

# Legume-based crop diversification reinforces soil health and carbon storage driven by microbial biomass and aggregates

Zhengjun Yan<sup>a</sup>, Jie Zhou<sup>a</sup>, Chunyan Liu<sup>a</sup>, Rong Jia<sup>a</sup>, Kevin Z. Mganga<sup>b</sup>, Lei Yang<sup>a</sup>, Yadong Yang<sup>a</sup>, Leanne Peixoto<sup>c</sup>, Huadong Zang<sup>a,d,e,\*</sup>, Zhaohai Zeng<sup>a,d,e,\*</sup>

<sup>a</sup> College of Agronomy and Biotechnology, China Agricultural University, Beijing, China

<sup>b</sup> Copernicus Institute of Sustainable Development, Utrecht University, the Netherlands

<sup>c</sup> Department of Agroecology, Aarhus University, Blichers Allé 20, Tjele, DK, 8830, Denmark

<sup>d</sup> Scientific Observing and Experimental Station of Crop High Efficient Use of Water in Wuqiao, the Ministry of Agriculture and Rural Affairs, Wuqiao 061802, China

<sup>e</sup> Innovation Center of Agricultural Technology for Lowland Plain of Hebei, Wuqiao 061802, China

## ARTICLE INFO

### Keywords:

Diversified cropping  
Legumes  
Aggregates  
Microbial biomass  
C sequestration  
Soil quality

## ABSTRACT

Diversified cropping is a crucial management practice for increasing carbon (C) and nitrogen (N) sequestration in agroecosystems. However, knowledge gaps regarding the mechanisms by which diversified cropping affects C sequestration and soil quality remain unclear. Herein, a field experiment across six-year was performed to explore the effect of three contrasting cropping systems (i.e., winter wheat/summer maize, winter wheat/summer maize-early soybean, and nature fallow) on soil quality and C sequestration, as well as their main drivers at both 0–20 cm and 20–40 cm soil depths. Diversified cropping increased soil organic C (SOC) stock by 9% and the majority of the soil biochemical metrics in the topsoil despite a 40% reduction in the fertilizer application relative to wheat/maize. This was attributed to increased SOC content in large macroaggregates and enhanced microbial turnover due to diverse fresh residue inputs under diversified cropping. Alternatively, the soil organic C, microbial biomass C, C-acquisition enzyme activity, and N-acquisition enzyme activity in large macroaggregates (> 2 mm) were increased by 15%, 15%, 32%, and 16% in the topsoil under diversified cropping versus wheat/maize, respectively. Partial least squares path model displayed that increased C sequestration was mainly driven by microbial biomass C irrespective of the bulk- and aggregate scale. Furthermore, diversified cropping increased the soil quality index by 1- to 2-fold relative to maize/wheat since increased aggregate stability benefited soil structure and nutrient cycling regardless of soil depth. Overall, increased SOC stock is dominantly driven by microbial biomass C, and the improved soil quality is mainly impelled by soil organic C and aggregate stability in responding to diversified cropping were expected to create win-win scenarios for agroecosystems.

## 1. Introduction

Soil holds more carbon (C) than the atmospheric and vegetation C pools combined, and displays a pivotal role in crop production, and mitigating the impacts of climate change (Foley et al., 2011; Jansson et al., 2020). For instance, a 2% increment of soil organic C (SOC) could increase wheat and maize yield by 10% and 23%, respectively (Oldfield et al., 2019). However, global SOC stock in intensified agroecosystems has decreased by 20–69% (Lal, 2019), which may threaten crop

production and climate mitigation. As such, implementing targeted crop management strategies (e.g., diversified cropping) could moderate such circumstances and benefit soil C sequestration (Lal et al., 2015; Zhao et al., 2022). However, a lack of systematic research on the mechanisms of C sequestration under diversified cropping, particularly in aggregate-scale.

Soil microbes are fundamental for C sequestration, which govern the balance between C loss via mineralization and C gains via microbial anabolism and necromass formation (Sokol and Bradford, 2019; Liang

**Abbreviations:** BG,  $\beta$ -1, 4-glucosidase; NAG,  $\beta$ -1,4-N-acetylglucosaminidase; TN, Total nitrogen; TDN, Total dissolved nitrogen; MBN, Microbial biomass nitrogen; C-acq, Carbon acquisition enzyme; MWD, Mean weight diameter; WMEB, Winter wheat/summer maize-early soybean; LAP, Leucine aminopeptidase; SOC, Soil organic carbon; DOC, Dissolved organic carbon; MBC, Microbial biomass carbon; SQI, Soil quality index; N-acq, Nitrogen acquisition enzyme; WM, Winter wheat/summer maize; F, Nature fallow.

\* Corresponding authors at: College of Agronomy and Biotechnology, China Agricultural University, Beijing, China.

E-mail addresses: [zanghuadong@cau.edu.cn](mailto:zanghuadong@cau.edu.cn) (H. Zang), [zengzhaohai@cau.edu.cn](mailto:zengzhaohai@cau.edu.cn) (Z. Zeng).

<https://doi.org/10.1016/j.still.2023.105848>

Received 18 February 2023; Received in revised form 6 June 2023; Accepted 23 July 2023

Available online 5 August 2023

0167-1987/© 2023 Elsevier B.V. All rights reserved.

et al., 2019; Wang et al., 2021). Since a series of microbial-mediated biochemical reactions depended on spatially heterogeneous aggregates, such stabilization is largely governed by the physical occlusion within soil aggregates for reducing mineralization (Trivedi et al., 2015; Xu et al., 2021). Here, diversified cropping creates a favorable soil microenvironment benefiting microbial diversity and activity and the potential for increased microbial-derived by-products (e.g., metabolites and enzymes), as well as necromass formation (Zhang et al., 2021). Consequently, the higher microbial-derived products might impel microbial functions (e.g., soil aggregate stability and nutrient cycling), which are dominantly responsible for C sequestration (Kallenbach and Grandy, 2011; Singh et al., 2019). For instance, twelve years of legume-based diversified cropping increased the SOC content, which was driven by interactions between diversified cropping and microbial diversity (Tiemann et al., 2015). Therefore, to make informed decisions about how to promote soil quality and C sequestration in response to diversified cropping, a comprehensive mechanistic understanding regarding the crucial driving factors on microbial-mediated C sequestration is imperative.

Soil quality is vital to the health of economies, ecosystems, as well as human populations (Fierer et al., 2021; Nayab et al., 2022), which can be evaluated by a series of indicators that encompass physical, and biochemistry soil properties (Williams et al., 2020; Jia et al., 2022). Diversified cropping benefits soil quality due to increased aggregate stability, and thus reducing soil erosion (Abid and Lal, 2008; Feng et al., 2020). Moreover, high crop diversification is responsible for the higher microbial diversity, which is involved in secreting various exo-enzymes that mediate most of the soil functions and processes (Congreves et al., 2015). Despite these findings, the precise mechanism by which soil health is impacted under diversified cropping has not been fully elucidated, especially the regulatory mechanisms and pathways involved in these processes still largely elude our comprehension.

The North China Plain comprises about 39% of the national arable land where the winter wheat-summer maize cropping system is widespread (Wu et al., 2006, 2008). However, the long-term continuous wheat-maize monoculture cropping system combined with the increase in extensive N fertilization, which is responsible for soil acidification and threatens agricultural sustainability (Yu et al., 2019). Alternative cropping systems (e.g., diversified cropping and nature fallow) and better management thus promise to deliver new solutions to reduce N fertilization, promote C sequestration, and increase soil quality for sustainable agricultural development (Toivonen et al., 2013; Gao et al., 2015; Lal, 2015). While large knowledge gaps remain regarding quantitative and mechanistic research on the changes in soil quality and C sequestration, particularly in aggregate scale, in response to alternative cropping systems. Thus, three field cropping systems (i.e., winter wheat/summer maize, winter wheat/summer maize-early soybean, and natural fallow) across six-year were utilized to probe the mechanisms of soil quality and C sequestration, as well as their main drivers. We aimed to 1) evaluate whether the soil aggregate stability, microbial biomass C, SOC mineralization, as well as enzyme activities varied, and if so, how to mediate C sequestration under diversified cropping; 2) determine the effect of diversified cropping on soil quality. We hypothesized that: 1) legume inclusion could increase SOC stock due to the occlusion physically within macroaggregates and increased microbial biomass; 2) legume inclusion could enhance the soil quality attributing to the diverse fresh residues benefited soil aggregate stability and nutrient cycling.

## 2. Materials and methods

### 2.1. Site description

The field experiment was established on a loamy Mollisol (37°62' N, 116°43' E) in October 2015, which belongs to the Wujiao Experimental Station of China Agricultural University, Hebei Province, China. The

mean annual temperature and mean annual precipitation are 13.8 °C and 547 mm in this region, respectively. The climate belongs to a sub-humid continental monsoon (Wang et al., 2022). The 0–20 cm soil characteristics in 2015 were: 9.0 g kg<sup>-1</sup> of SOC, 1.3 g kg<sup>-1</sup> of total nitrogen (TN), 1.72 g kg<sup>-1</sup> of total phosphorus (P), 89.8 mg kg<sup>-1</sup> of available P, and soil pH = 7.74. The 20–40 cm soil characteristics in 2015 were: 3.48 g kg<sup>-1</sup> of SOC, 0.45 g kg<sup>-1</sup> of TN, and soil pH = 8.25. The texture of the 20–40 cm soil comprised: sand = 17.98%, silt = 67.67%, and clay = 14.35%.

