mid-June and harvested in early October, respectively; the soybean was sown in early May and harvested in mid-September every 2 years from 2015 to 2021. Rotary tillage with residue incorporation was performed for winter wheat and soybean, and no-till with residue mulching was done for summer maize, respectively. Winter wheat was sown with a 15-cm row spacing. Summer maize was sown with a 60-cm row spacing and a 24-cm plant distance in a row. Soybean was sown with a 40-cm row spacing and a 15-cm plant distance in a row. While natural fallow was mainly growing grass naturally where the aboveground biomass is much less than diversified cropping and wheat/maize cropping biomass. The aboveground residues are returned to the field for fallow without additional agronomic practices. All other treatments followed typical agronomic and management practices used by practitioners from the study area.

2.3. Soil sampling and analysis

Bulk soil and aggregate samples were collected after the wheat harvest in early June 2021 with four field replicates. A soil auger (8 cm diameter) was used to collect three sub-samples from each plot at 0–20 cm (topsoil) and 20–40 cm (subsoil). For each depth interval, five field replicates along an S-curve transect were pooled to form a composite soil sample.

Soil samples (300 g each) were air-dried to approximately 15% gravimetric water content across 24 h at room temperature (~22°C), and were then manually crumbled through a < 8 mm mesh for aggregate size fractionation (Bach and Hofmockel, 2014). Subsequently, the samples were shaken on the Vibratory Sieve Shaker machine (ZD-ZDS, China) for 5 min at approximately 600 rpm through 2 mm and 0.25 mm pore sizes. Three aggregate size classes: > 2 mm (macroaggregates), 0.25 2 mm (mesoaggregates), and < 0.25 mm (microaggregates) were obtained.

2.4. Laboratory incubation

Bulk soil and the three aggregate size fractions (each 20 g) were adjusted to 70% water holding capacity (WHC) by adding distilled water under 15°C and 25°C and were placed in a thermostatic incubator (BIC-300) with 4 replications for 90 days. Here, 15°C represents the mean temperature of the wheat season, and 25°C represents the temperature increase of 10°C. After 7 days pre-incubation, a beaker containing NaOH (5 mL, 1 M) was placed into the sealing vitreous bottles (250 mL) to absorb the CO_2 evolved. The alkali traps were replaced after 1, 3, 5, 7, 14, 21, 30, 40, 60, and 90 days after incubation. Subsequently, SOM mineralization was determined by titrating the remaining NaOH with HCl (1 M), which is expressed as mg C kg^{-1} soil. The temperature sensitivity (Q_{10}) of SOM mineralization was calculated as follows:

$$Q_{10} = R_{25}/R_{15} \tag{1}$$

where R_{25} and R_{15} refer to the absolute SOM mineralization at 25°C and 15°C, respectively; Q_{10} represents the temperature sensitivity.

2.5. Analysis of soil physicochemical parameters

After incubation, the bulk soil and the three aggregate size fractions (> 2, 0.25–2, and < 0.25 mm) were used to measure soil microbial biomass C (MBC) and N (MBN) based on the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, two subsamples of 5 g fresh weight of sieved soil were used for each sample. One subsample was fumigated for 24 h with ethanol-free chloroform and extracted using 0.5 M K_2SO_4 (4: 1 v: w; fresh soil) after shaking for 30 min at 300 rpm. The other subsample was directly extracted following the same protocol without fumigating. The extracts were filtered through a 0.45 μm millipore filter. Finally, the MBC and MBN were analyzed using a TOC/TN analyzer (Shimadzu Co., Ltd., Japan). The dissolved organic C (DOC) and total dissolved N (TDN) were measured in the 0.5 M K_2SO_4 non-fumigated extracts using the TOC/TN analyzer as described above.

Activities of three enzymes associated with C (β-1, 4-glucosidase, BG), and N (β-1, 4-*N*-acetylglucosaminidase, NAG; Leucine aminopeptidase, LAP) cycles were determined using 4-methylumbelliferone (MUF for BG and NAG) and 7-amino-4-methylcoumarin (AMC for ALP) (Razavi et al., 2016; Zang et al., 2020). Briefly, 1 g fresh soil was mixed with 50 mL sterile water in a glass bottle and shaken for half an hour using the oscillator. 50 μL of suspension, 50 μL of buffer, and 100 μL corresponding substrate (400 μmol g^{-1}) were added to a 96-well microplate reader. Fluorescence values were read using a Thermo1510, MULTISKAN Sky fluorometer (Ex. 360 nm; Em. 450 nm; 142 Thermo Fisher Scientific, USA) after incubation at 0, 0.5, 1, and 2 h time intervals and calculated in μmol g^{-1} h^{-1}.

Considering that NAG and LAP display similar functions, the following equation was used to normalize the enzyme activities (Jia et al., 2022):

$$N - acq = \sqrt[2]{(NAG \cdot LAP)} \tag{2}$$

where N-acq represents the N-acquisition enzyme activities, respectively.

Effects of WM-soybean and fallow on bulk soil physicochemical parameters were quantified as effect sizes:

$$\text{Effect size} = ln(E_{WM-soybean/fallow}) - ln(E_{WM}) \tag{3}$$

where $E_{WM-soybean/fallow}$ refers to the bulk soil physicochemical parameters under WM-soybean and fallow, E_{WM} refers to the bulk soil physicochemical parameters under WM.

2.6. Statistical analysis

Normal distribution and equality of variance (Levene) were ensured before performing the analyses. Mean differences in the treatments (cropping system) were examined using one-way analysis of variance (ANOVA) by SPSS version 25.0 (IBM SPSS Software Inc., Armonk, NY, USA) to identify the effect of cropping systems on soil properties. The R (Version 4.0.3) package "GGally" was used to perform Pearson correlations between SOM mineralization and other soil properties. Further, the statistical relationship among cropping systems, soil properties, and SOM mineralization was explored by using partial least squares path modeling (PLS-PM) (Latan et al., 2017). Alternatively, variation partition analysis (VPA) was carried out to identify the effect of different categories of soil properties on SOM mineralization.

3. Results

3.1. Effect of cropping system and temperature on SOM mineralization

Soybean inclusion decreased the SOM mineralization regardless of temperature across six-year cropping strategies, especially in the topsoil ($p < 0.05$, Figs. 1 and 7). The SOM mineralization decreased by 24% at 15°C, and decreased by 17% at 25°C in the topsoil under diversified cropping versus wheat/maize, respectively ($p < 0.05$, Fig. 1). Fallow and wheat/maize exhibited comparable SOM mineralization at 15°C and 25°C in the topsoil (Fig. 1). However, there were no significant changes regarding SOM mineralization at 15°C and 25°C in the subsoil among three cropping systems (Fig. 1). Overall, soybean inclusion exerted a stronger effect on reducing SOM mineralization in the topsoil.

3.2. Effect of cropping system and temperature on bulk soil properties and their relationships after incubation

Cropping system and temperature imposed a significant effect on
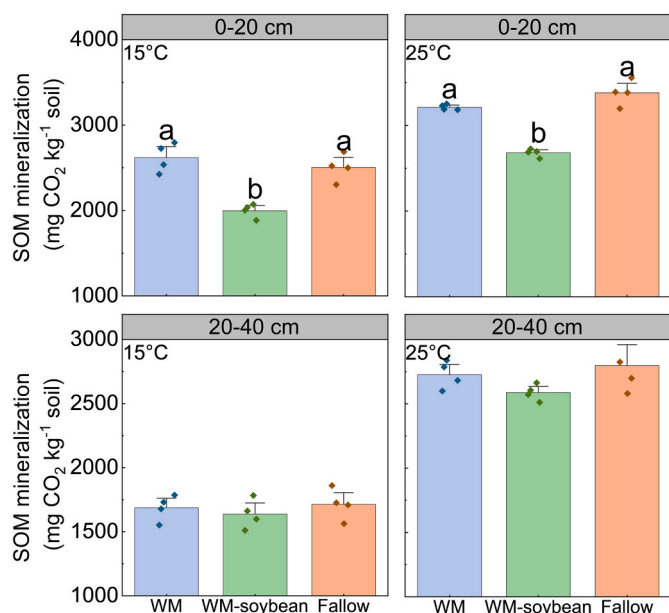
Fig. 1. Soil organic matter (SOM) mineralization at 0–20 and 20–40 cm soil depths after 90 days incubation at 15°C and 25°C under three cropping systems: winter wheat/summer maize (WM), winter wheat/summer maize-soybean (WM-soybean), and fallow. Different lowercase letters denote significant differences between cropping systems ($p < 0.05$). Values are average ($\pm$ SE) of the four replicates.