### 2.2. Experimental design and management

Three cropping systems were utilized as follows: (1) conventional winter wheat (*Triticum aestivum* L.; Jimai 22)/summer maize (*Zea mays* L.; Zhengdan 958) (WM) with two harvests in one year in the North China Plain as the control treatment; (2) optimized winter wheat/summer maize-early soybean (*Glycine max* L.; Xudou 20) (WMEB) with three harvests in two years as the diversified cropping system to achieve sustainable development for agroecosystem; and (3) natural fallow (F). Each cropping system (10 m × 7.2 m) was replicated thrice with a randomized design completely. The farming operations (e.g., fertilization, sown, harvest, irrigation, seeding rate, and tillage) of three cropping systems were shown in Table S1. For all treatments, the agronomic and management practices were performed according to normal practices in this region.

### 2.3. Collection of soil sample and preparation

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) soil depths after the wheat harvest in early June 2021. Soil samples were collected from 0–20 cm and 20–40 cm depth of three cropping system with three field replicates. Three random individual soil cores were collected from each plot, and mixed as one sample. Fine roots and other plant residues were manually removed from soil samples. The samples were then placed in a marked plastic bag ensuring no pressure was applied. The sampled topsoil represents the plough horizon (Ap). The subsoil was sampled to estimate the soil organic carbon accumulation, which was affected by topsoil management. All visible roots and crop residues (e.g., mixed during sampling from recent crop harvest) were manually removed. Additionally, three soils sampled with 100 cm<sup>3</sup> cylinders for both top and subsoil were used to determine bulk density based on the volumetric ring method (Li et al., 2014). The samples were oven-dried (105 °C), left for cooling in a desiccator, and weighed for subsequent analyses (Yan et al., 2021).

### 2.4. Aggregate size separation

Soil samples (300 g each) with a 15% gravimetric water content were used to separate aggregates (Yan et al., 2022). Briefly, samples were broken apart < 8 mm manually, and were then sieved through mesh sizes (0.25 mm and 2 mm) on the Vibratory Sieve Shaker machine (ZD-ZDS, China) at 600 rpm for 5 min. Subsequently, the > 2 mm (large macroaggregates), 0.25–2 mm (small macroaggregates) and < 0.25 mm (microaggregates) were obtained. The detailed operations were conducted according to Yan et al. (2022). Optimal-moisture sieving was used as it reduces microbial disturbance, minimizes aggregate abrasion, and avoids water-soluble C and N losses (Dorodnikov et al., 2009; Yan et al., 2022). The aggregate stability as indicated by mean weight diameter (MWD) was calculated according to Meng et al. (2014).

### 2.5. Laboratory incubation

Both bulk soil and aggregates (> 2, 0.25–2, and < 0.25 mm) were used for laboratory incubation to determine SOC mineralization. In brief, fresh samples (20 g) were pre-incubated for 5 days at 70% water

holding capacity in constant 15 °C temperature (corresponding to the wheat season temperature) within a thermostatic incubator (BIC-300). Subsequently, an alkali traps (5 ml, 1 M NaOH) were placed in a closed bottle containing a soil sample and used to absorb the evolved CO<sub>2</sub> at a regular interval during the incubation (Zang et al., 2020). Further yet, three empty bottles (controls/blanks) were used to eliminate the effects of CO<sub>2</sub> in the atmosphere during incubation. The NaOH was periodically changed after 1, 3, 5, 7, 14, 21, 30, 40, 60, and 90 days of incubation and the solution was transferred to a conical flask with 2–3 washing of the vial with distilled water. The excess amounts of carbonates were removed by adding a few drops of 1 M BaCl<sub>2</sub> to saturate the solution, where Na<sub>2</sub>CO<sub>3</sub> was converted into BaCO<sub>3</sub>. The remaining (un-reacted) amount of NaOH was titrated with 0.1 M HCl in the presence of phenolphthalein indicator, and finally the specific mineralization is expressed as mg CO<sub>2</sub> kg<sup>-1</sup> SOC.

## 2.6. Analysis of soil physicochemical parameters

The SOC and TN concentrations of both bulk soil and aggregates (> 2, 0.25–2, and < 0.25 mm) were measured following the protocol by Bao (2000). The chloroform fumigation-extraction method was utilized to measure soil microbial biomass C (MBC) and microbial biomass N (MBN) (Vance et al., 1987). The SOC and TN stocks for a corresponding soil layer were calculated using the following formula:

$$C_{stock} = SOC \times BD_i \times H_i \quad (1)$$

$$N_{stock} = TN \times BD_i \times H_i \quad (2)$$

Where  $C_{stock}$  and  $N_{stock}$  indicate the SOC and TN stocks (kg m<sup>-2</sup>), respectively;  $BD_i$  and  $H_i$  mean the soil bulk density (kg m<sup>-3</sup>) and the thickness (m) in the corresponding soil layer, respectively.

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were analyzed from samples in the incubation beakers by using the chloroform-fumigation-extraction method (Vance et al., 1987). Soil field moisture content was adjusted to 50% of their water-holding capacity (WHC) before microbial analysis. Then, 5 g soil with oven-dry weight was transferred separately into Petri-dishes within a desiccator. Sub-samples were fumigated with ethanol-free chloroform (CHCl<sub>3</sub>) for 24 h at 25 °C. Samples were extracted for MBC and MBN by adding 20 ml of 0.05 M K<sub>2</sub>SO<sub>4</sub>, shaking for 30 min at 300 rpm, and then filtered through a 0.45 μm millipore filter. Three 5 g nonfumigated soil sub-samples were processed in the same manner. Finally, the MBC and MBN were analyzed using a wet combustion-based C/N analyzer (Shimadzu Inc.,).

The dissolved organic C (DOC) and total dissolved N (TDN) were extracted by shaking 5 g of soil in deionized water (20 ml) mixture at 300 rpm for 30 min and centrifuging at 5000 rpm for 10 min (Haynes, 2005). The supernatants were filtered through a 0.45 μm millipore filter. Finally, the TOC analyzer (TOC-L CPN, Shimadzu, Japan) was utilized to measure the DOC and TDN.

## 2.7. Enzyme activity assays

Enzyme activities of β-1, 4-glucosidase (BG), β-1, 4-N-acetylglucosaminidase (NAG), and Leucine aminopeptidase (LAP) were determined via 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC) (Razavi et al., 2016; Ma et al., 2022). Briefly, fresh soil (1 g) was added to the 50 ml distilled water in a glass bottle shaken for half-hour. Afterwards, suspension (50 μl), buffer (50 μl), combined with corresponding substrates (100 μl, 400 μmol g<sup>-1</sup>) were dispensed into a 96-well microplate reader. Fluorescence values were read via a Thermo1510, MULTISKAN Sky fluorometer (Ex. 360 nm; Em. 450 nm; 142 Thermo Fisher Scientific, USA) after incubation and calculated in μmol g<sup>-1</sup> h<sup>-1</sup> (Chu et al., 2022).

Considering that NAG and LAP display similar functions, the following equation was used to normalize the enzyme activities (Jia

et al., 2022):

$$N - acq = \sqrt[3]{(NAG \bullet LAP)} \quad (3)$$

Where N-acq represents the N-acquisition enzyme activity, NAG and LAP refer to the β-1, 4-N-acetylglucosaminidase and Leucine aminopeptidase, respectively.

## 2.8. Soil quality index

All soil metric datas were calculated to transform into dimensionless scores ranging from 0 to 1 to acquire the soil quality index (SQI) for three cropping systems as follows:

$$SL = \frac{x - L}{H - L} \quad (4)$$

$$SL = 1 - \frac{x - L}{H - L} \quad (5)$$

Where  $S_L$  indicated the linear score (0–1),  $x$  stands for the measured value of the index;  $H$  and  $L$  referred to the highest and lowest values of the index, respectively. Specifically, if a parameter was a positive indicator and benefited soil quality (e.g., SOC), then it was normalized by Eq. (4). Otherwise, the parameter was normalized by Eq. (5) (e.g., mineralization). The SQI score was assessed via an SQI-length approach based on the length of a chord diagram calculated according to the transformed soil metrics (Kuzyakov et al., 2020):

$$SQI_{-length} = 0.5 \times \sum_1^n \times S_L^2 \sin \frac{2\pi}{n} \quad (6)$$

Where SQI-length indicates the SQI score, and  $n$  represents the number of soil metrics applied for the SQI-length.