bulk soil properties, especially in the topsoil (Fig. 2). Within the topsoil, the MBC, MBC/MBN, DOC, and C-acq were higher under diversified cropping relative to wheat/maize at 15°C (Fig. 2; $p < 0.05$). Here fallow
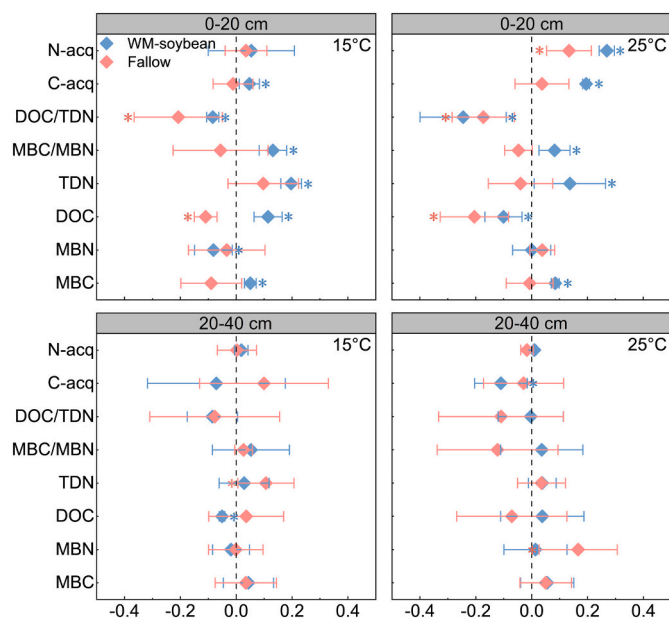
Fig. 2. Effect sizes of winter wheat/summer maize-soybean and fallow on C-acq, N-acq, DOC/TDN, DOC, MBC/MBN, and MBC relative to wheat/maize at 0–20 and 20–40 cm soil depths after 90 days incubation at 15°C and 25°C. The soil properties were: β-1, 4-glucosidase (C-acq), N-acquisition enzyme activities (N-acq), dissolved organic C/total dissolved N (DOC/TDN), dissolved organic C (DOC), total dissolved N (TDN), microbial biomass C/microbial biomass N (MBC/MBN), microbial biomass C (MBC), and microbial biomass N (MBN). Values are average ($\pm$ CI) of four replicates. Asterisks indicate statistically significant differences of winter wheat/summer maize-soybean and fallow relative to wheat/maize ($p < 0.05$).

decreased the DOC and DOC/TDN relative to wheat/maize at 15°C (Fig. 2; $p < 0.05$). Further, MBC, MBC/MBN, C-acq, and N-acq were higher under diversified cropping compared to wheat/maize at 25°C (Fig. 2; $p < 0.05$). Compared to wheat/maize, fallow increased the N-acq but reduced the DOC and DOC/TDN at 25°C (Fig. 2; $p < 0.05$). In the subsoil, diversified cropping and fallow displayed almost no effect on bulk soil properties regardless of temperature (Fig. 2; $p > 0.05$).

The PLS-PM showed that cropping system (-0.46) and substrate availability (-0.59) had strong effects on SOM mineralization in the topsoil, and the substrate availability (-0.94) held a pronounced effect on SOM mineralization in the subsoil (Fig. 3). Further yet, the total effects indicated that substrate availability had the dominant effect on mediating SOM mineralization regardless of soil depth. Overall, the cropping system exerted a much stronger effect on SOM mineralization, which was dominantly driven by the substrate availability.

3.3. Effect of cropping system and temperature on aggregate-scale SOM mineralization

Cropping systems exhibited a significant effect on the aggregate-scale SOM mineralization at both soil depths (Figs. 4 and 7). Within the topsoil, soybean inclusion decreased the SOM mineralization irrespective of aggregate sizes and temperature, while the effect sizes were weakened with the aggregate size decreasing relative to wheat/maize (Fig. 4a and b). Fallow only decreased the SOM mineralization of macroaggregates irrespective of temperature relative to wheat/maize (Fig. 4a). In the subsoil, SOM mineralization was lower under diversified cropping irrespective of aggregate sizes, while fallow had no observable effect sizes at 15°C compared to wheat/maize (Fig. 4a and b). Further yet, variation partitioning analysis (VPA) demonstrated that microbial SOM mineralization was depended on the microbial biomass, substrate availability, and enzyme activities within three aggregate classes (Fig. 4c). Specifically, the substrate availability held the highest effect on microbial SOM mineralization regardless of aggregate sizes, followed by microbial biomass and enzyme activities (i.e., in the order substrate availability > microbial biomass > enzyme activities) (Fig. 4c). In the macroaggregates, the interaction between microbial biomass and substrate availability had a prominent effect on microbial SOM mineralization (Fig. 4c). While the interaction among microbial biomass, substrate availability, and enzyme activities was more pronounced in mesoaggregates and microaggregates (Fig. 4c). Overall, the substrate availability drives the SOM mineralization mainly at aggregate-scale where the reduced SOM mineralization within macroaggregates determined the decreased bulk soil SOM mineralization under diversified cropping.

3.4. Effect of cropping system on the temperature sensitivity of soil properties

The cropping system exerted a remarkable effect on the temperature sensitivity of topsoil properties (Figs. 5 and 7). Specifically, within the topsoil, soybean inclusion increased the Q_{10} of SOM mineralization and C-acq by 9% and 16% relative to wheat/maize, respectively ($p < 0.05$; Fig. 5). However, the Q_{10} of DOC was decreased by 19% under diversified cropping versus wheat/maize ($p < 0.05$; Fig. 5). Fallow increased the Q_{10} of MBC by 9% compared to wheat/maize ($p < 0.05$; Fig. 5). In the subsoil, there were no significant differences among three cropping systems. Additionally, in the topsoil, the Q_{10} of MBC was positively correlated (0.88; $p < 0.05$) with the Q_{10} of SOM mineralization, whereas the Q_{10} of DOC had a negative effect (-0.97; $p < 0.05$) on the Q_{10} of SOM mineralization (Fig. 5). Further, random forest showed that the Q_{10} of DOC exerted the greatest effect on the Q_{10} of SOM mineralization. At the aggregate-scale, mesoaggregates and microaggregates dominated the Q_{10} of SOM mineralization in the topsoil under diversified cropping and fallow versus wheat/maize (Fig. 6). Overall, increased Q_{10} of SOM mineralization was largely observed on mesoaggregates and
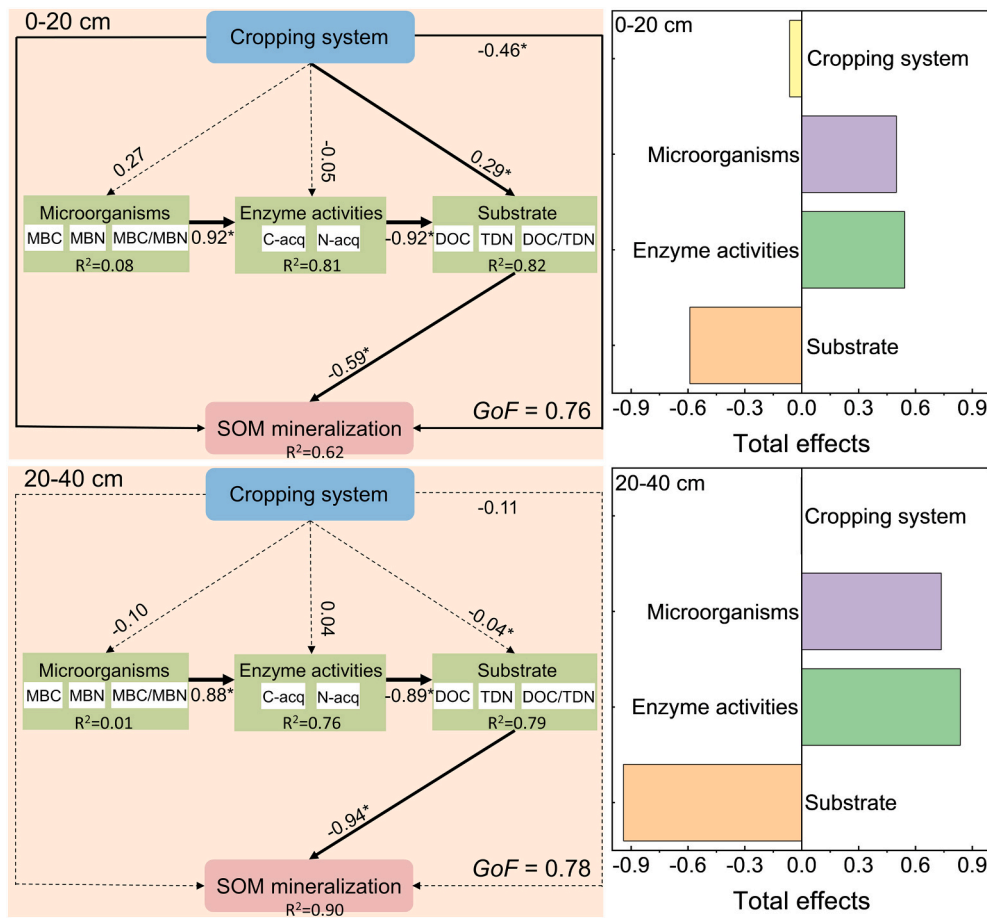
Fig. 3. Directed graph of the partial least squares path model. Each rectangle represented an observed variable. Path coefficients were calculated after 1000 bootstraps and reflected in the width of the arrow, with solid and dashed lines indicating whether there is a significant effect, respectively. The "*" showed that coefficients differ significantly ($p < 0.05$). The model was assessed using the Goodness of Fit (*GoF*) statistic, and the *GoF* value was 0.76 at 0–20 cm and 0.78 at 20–40 cm, respectively. The measured bulk soil biochemical metrics were: Cropping system, three cropping systems; microorganisms, microbial biomass C, microbial biomass N, and microbial biomass C/microbial biomass N; substrate availability, dissolved organic C, total dissolved N, and dissolved organic C/total dissolved N; enzyme activities, β-1, 4-glucosidase, and N-acquisition enzyme activities. Total effect represented relative importance of soil properties on SOM mineralization.

microaggregates, and were stimulated by the Q_{10} of DOC under diversified cropping and fallow.

4. Discussion

### 4.1. Effect of cropping system on SOM mineralization

In line with our first hypothesis, our results showed that soybean inclusion decreased SOM mineralization (Fig. 1). Such a result was congruent with Ghimire et al. (2019) who advocated legume-based cropping as a strategy to decrease SOM mineralization. The underlying mechanism is likely that soybean inclusion increased the N availability (e.g., TDN) due to the N_2 fixation (Fig. 2), which could be utilized readily by microorganisms and decreasing N limitation. Consequently, the additional N resource requirement obtained from SOM mineralization is reduced based on the "microbial N mining" theory (Meyer et al., 2017; Shahbaz et al., 2018). Additionally, the relatively low C/N ratio of legume residue and increased substrate availability (e.g., DOC) were readily utilized by microbes without investing more energy via microbial respiration to mine nutrients from recalcitrant SOM, thus leading to less SOM mineralization under diversified cropping (Franke et al., 2008; Mganga et al., 2022). The PLS-PM further demonstrated that the cropping system and substrate availability exhibited a closely negative effect on SOM mineralization, with substrate availability exerting the greatest influence in restricting SOM mineralization (Fig. 3).