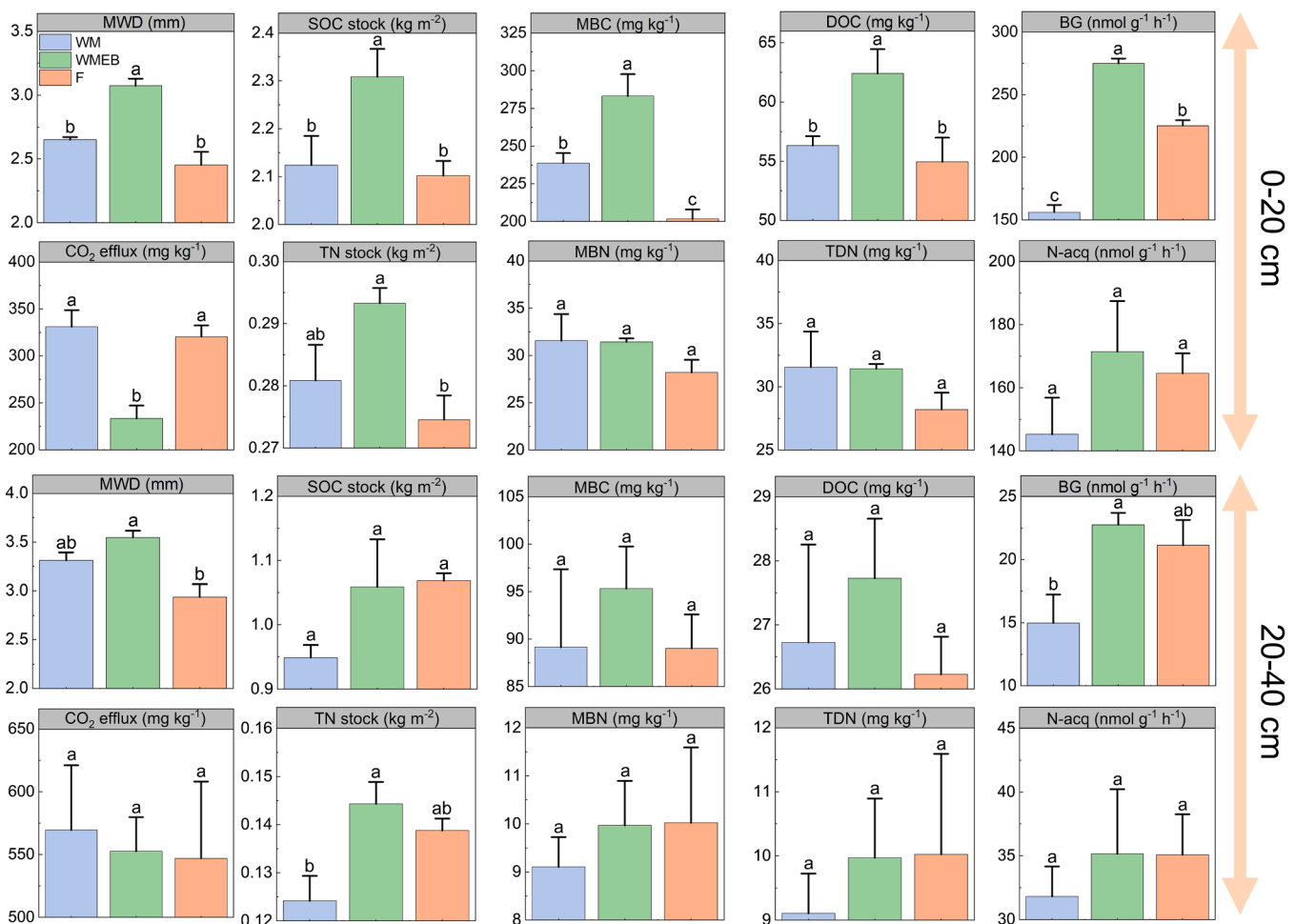
## 2.9. Statistical analysis

The normality and homogeneity of variance were checked by the Shapiro-Wilk test ( $p > 0.05$ ) and Levene test ( $p > 0.05$ ) before statistical analysis, respectively. One-way analysis of variance (ANOVA) together with Fisher's Least Significant Difference test ( $p < 0.05$ ) was performed via SPSS version 25.0 (IBM SPSS Software Inc., Armonk, NY, USA) to identify the effect of cropping systems on soil metrics. The Pearson's correlations between SQI and other parameters were calculated via the R package "GGally" (Version 4.0.3). Also, the partial least squares path modeling (PLS-PM) was used to explore the statistical relationship among soil physicochemical properties, SQI, and SOC stock (Latan et al., 2017).

## 3. Results

### 3.1. Effect of cropping systems on bulk soil biochemical metrics

Diversified cropping altered nearly all measured soil metrics, especially in the topsoil across six-year cropping strategies (Figs. 1 and 6). In the topsoil, the SOC stock increased by 9% under diversified cropping relative to wheat/maize ( $p < 0.05$ ), while fallow and wheat/maize displayed comparable SOC stock in the topsoil (Fig. 1). Further, the MWD, MBC, DOC, BG, and TN stock were higher and SOC mineralization was lower under diversified cropping relative to wheat/maize ( $p < 0.05$ ; Fig. 1). Here fallow increased the BG, but decreased the MBC relative to wheat/maize ( $p < 0.05$ ; Fig. 1). In the subsoil, however, there were few changes in measured soil metrics among the three cropping systems, whilst diversified cropping increased the BG and TN stock relative to wheat/maize ( $p < 0.05$ ; Fig. 1). Overall, diversified cropping changed nearly all measured soil properties in the topsoil and exhibited minor effects in the subsoil.



**Fig. 1.** Mean weight diameter (MWD), CO<sub>2</sub> efflux, SOC stock, TN stock, microbial biomass C (MBC), microbial biomass N (MBN), dissolved organic C (DOC), total dissolved N (TDN),  $\beta$ -1, 4-glucosidase (BG), and nitrogen-acquisition enzyme activity (N-acq) at 0–20 and 20–40 cm soil depths under three cropping systems: winter wheat/summer maize (WM), winter wheat/summer maize-early soybean (WMEB), and nature fallow (F). Different lowercase letters denote significant differences between cropping systems ( $p < 0.05$ ). Values are average ( $\pm$  SE) of the three replicates ( $n = 3$ ).

### 3.2. Effect of cropping systems on aggregate-scale biochemical metrics

Cropping systems displayed a remarkable effect on the aggregate-scale biochemical metrics in the topsoil, especially for large macroaggregates ( $> 2$  mm) (Fig. 2 and S2). In the topsoil, the SOC, MBC, BG, and N-acq in large macroaggregates were all increased by 15%, 15%, 32%, and 16% in the topsoil under diversified cropping relative to wheat/maize, respectively ( $p < 0.05$ ; Fig. 2). While fallow decreased the TN and N-acq by 14% and 27% in large macroaggregates versus wheat/maize, respectively ( $p < 0.05$ ; Fig. 2). In the subsoil, diversified cropping and fallow increased both the TN and MBN contents in large macroaggregates relative to wheat/maize, respectively ( $p < 0.05$ ; Fig. 2). Overall, diversified cropping increased the majority of soil metrics within the large macroaggregates in the topsoil but exhibited minor effects in the subsoil.

### 3.3. Effect of cropping systems on soil quality index

Diversified cropping changed the soil quality index (SQI) across six-year cropping strategies (Figs. 3 and 6). Compared to wheat/maize, diversified cropping increased the SQI by 197% in the topsoil, and by 113% in the subsoil ( $p < 0.05$ ; Fig. 3). However, there were no significant differences regarding the SQI between fallow and wheat/maize for both soil depths (Fig. 3). Overall, diversified cropping enhanced the SQI regardless of soil depth.

### 3.4. The relationships between C sequestration, and other biochemical metrics at aggregate-scale

For both soil depths, the PLS-PM indicated that aggregate sizes and MBC held an imperative direct effect (0.44 and 0.55), whilst CO<sub>2</sub> efflux alone exerted a negative direct effect ( $-0.39$ ) on C sequestration ( $p < 0.05$ ; Fig. 4a and b). In addition, BG had a significant indirect effect by mediating DOC and MBC resulting in C sequestration (Fig. 4a and b). Collectively, diversified cropping benefited C sequestration which was dominantly driven by MBC at the aggregate-scale.

### 3.5. The relationships between soil quality, SOC stock, and other bulk soil biochemical metrics

Pearson correlation analysis displayed a strong correlation between soil biochemical metrics and SQI and SOC stock, especially in the topsoil ( $p < 0.05$ ; Fig. 5a). The SOC and TN stocks, MWD, MBC, BG, and DOC were positively correlated with SQI, while there was a negative correlation between CO<sub>2</sub> efflux and SQI ( $p < 0.05$ ; Fig. 5a). In the subsoil, only the N stock and MWD were closely correlated with SQI, along with the positive correlation between C and N stock ( $p < 0.05$ ; Fig. 5a). For both soil depths, the PLS-PM showed that SOC stock and MWD had strong positive effects (0.71 and 1.02) on SQI ( $p < 0.05$ ; Fig. 5b). Furthermore, the MBC content held a much promotional effect (0.47) directly on the SOC stock ( $p < 0.05$ ; Fig. 5b). Collectively, the MWD and

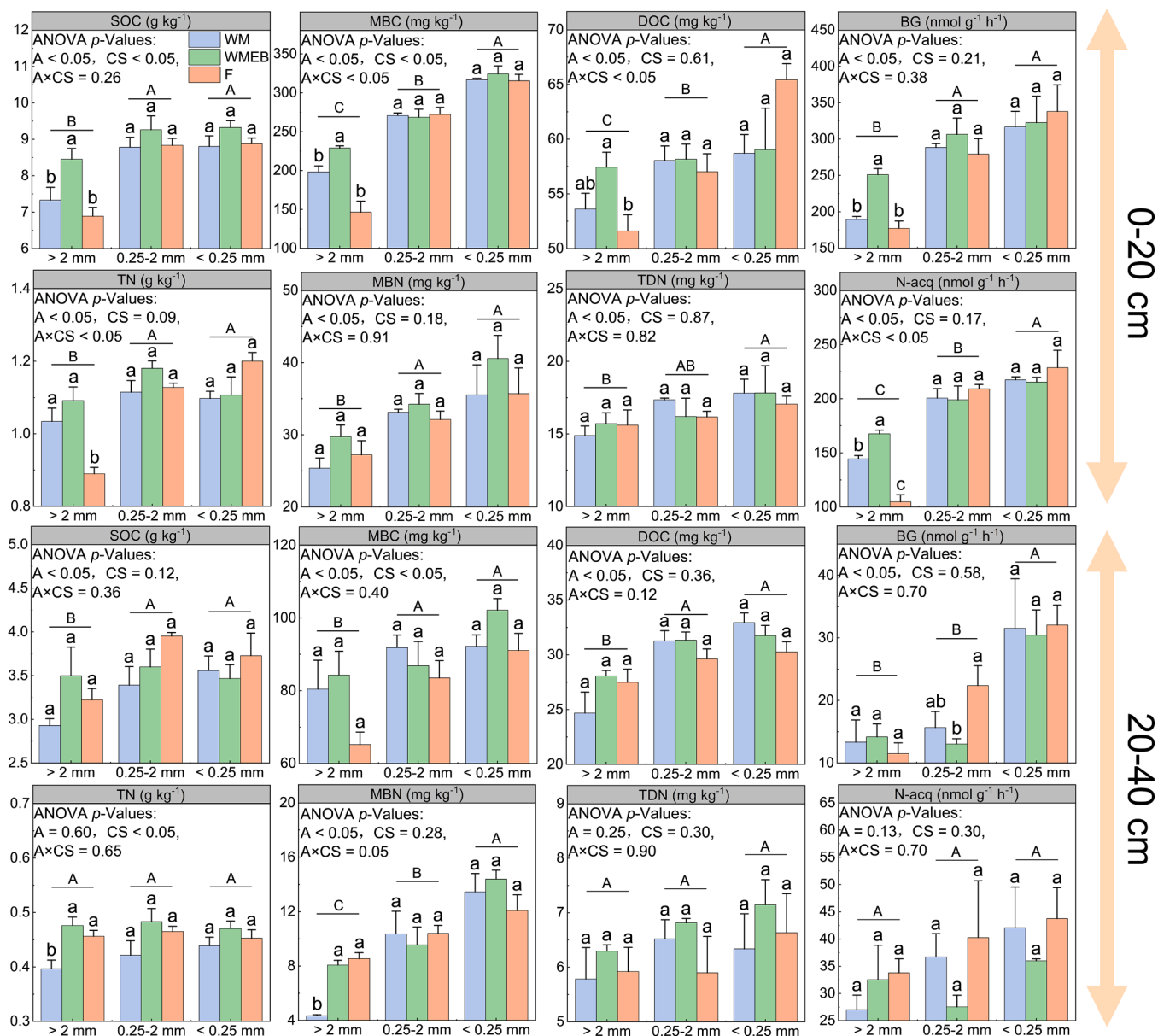


Fig. 2. Soil organic C (SOC), total nitrogen (TN), microbial biomass C (MBC), microbial biomass N (MBN), dissolved organic carbon (DOC), total dissolved nitrogen (TDN),  $\beta$ -1, 4-glucosidase (BG), and nitrogen-acquisition enzyme activity (N-acq) within aggregates at 0–20 and 20–40 cm soil depths under three cropping systems: winter wheat/summer maize (WM), winter wheat/summer maize-early soybean (WMEB), and nature fallow (F). Different lowercase letters denote significant differences between cropping systems ( $p < 0.05$ ). Values are average ( $\pm$  SE) of the three replicates ( $n = 3$ ).

SOC stock played pivotal roles in mediating the SQI.

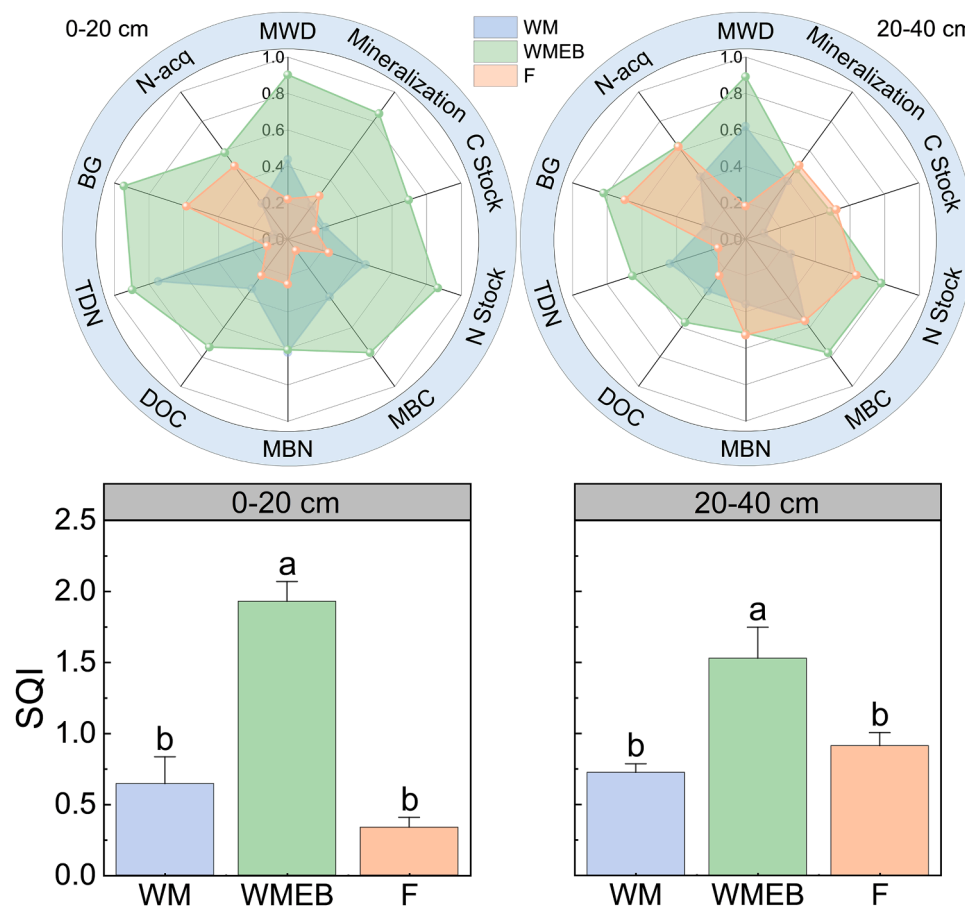
## 4. Discussion

### 4.1. Diversified cropping increased SOC stock

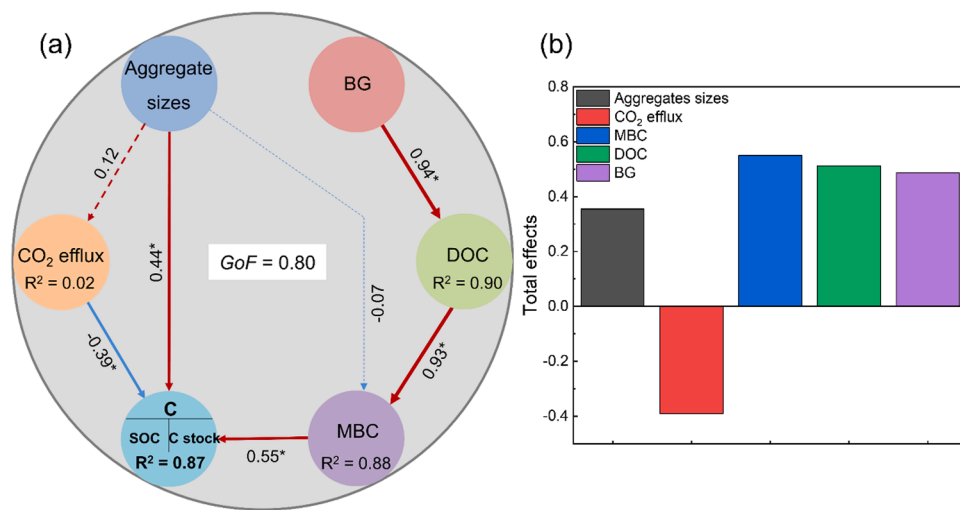
The six-year diversified cropping benefited soil C sequestration in the topsoil, despite a 40% reduction in the N fertilizer compared to wheat/maize (Figs. 1 and 6). This was partly attributed to the large input of senescent roots and nodules of legumes that were responsible for the increased SOC stock (Rosenstock et al., 2014; Meena and Lal, 2018; Kazmierczak et al., 2020). Moreover, our results indicated that diversified cropping increased the TN stock, which might be due to the N-enriched legume residues decomposition into soil easily combined with biological N<sub>2</sub> fixation of soybean, thus resulting in a high potential for N sequestration (Diekow et al., 2005; Martin et al., 2019). Such a result may increase the microbial C limitation and thus stimulate

increased C-acquisition enzyme activities (Fig. 1) to decompose crop residues in diversified cropping. Alternatively, legume residue as easily available substrates for microbes (i.e., lower C/N ratio) offset potential priming of existing SOC under diversified cropping (Franke et al., 2008; Zhang et al., 2019).