Additionally, soybean inclusion decreased SOM mineralization within aggregates in the topsoil where the effect sizes were weakened with the aggregate size decreasing relative to wheat/maize (Fig. 4a, b). This is attributed to the relatively higher macroaggregates (Fig. S1) physically protecting SOM against microbial attack, and thereby decreasing SOM mineralization under diversified cropping, as evidenced by Wang et al. (2017). Variation in SOM mineralization within aggregates is mainly impelled by substrate availability irrespective of aggregate size (Fig. 4c). Since soybean inclusion increased the substrate availability in spatially heterogeneous aggregates, the increased enzyme activities might catalytically degrade these substrates for microbial growth (Fig. S2). Consequently, the microbial activities and production of microbial-derived binding agents (e.g., glomalin) might be increased, which are imperative for soil aggregation (Bossuyt et al., 2001; Zhou et al., 2020), ultimately physically hindering microbial degrading of the SOM (Yan et al., 2023). Taken together, the reduced SOM mineralization within macroaggregates determined the decreased bulk soil CO_2 efflux in the topsoil, and is mainly impelled by substrate availability under diversified cropping.

Furthermore, our results showed that soybean inclusion had no significant effect on SOM mineralization in the subsoil across six years (Fig. 1). This is because most of the soybean roots (66%) were distributed in the topsoil (Li et al., 2017), short-term soybean inclusion might not significantly affect the subsoil, thus leading to similar SOM mineralization versus wheat/maize. Further, since the plant-derived C is
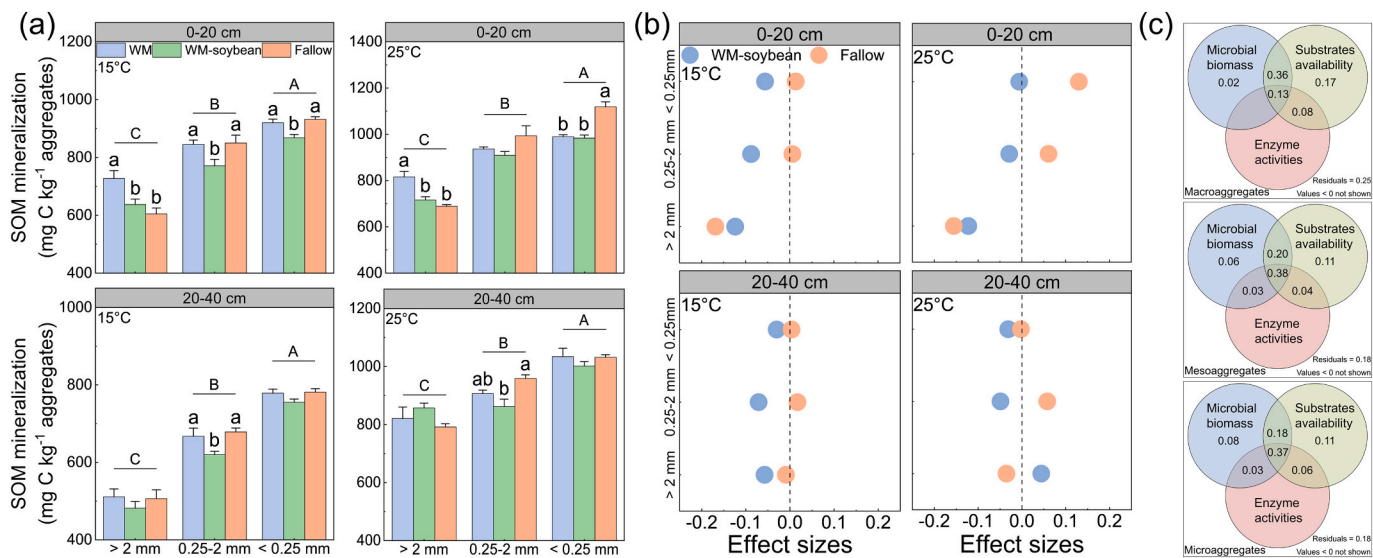
Fig. 4. (a) Soil organic matter (SOM) mineralization within aggregates at 0–20 and 20–40 cm soil depths after 90 days incubation at 15°C and 25°C under three cropping systems: winter wheat/summer maize (WM), winter wheat/summer maize-soybean (WM-soybean), and fallow. Different lowercase letters denote significant differences between cropping systems ($p < 0.05$). Values are average ($\pm$ SE) of the four replicates. (b) Effect sizes of diversified cropping (winter wheat/summer maize-soybean) and fallow on soil organic matter (SOM) mineralization within aggregates relative to wheat/maize at 0–20 and 20–40 cm soil depths after 90 days