Further yet, our results showed that diversified cropping had no significant effect on SOC stock in the subsoil across six-year (Fig. 1). This is because 66% of the soybean roots were distributed in topsoil (Li et al., 2017), short-term diversified cropping might not favor the majority of plant-derived C inputs to subsoil, thus leading to similar SOC stock versus wheat/maize. Similar results were also observed by Blanco-Canqui et al. (2017) who found that legume-based cropping did not affect SOC stock in the subsoil over two decades. Further, since the C is 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), thus leading to comparable MBC content versus



**Fig. 3.** The radar graphs show the relative responses of soil biochemical metrics to the cropping system at 0–20 and 20–40 cm soil depth. The soil quality index (SQI) in response to cropping system at 0–20 and 20–40 cm soil depth (c). Different lowercase letters denote significant differences between cropping systems ( $p < 0.05$ ). Values are average ( $\pm$  SE) of the three replicates ( $n = 3$ ).

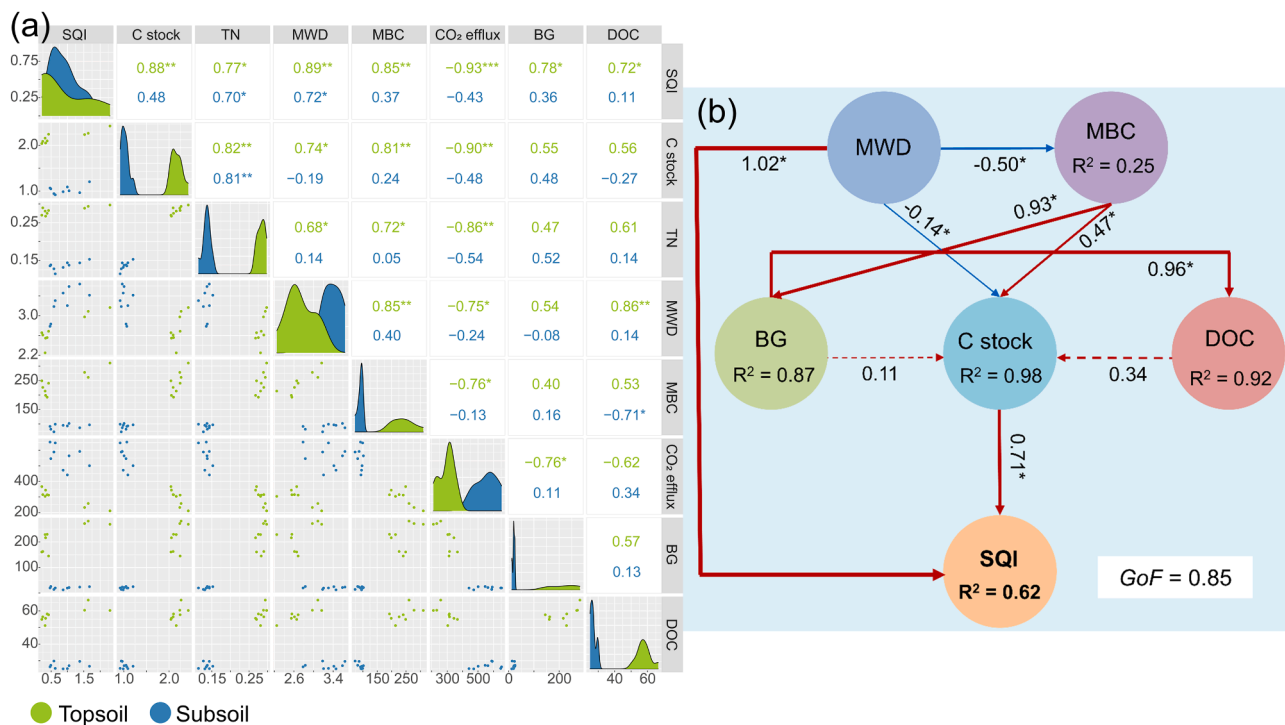


**Fig. 4.** (a) Directed graph of the partial least squares path model. Each circle represents an observed variable. Path coefficients are calculated after 1000 bootstraps and reflected in the width of the arrow, with red and blue indicating positive and negative effects, respectively. The “\* ” showed that coefficients differ significantly ( $p < 0.05$ ). The model was assessed using the Goodness of Fit ( $GoF$ ) statistic, and the  $GoF$  value was 0.80. (b) Total effect represented the relative importance of soil biochemical metrics on C stock at the aggregate-scale.

wheat/maize (Fig. 1). Such results indicated that diversified cropping unawakened the microbial-driven carbon turnover in the subsoil, thus leading to slight changes in C sequestration (Wright et al., 2008; Gentsch et al., 2020).

As macroaggregates are largely formed around fresh residues (including roots and exudates), diversified cropping provides more diverse and dissolved C input to the soil by creating more resource

niches for soil microorganisms, which often increases the microbial biomass (Fig. 1), microbial functioning and biodiversity (McDaniel et al., 2014; Mooshammer et al., 2022). This could simultaneously enhance microbial activities and production of microbial-derived binding agents (e.g., glomalin), which are imperative for promoting soil aggregation (Bossuyt et al., 2001; Zhou et al., 2020, 2022a). Further yet, due to the less physical disturbance to soil aggregates during the three



**Fig. 5.** (a) The Pearson correlation between SQI and other bulk soil biochemical metrics at 0–20 cm 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. (b) Directed graph of the partial least squares path model. Each circle represented an observed variable. Path coefficients were calculated after 1000 bootstraps and reflected in the width of the arrow, with red and blue indicating positive and negative effects, 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 0.85.

crop harvests over two years (Jin et al., 2021), thus soil aggregate stability was larger under diversified cropping versus wheat/maize. Here SOC is physically occluded by increased aggregate stability hindering microbial degradation, thus enhancing SOC stock (Fig. 1). This was further supported by the higher MBC content (Fig. 1) and the positive correlation between MBC, MWD, and SOC stock (Fig. 5). Such results compare well with those obtained by Kumar et al. (2018) who documented that legume-based cropping systems improved SOC content by increasing microbial biomass that promoted soil aggregation. The PLS-PM further confirmed that the MBC displayed a positive effect on SOC stock (Fig. 5b). Furthermore, diversified cropping decreased the SOC mineralization (Fig. 1), which was due to the higher soil aggregate stability as indicated by the increased MWD (Haynes and Francis, 1993). The physical protection of large macroaggregates reduced microbial accessibility to the SOC (Six et al., 2000), and thus promoted C sequestration as evidenced by the negative correlation between CO<sub>2</sub> efflux and SOC stock (Fig. 5a).

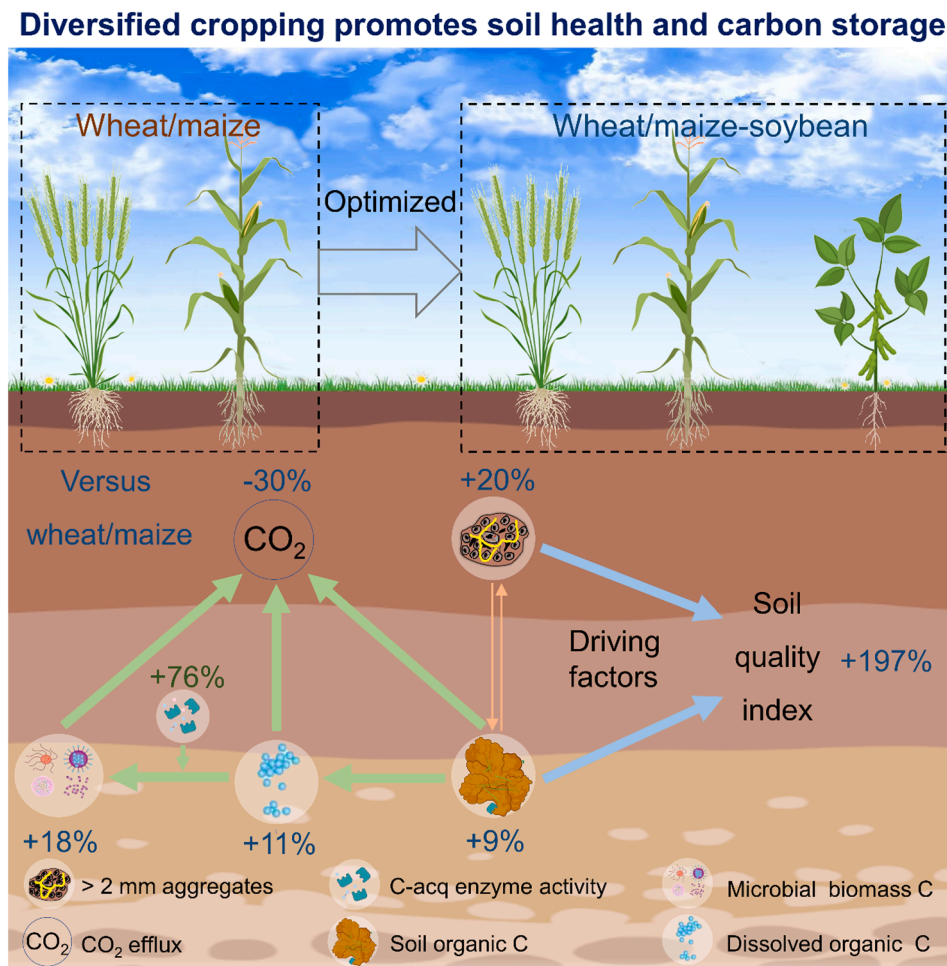
In our study, the SOC and TN stocks under natural fallow were comparable to the wheat/maize (Fig. 1). Nature fallow reduced frequent soil disturbance by intensive tillage, which could increase aggregate stability, thus benefiting SOC stock (Jiang and Xie, 2009). Nevertheless, the minimal diversity of residue input could be responsible for the lower microbial biomass (Fig. 1) and turnover rate, which might not result in higher MWD physically protecting SOC and TN content (Nielsen and Calderon, 2011; Kumar et al., 2018). These two opposing effects might in part counteract each other and resulted in similar SOC stock as wheat/maize.

#### 4.2. Diversified cropping increased soil C sequestration at the aggregate-scale

The higher proportion of large macroaggregate (> 2 mm) and its C concentration were essential for C stabilization and long-term

sequestration (Six et al., 2002; Kumar et al., 2019). In our study, diversified cropping increased the SOC content within > 2 mm aggregates relative to wheat/maize (Figs. 2 and S2). Here, the MBC was higher in large macroaggregates under diversified cropping (Fig. 2), which is supported by the fact that larger pores favor microorganisms proliferation (Tisdall and Oades, 1982; Chenu et al., 2001). Further, increased enzyme activities could catalytically degrade the higher labile substrates in > 2 mm aggregates (Fig. 2), which were utilized by microorganisms to build up their biomass in large macroaggregates rather microaggregates under diversified cropping (Liang et al., 2017; Wang et al., 2017). Such a result could likely contribute to increasing C sequestration in large macroaggregates after a long-term microbial turnover (Mooshammer et al., 2022). Moreover, the PLS-PM further demonstrated that aggregate sizes and MBC displayed promotional effects on soil C sequestration (Fig. 4a) where the MBC held evidence-based greatest benefits on C sequestration (Fig. 4b). Further yet, the SOC stock was mainly dominated by the > 2 mm SOC stock (Fig. S2). These results reinforce that the increased SOC stock was driven by the SOC content in large macroaggregates (Novelli et al., 2011). In our study, there were virtually no significant differences regarding SOC concentration within small macroaggregates and microaggregates under three cropping systems (Fig. 2). In the scenario of legume-based diversified cropping, the more diverse and dissolved C undergoes microbial metabolic decomposition to form larger soil particles and large macroaggregates and has little effect on smaller aggregates relatively (Six et al., 2000; Six and Paustian, 2014). Such a result could explain why the legume-based diversified cropping had a greater impact on the large macroaggregate. Taken together, the increased MBC content in > 2 mm aggregates is responsible for the C sequestration at the aggregate-scale, thus contributing to the increased SOC stock in the topsoil under diversified cropping, even reducing N fertilizer application.

We also acknowledge some limitations in our study. First, the dynamics of soil C pools depend on the balance of plant C input and



**Fig. 6.** Graphical abstract illustrating diversified cropping stimulating soil quality and carbon sequestration. The wheat/maize-soybean cropping system increased the soil C stock by 9% relative to wheat/maize. Alternatively, diversified cropping improved soil quality, which was mainly driven by aggregates and soil organic C.

microbial decomposition (Wang et al., 2021). Only considering changes in microbial C pool, metabolic activities, and enzyme activities under diversified cropping versus wheat-maize in our study cannot fully explain the changes in soil C pools. Second, since the recognized importance of the contribution of microbial necromass to soil C sequestration (Liang et al., 2019). Further research is required to investigate the effect of diversified cropping on microbial necromass when considering the microbial mechanisms regulating the SOC dynamics, which is paramount for a sustainable agroecosystem.

#### 4.3. Diversified cropping enhanced the soil quality index

Diversified cropping benefited soil quality, which was mainly driven by an increased SOC stock and MWD as supported by the PLS-PM (Figs. 3 and 5). Stabilization of soil aggregation in the legume-based diversified cropping is characterized by an increased proportion of large macro-aggregates (Fig. 2, Fig. S1) and stimulation of microbial biomass as larger pores favor microorganisms to proliferate (Tisdall and Oades, 1982; Bruns and Couradeau, 2014). Such a result combined with stimulated microbial functioning and biodiversity via diverse fresh residues improved soil functions (e.g., soil aggregation and nutrient cycling) and related ecosystem services, as well as consequently soil quality (Gupta and Germida, 2015; Nunes et al., 2018; Sánchez et al., 2022). These findings concur well with results obtained by Feng et al. (2020) and Williams et al. (2020), who reported that diversified cropping is crucial for enhancing soil quality due to increased aggregate stability providing water infiltration and retention, biodiversity, and C sequestration.

Improved soil aggregation benefits the spatial and temporal distribution of air, water, and solute flow, thus affecting root and microbial respiration, penetration, and seedling emergence. These improvements further benefit soil microbial activities and stimulate the microbial-derived by-product (Blankinship et al., 2016; Zhou et al., 2022b). Furthermore, the increased soil aggregation physically stabilizes SOC in the long term under diversified cropping (Yan et al., 2022). Consequently, diversified cropping improved soil quality via enhancing biochemical metrics, SOC stock, and aggregate stability.

#### 5. Conclusion

The six-year diversified cropping increased the SOC stock (~9%) and nearly all soil biochemical metrics relative to wheat/maize in the topsoil. The SOC, microbial biomass C, C-acquisition enzyme activity, and dissolved organic C concentrations within > 2 mm aggregates all increased in the topsoil under diversified cropping versus wheat/maize. The partial least squares path model suggested that microbial biomass C was the main driver for C sequestration regardless of bulk- and aggregate-scale. Moreover, diversified cropping enhanced the soil quality index due to increased aggregate stability benefiting soil microbial proliferation and C sequestration. The SOC stock and soil quality index under natural fallow were comparable to wheat/maize, and might not be wise for agriculture management at the expense of crop production. Overall, our results illustrate the mechanism of soybean inclusion in SOC stock and soil quality improvement in wheat-maize cropping system, while also suggesting the potential for reducing N



inputs in intensive high-yield cropping systems. Therefore, we advocated that legume-based diversified cropping was expected to create win-win scenarios for enhancing soil quality and advancing sustainable intensification in agroecosystems.

### 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.

### Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (32101850), the Joint Funds of the National Natural Science Foundation of China (U21A20218), and the National Key Research & Development Program of China (2021YFD1901002).

### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.still.2023.105848](https://doi.org/10.1016/j.still.2023.105848).