incubation at 15°C and 25°C. Values are average of the four replicates. (c) Variation partitioning analysis (VPA) shows the effects of soil microbial biomass, substrates availability, and enzyme activities on microbial SOM mineralization in macroaggregates, mesoaggregates, and microaggregates. During data analysis, microbial biomass C, microbial biomass N, and their stoichiometry were used for microbial biomass; dissolved organic C, total dissolved N, and their stoichiometry were used for substrates availability; β-1, 4-glucosidase, N-acquisition enzyme activities, and their stoichiometry were used for enzyme activities.
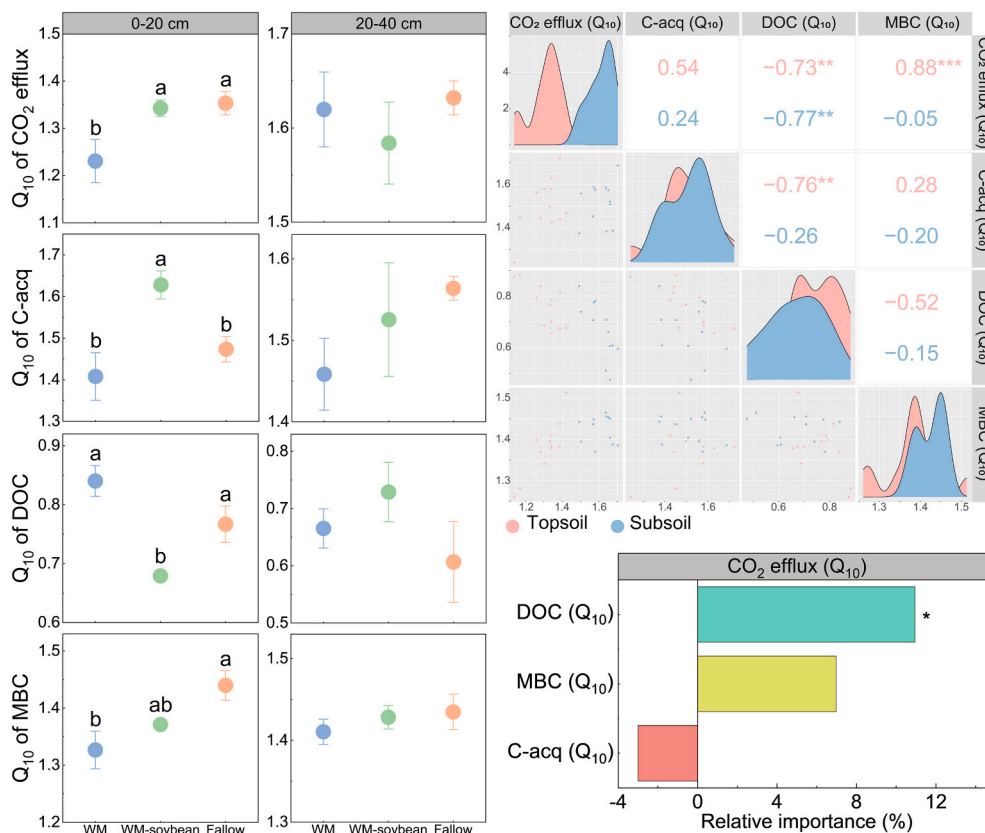
Fig. 5. Temperature sensitivity (Q_{10}) of soil organic matter mineralization (CO_2 efflux), β-1, 4-glucosidase (C-acq), dissolved organic C (DOC), and microbial biomass C (MBC) at 0–20 and 20–40 cm soil depths after 90 days incubation under three cropping systems: winter wheat/summer maize (WM), winter wheat/summer maize-soybean (WM-soybean), and fallow. Different lowercase letters denote significant differences between cropping systems ($p < 0.05$). Values are average ($\pm$ SE) of the four replicates. The Pearson correlation between temperature sensitivity of CO_2 efflux and temperature sensitivity of C-acq, DOC, and MBC at 0–20 and 20–40 cm soil depths. The "*, **, and ***" indicate significant differences between the parameters at $p < 0.05$, $p < 0.01$, $p < 0.001$ level. Random forest showed the relative importance (%) of Q_{10} of C-acq, DOC, and MBC on Q_{10} of CO_2 efflux. The "*" indicates significant differences at $p < 0.05$ level.
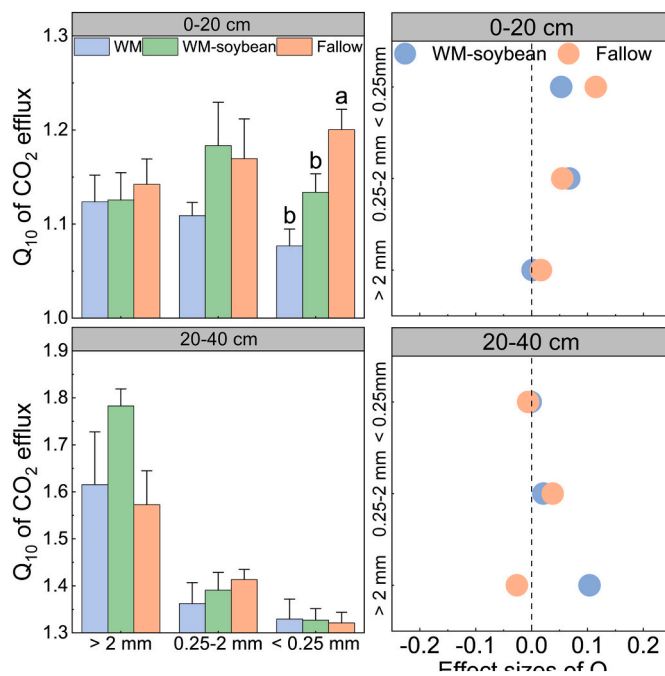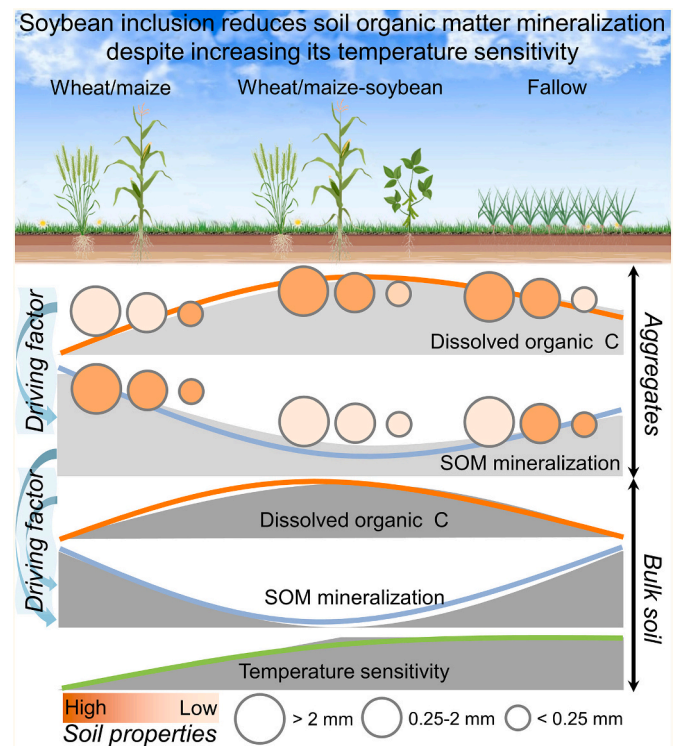
Fig. 6. Temperature sensitivity (Q_{10}) of soil organic matter mineralization (CO_2 efflux) within aggregates at 0–20 and 20–40 cm soil depths after 90 days incubation under three cropping systems: winter wheat/summer maize (WM), winter wheat/summer maize-soybean (WM-soybean), and fallow. Different lowercase letters denote significant differences between cropping systems ($p < 0.05$). Values are average ($\pm$ SE) of the four replicates. Effect sizes of winter wheat/summer maize-soybean and fallow on Q_{10} of CO_2 efflux within aggregates relative to wheat/maize at 0–20 and 20–40 cm soil depths after 90 days incubation. Values are average of the four replicates.