### References

- Abid, M., Lal, R., 2008. Tillage and drainage impact on soil quality: I. Aggregate stability, carbon and nitrogen pools. *Soil Tillage Res.* 100, 89–98.
- Bao, S.D., 2000. *Soil Agrochemical Analysis*, third ed. China Agricultural Press, Beijing, China.
- Blanco-Canqui, H., Francis, C.A., Galusha, T.D., 2017. Does organic farming accumulate carbon in deeper soil profiles in the long term? *Geoderma* 288, 213–221.
- Blankinship, J.C., Fonte, S.J., Six, J., Schimel, J.P., 2016. Plant versus microbial controls on soil aggregate stability in a seasonally dry ecosystem. *Geoderma* 272, 39–50.
- Bossuyt, H., Deneff, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. *Appl. Soil Ecol.* 16, 195–208.
- Bruns, M.A., Couradeau, E., 2014. Sustainable soil health. In: Ricroch, A., Chopra, S., Fleischer, S.J. (Eds.), *Plant Biotechnology: Experience and Future Prospects*. Springer International Publishing, Switzerland, pp. 209–223.
- Chenu, C., Hassink, J., Bloem, J., 2001. Short-term changes in the spatial distribution of microorganisms in soil aggregates as affected by glucose addition. *Biol. Fert. Soils* 34, 349–356.
- Chu, J.C., Zhou, J., Wang, Y., Jones, D.L., Ge, J.Y., Yang, Y.D., Brown, R.W., Zang, H.D., Zeng, Z.H., 2022. Field application of biodegradable microplastics has no significant effect on plant and soil health in the short term. *Environ. Pollut.* 316, 120556.
- Congreves, K.A., Hayes, A., Verhallen, E.A., Van Eerd, L.L., 2015. Long-term impact of tillage and crop rotation on soil health at four temperate agroecosystems. *Soil Tillage Res.* 152, 17–28.
- Diekow, J., Mielniczuk, J., Knicker, H., Bayer, C., Dick, D.P., Kögel-Knabner, I., 2005. Soil C and N stocks as affected by cropping systems and nitrogen fertilisation in a southern Brazil Acrisol managed under no-tillage for 17 years. *Soil Tillage Res.* 81, 87–95.
- Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Marhan, S., Fangmeier, A., Kuzyakov, Y., 2009. Stimulation of microbial extracellular enzyme activities by elevated CO<sub>2</sub> depends on soil aggregate size. *Glob. Chang. Biol.* 15, 1603–1614.
- Feng, H., Abagandura, G.O., Senturklu, S., Landblom, D.G., Lai, L., Ringwall, K., Kumar, S., 2020. Soil quality indicators as influenced by 5-year diversified and monoculture cropping systems. *J. Agric. Sci.* 158, 594–605.
- Fierer, N., Wood, S.A., de Mesquita, C.P.B., 2021. How microbes can, and cannot, be used to assess soil health. *Soil Biol. Biochem.* 153, 108111.
- Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, J.S., O'Connell, C., Ray, D.K., West, P.C., 2011. Solutions for a cultivated planet. *Nature* 478, 337–342.
- Franke, A.C., Laberge, G., Oyewole, B.D., Schulz, S., 2008. A comparison between legume technologies and fallow, and their effects on maize and soil traits, in two distinct environments of the west african savannah. *Nutr. Cycl. Agroecosyst.* 82, 117–135.
- Gao, B., Ju, X.T., Meng, Q.F., Cui, Z.L., Christie, P., Chen, X.P., Zhang, F.S., 2015. The impact of alternative cropping systems on global warming potential, grain yield and groundwater use. *Agric. Ecosyst. Environ.* 203, 46–54.
- Gentsch, N., Boy, J., Batalla, J.D.K., Heuermann, D., von Wiren, N., Schweneker, D., Feuerstein, U., Gross, J., Bauer, B., Reinhold-Hurek, B., Hurek, T., Cespedes, F.C., Guggenberger, G., 2020. Catch crop diversity increases rhizosphere carbon input and soil microbial biomass. *Biol. Fert. Soils* 56, 943–957.
- Gupta, V.V., Germida, J.J., 2015. Soil aggregation: influence on microbial biomass and implications for biological processes. *Soil Biol. Biochem.* 80, A3–A9.
- Haynes, R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: an overview. *Adv. Agron.* 5, 221–268.
- Haynes, R.J., Francis, G.S., 1993. Changes in microbial biomass C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. *Soil Sci.* 44, 665–675.
- Jansson, J.K., Hofmockel, K.S., 2020. Soil microbiomes and climate change. *Nat. Rev. Microbiol.* 18, 35–46.
- Jia, R., Zhou, J., Chu, J.C., Shahbaz, M., Yang, Y.D., Jones, D.L., Zang, H.D., Razavi, B.S., Zeng, Z.H., 2022. Insights into the associations between soil quality and ecosystem multifunctionality driven by fertilization management: a case study from the North China Plain. *J. Clean. Prod.* 362, 132265.
- Jiang, X.J., Xie, D.T., 2009. Combining ridge with no-tillage in lowland rice-base cropping system: long-term effect on soil and rice yield. *Pedosphere* 19, 515–522.
- Jin, V.L., Wienhold, B.J., Mikha, M.M., Schmer, M.R., 2021. Cropping system partially offsets tillage-related degradation of soil organic carbon and aggregate properties in a 30-yr rainfed agroecosystem. *Soil Tillage Res.* 209, 104968.
- Kallenbach, C., Grandy, A.S., 2011. Controls over soil microbial biomass responses to carbon amendments in agricultural systems: a meta-analysis. *Agric. Ecosyst. Environ.* 144, 241–252.
- Kazmierczak, T., Yang, L., Boncompagni, E., Meilhoc, E., Frugier, F., Frendo, P., Bruand, C., Gruber, V., Brouquisse, R., 2020. Legume nodule senescence: a coordinated death mechanism between bacteria and plant cells. *Adv. Bot. Res.* 94, 182–203.
- Kumar, N., Nath, C.P., Hazra, K.K., Das, K., Venkatesh, M.S., Singh, M.K., Singh, S.S., Praharaj, C.S., Singh, N.P., 2019. Impact of zero-till residue management and crop diversification with legumes on soil aggregation and carbon sequestration. *Soil Tillage Res.* 189, 158–167.
- Kumar, S., Meena, R.S., Lal, R., Singh Yadav, G., Mitran, T., Meena, B.L., Dotaniya, M.L., EL-Sabagh, A., 2018. Role of legumes in soil carbon sequestration. In: Meena, R.S., Das, A., Yadav, G.S., Lal, R. (Eds.), *Legumes for Soil Health and Sustainable Management*. Springer, Singapore, pp. 109–138.
- Kuzyakov, Y., Gunina, A., Zamanian, K., Tian, J., Luo, Y., Xu, X.L., Yudina, A., Aponte, H., Alharbi, H., Ovsepyan, L., Kurganova, I., Ge, T.D., Guillaume, T., 2020. New approaches for evaluation of soil health, sensitivity and resistance to degradation. *Front. Agric. Sci. Eng.* 7, 282–288.
- Lal, R., 2015. Sequestering carbon and increasing productivity by conservation agriculture. *J. Soil Water Conserv.* 70, 55A–62A.
- Lal, R., 2019. Carbon cycling in global drylands. *Curr. Clim. Chang. Rep.* 5, 221–232.
- Latan, H., Noonan, R., Matthews, L., 2017. Partial least squares path modeling. *Partial least squares path modeling: basic concepts, methodological issues and applications*.
- Li, H., Mollier, A., Ziadi, N., Shi, Y., Parent, L.E., Morel, C., 2017. Soybean root traits after 24 years of different soil tillage and mineral phosphorus fertilization management. *Soil Tillage Res.* 165, 258–267.
- Li, Y.Y., Dong, S.K., Wen, L., Wang, X.X., Wu, Y., 2014. Soil carbon and nitrogen pools and their relationship to plant and soil dynamics of degraded and artificially restored grasslands of the Qinghai-tibetan plateau. *Geoderma* 213, 178–184.
- Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. *Nat. Microbiol.* 2, 1–6.
- Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. *Glob. Chang. Biol.* 25, 3578–3590.
- Lynch, J.P., Wojciechowski, T., 2015. Opportunities and challenges in the subsoil: pathways to deeper rooted crops. *J. Exp. Bot.* 66, 2199–2210.
- Ma, H.Y., Zhou, J., Ge, J.Y., Nie, J.W., Zhao, J., Xue, Z.Q., Hu, Y.G., Yang, Y.D., Peixoto, L., Zang, H.D., Zeng, Z.H., 2022. Intercropping improves soil ecosystem multifunctionality through enhanced available nutrients but depends on regional factors. *Plant Soil* 480, 71–84.
- Martin, A.R., Hayes, F.J., Borden, K.A., Buchanan, S.W., Gordon, A.M., Isaac, M.E., Thevathasan, N.V., 2019. Integrating nitrogen fixing structures into above- and belowground functional trait spectra in soy (*Glycine max*). *Plant Soil* 440, 53–69.
- McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecol. Appl.* 24, 560–570.
- Meena, R.S., Lal, R., 2018. Legumes and sustainable use of soils. In: *Legumes for Soil Health and Sustainable Management*. Springer, Singapore, pp. 1–31.
- Mooshammer, M., Grandy, A.S., Calderón, F., Culman, S., Deen, B., Drijber, R.A., Dunfield, K., Jin, V.L., Lehman, R.M., Osborne, S.L., Schmer, M., Bowles, T.M., 2022. Microbial feedbacks on soil organic matter dynamics underlying the legacy effect of diversified cropping systems. *Soil Biol. Biochem.* 167, 108584.
- Nayab, G., Zhou, J., Jia, R., Lv, Y.H., Yang, Y.D., Brown, R.W., Zang, H.D., Jones, D.L., Zeng, Z.H., 2022. Climate warming masks the negative effect of microplastics on plant-soil health in a silt loam soil. *Geoderma* 425, 116083.
- Nielsen, D.C., Calderon, F.J., 2011. Fallow effects on soil. In: Hatfield, J.L., Sauer, T.J. (Eds.), *Soil Management: Building a Stable Base for Agriculture*. American Society of Agronomy and Soil Science Society of America, Madison, WI, pp. 287–300.
- Novelli, L.E., Caviglia, O.P., Melchiori, R.J.M., 2011. Impact of soybean cropping frequency on soil carbon storage in Mollisols and Vertisols. *Geoderma* 167, 254–260.
- Nunes, M.R., van Es, H.M., Schindelbeck, R., Ristow, A.J., Ryan, M., 2018. No-till and cropping system diversification improve soil health and crop yield. *Geoderma* 328, 30–43.
- Oldfield, E.E., Bradford, M.A., Wood, S.A., 2019. Global meta-analysis of the relationship between soil organic matter and crop yields. *Soil* 51, 15–32.