Fig. 7. Graphical abstract illustrated the effect of three cropping systems on soil organic matter (SOM) mineralization and its temperature sensitivity. The wheat/maize-soybean cropping system decreased SOM mineralization by reducing the large macroaggregates SOM mineralization mainly relative to wheat/maize. The decreased SOM mineralization was dominantly driven by dissolved organic C under wheat/maize-soybean cropping system. Further, wheat/maize-soybean and fallow increased the temperature sensitivity of SOM mineralization versus wheat/maize.

seldom transported to the subsoil due to the fewer soybean roots distributed in the subsoil, which had little effect on the subsoil microbial community (Lynch and Wojciechowski, 2015; Li et al., 2017; Mooshammer et al., 2022). This might lead to comparable MBC content and enzyme activities between diversified cropping and wheat/maize (Fig. 2), thus leading to slight changes in SOM mineralization.

In addition, fallow reduced frequent soil disturbance through intensive tillage practices, which could increase aggregate stability, thus reducing SOM losses (Jiang and Xie, 2009). Nevertheless, the reduced residue production input under fallow vs. wheat/maize could affect the formation of soil aggregation (Blanco-Canqui and Schlegel, 2013). Such a scenario reduced the physical protection of macroaggregates and caused higher SOM mineralization (Nielsen and Calderón, 2011; Yan et al., 2023). These two opposing effects might in part counteract each other and result in similar SOM mineralization as wheat/maize. Further, given the pivotal role of microbial biomass and enzyme activities in mediating CO_2 efflux (Min et al., 2019), our study reveals comparable levels of these parameters between fallow and wheat/maize (Fig. 2), thus leading to similar SOM mineralization. Congruent results were also presented by Zhang et al. (2019) who advocated the comparable SOM mineralization between fallow and wheat-based cropping systems after nine years.

4.2. Effect of cropping system on the temperature sensitivity of SOM mineralization

Contrary to our second hypothesis, soybean inclusion increased the Q_{10} of SOM mineralization, especially in the topsoil (Fig. 5). The increased Q_{10} of C-acq catalyzes quick degradation of substrate, thus restricting resource availability (e.g., decreased Q_{10} of DOC) (Fig. 5) and leading to passive microbial processes under diversified cropping

(Walker et al., 2018). Subsequently, microorganisms have to change their resource allocation under the lower resource availability, and devote more energy to decompose the SOM existing for nutrient acquisition, which remarkably increases soil respiration at a higher temperature (25°C vs. 15°C) (Spohn et al., 2016; Öquist et al., 2017). The observed negative correlation between the Q_{10} of DOC and Q_{10} of SOM mineralization as an evidence-based indicator, indicating that the decreased substrate availability is responsible for the increased SOM mineralization (Fig. 5). Random forest further demonstrated that the Q_{10} of DOC was dominant in the Q_{10} of SOM mineralization.

At the aggregate-scale, diversified cropping increased the Q_{10} of SOM mineralization where mesoaggregates and microaggregates recorded the higher Q_{10} (Fig. 6). The SOM within macroaggregates is more recalcitrant than that in microaggregates due to the physical occlusion that hinders microbial degradation, thus leading to a lower Q_{10} of SOM mineralization in macroaggregates (Xie et al., 2017; Kan et al., 2020). Further, soybean inclusion increases substrate availability (Zhang et al., 2021), which is expected to be more exposed to microorganisms in the smaller aggregates due to the larger surface area (Bimüller et al., 2016; Reeves et al., 2019). Therefore, the Q_{10} of SOM mineralization is increased within the smaller aggregates versus wheat/maize (Fig. 6).

Furthermore, the Q_{10} of SOM mineralization was higher under fallow relative to wheat/maize (Fig. 5). Since fallow reduced the residue input, the instability of SOM (e.g., DOC) was decreased across six years (Fig. 2). Given that intrinsically chemically recalcitrant organic compounds are considered a more stabilized form and depleted more slowly but more susceptible to environmental change (e.g., temperature) (Conant et al., 2011; Sierra, 2012; Bimüller et al., 2016). Such a result led to higher Q_{10}

of SOM mineralization in response to higher temperatures under fallow (Fig. 5). This was further supported by Lefèvre et al. (2014) who documented the increased temperature sensitivity for stable SOM under fallow. Additionally, fallow held the lowest macroaggregate proportions among the three cropping systems (Fig. S1), which was responsible for the higher Q_{10} of SOM mineralization (Sey et al., 2008). As fine particles associated with microaggregates could strongly adsorb SOM by several mechanisms (e.g. ligand exchange, polyvalent cation bridges, and large surface areas) (Sollins et al., 1996). The higher activation energy was required to decompose recalcitrant substrate versus labile substrate, which further resulted in higher Q_{10} of SOM mineralization within the smaller aggregates with increasing temperatures (Kleber, 2010; Conant et al., 2011; Ghosh et al., 2016). Overall, soybean inclusion and fallow increase the Q_{10} of SOM mineralization, which is mainly attributed to the enhanced Q_{10} within the mesoaggregates and microaggregates.

5. Conclusion

The six-year diversified cropping decreased SOM mineralization by 17%–24%, regardless of temperature relative to wheat/maize in the topsoil. Further, the SOM mineralization within macroaggregates was 0.17–0.27 times lower than meso- and micro-aggregates and is responsible for the reduced bulk soil CO_2 efflux under diversified cropping. The increased substrate availability (e.g., DOC) was readily utilized by microbes without investing more energy to decompose the recalcitrant SOM, and was the main driver for SOM mineralization in both bulk soil and aggregate scales. Moreover, soybean inclusion and fallow increase the Q_{10} of SOM mineralization by 9% and 10% in meso- and micro-aggregates, respectively. Overall, we recommend that legume-based diversification should be advocated for decreasing SOM mineralization, and further benefiting C sequestration and mitigating climate change, which creates a win-win scenario for sustainable agricultural development in the North China Plain.

CRediT authorship contribution statement

Zhengjun Yan: Writing – original draft. **Rong Jia:** Writing – review & editing. **Jie Zhou:** Writing – review & editing. **Kazem Zamanian:** Writing – review & editing. **Yadong Yang:** Writing – review & editing. **Kevin Z. Mganga:** Writing – review & editing. **Zhaohai Zeng:** Methodology, Writing – review & editing. **Huadong Zang:** Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

We declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary information regarding aggregate proportions, and soil metrics within aggregate figure data under cropping systems can be found in the end. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171334.

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