- Razavi, B.S., Zarebanadkouki, M., Blagodatskaya, E., Kuzyakov, Y., 2016. Rhizosphere shape of lentil and maize: spatial distribution of enzyme activities. *Soil Biol. Biochem.* 96, 229–237.
- Rosenstock, T.S., Tully, K.L., Arias-Navarro, C., Neufeldt, H., Butterbach-Bahl, K., Verchot, L.V., 2014. Agroforestry with N<sub>2</sub>-fixing trees: sustainable development's friend or foe? *Curr. Opin. Environ. Sustain.* 6, 15–21.
- Sánchez, E., Zabaleta, R., Fabiani, M.P., Rodriguez, R., Mazza, G., 2022. Effects of the amendment with almond shell, bio-waste and almond shell-based biochar on the quality of saline-alkali soils. *J. Environ. Manag.* 318, 115604.
- Singh, A.K., Cabral, C., Kumar, R., Ganguly, R., Rana, K.H., Gupta, A., Lauro, M.R., Carbone, C., Reis, F., Pandey, A.K., 2019. Beneficial effects of dietary polyphenols on gut microbiota and strategies to improve delivery efficiency. *Nutrients* 11, 2216.
- Six, J., Paustian, K., 2014. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. *Soil Biol. Biochem.* 68, A4–A9.
- Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biol. Biochem.* 32, 2099–2103.
- Six, J., Conant, R., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant Soil* 241, 155–176.
- Sokol, N.W., Bradford, M.A., 2019. Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. *Nat. Geosci.* 12, 46–53.
- Tiemann, L.K., Grandy, A.S., Atkinson, E.E., Marin-Spiotta, E., McDaniel, M.D., 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecol. Lett.* 18, 761–771.
- Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. *Eur. J. Soil Sci.* 33, 141–163.
- Toivonen, M., Herzon, I., Helenius, J., 2013. Environmental fallows as a new policy tool to safeguard farmland biodiversity in Finland. *Biol. Conserv.* 159, 355–366.
- Trivedi, P., Rochester, I.J., Trivedi, C., Van Nostrand, J.D., Zhou, J., Karunaratne, S., Anderson, I.C., Singh, B.K., 2015. Soil aggregate size mediates the impacts of cropping regimes on soil carbon and microbial communities. *Soil Biol. Biochem.* 169–181.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707.
- Wang, B., An, S., Liang, C., Liu, Y., Kuzyakov, Y., 2021. Microbial necromass as the source of soil organic carbon in global ecosystems. *Soil Biol. Biochem.* 162, 108422.
- Wang, C., Qu, L.R., Yang, L.M., Liu, D.W., Morrissey, E., Miao, R.H., Liu, Z.P., Wang, Q. K., Fang, Y.T., Bai, E., 2021. Large-scale importance of microbial carbon use efficiency and necromass to soil organic carbon. *Glob. Chang. Biol.* 27, 2039–2048.
- Wang, X.Q., Jia, R., Zhao, J., Yang, Y.D., Zang, H.D., Zeng, Z.H., Olesen, J.E., 2022. Quantifying water footprint of winter wheat-summer maize cropping system under manure application and limited irrigation: an integrated approach. *Resour. Conserv. Recycl.* 183, 106375.
- Wang, Y., Li, C., Tu, C., Hoyt, G.D., DeForest, J.L., Hu, S., 2017. Long-term no-tillage and organic input management enhanced the diversity and stability of soil microbial community. *Sci. Total Environ.* 609, 341–347.
- Williams, H., Colombi, T., Keller, T., 2020. The influence of soil management on soil health: an on-farm study in southern Sweden. *Geoderma* 360, 114010.
- Wright, A.L., Hons, F.M., Lemon, R.G., McFarland, M.L., Nichols, R.L., 2008. Microbial activity and soil C sequestration for reduced and conventional tillage cotton. *Appl. Soil Ecol.* 38, 168–173.
- Wu, D., Yu, Q., Wang, E., Hengsdijk, H., 2008. Impact of spatial-temporal variations of climatic variables on summer maize yield in North China Plain. *Int. J. Plant Prod.* 2, 71–88.
- Wu, D.R., Yu, Q., Lu, C.H., Hengsdijk, H., 2006. Quantifying production potentials of winter wheat in the North China Plain. *Eur. J. Agron.* 24, 226–235.
- Xu, P.D., Zhu, J., Wang, H., Shi, L., Zhuang, Y., Fu, Q.L., Chen, J.Z., Hu, H.Q., Huang, Q. Y., 2021. Regulation of soil aggregate size under different fertilizations on dissolved organic matter, cellobiose hydrolyzing microbial community and their roles in organic matter mineralization. *Sci. Total Environ.* 755, 142595.
- Yan, Z.J., Zhou, J., Nie, J.W., Yang, Y.D., Zhao, J., Zeng, Z.H., Marshall, M.R., Peixoto, L., Zang, H.D., 2021. Do cropping system and fertilization rate change water-stable aggregates associated carbon and nitrogen storage? *Environ. Sci. Pollut. Res.* 28, 65862–65871.
- Yan, Z.J., Zhou, J., Yang, L., Gunina, A., Yang, Y.D., Peixoto, L., Zeng, Z.H., Zang, H.D., Kuzyakov, Y., 2022. Diversified cropping systems benefit soil carbon and nitrogen stocks by increasing aggregate stability: results of three fractionation methods. *Sci. Total Environ.* 824, 153878.
- Yu, C., Huang, X., Chen, H., Godfray, H.C.J., Wright, J.S., Hall, J.W., Gong, P., Ni, S., Qiao, S., Huang, G., Xiao, Y., Zhang, J., Feng, Z., Ju, X., Ciais, P., Stenseth, N.C., Hessen, D.O., Sun, Z., Yu, L., Cai, W., Fu, H., Huang, X., Zhang, C., Liu, H., Taylor, J., 2019. Managing nitrogen to restore water quality in China. *Nature* 567, 516–520.
- Zang, H.D., Zhou, J., Marshall, M.R., Chadwick, D.R., Wen, Y., Jones, D.L., 2020. Microplastics in the agroecosystem: are they an emerging threat to the plant-soil system? *Soil Biol. Biochem.* 148, 107926.
- Zhang, D.B., Yao, Z.Y., Chen, J., Yao, P.W., Zhao, N., He, W.X., Li, Y.Y., Zhang, S.Q., Zhai, B.N., Wang, Z.H., Huang, D.L., Cao, W.D., Gao, Y.J., 2019. Improving soil aggregation, aggregate-associated C and N, and enzyme activities by green manure crops in the loess plateau of China. *Eur. J. Soil Sci.* 70, 1267–1279.
- Zhang, K.L., Maltais-Landry, G., Liao, H.L., 2021. How soil biota regulate C cycling and soil C pools in diversified crop rotations. *Soil Biol. Biochem.* 156, 108219.
- Zhou, J., Zang, H.D., Loepmann, S., Gube, M., Kuzyakov, Y., Pausch, J., 2020. Arbuscular mycorrhiza enhances rhizodeposition and reduces the rhizosphere priming effect on the decomposition of soil organic matter. *Soil Biol. Biochem.* 140, 107641.
- Zhao, J., Chen, J., Beillouin, D., Lambers, H., Yang, Y.D., Smith, P., Zeng, Z.H., Olesen, J. E., Zang, H.D., 2022. Global systematic review with meta-analysis reveals yield advantage of legume-based rotations and its drivers. *Nat. Commun.* 13, 4926.
- Zhou, J., Gube, M., Holz, M., Song, B., Shan, I., Shi, L.L., Kuzyakov, Y., Dippold, M.A., Pausch, J., 2022a. Ectomycorrhizal and non-mycorrhizal rhizosphere fungi increase root-derived C input to soil and modify enzyme activities: a <sup>14</sup>C pulse labeling of *Picea abies* seedlings. *Plant Cell Environ.* 45, 3122–3133.
- Zhou, J., Jia, R., Brown, R.W., Yang, Y.D., Zeng, Z.H., Jones, D.L., Zang, H.D., 2022b. The long-term uncertainty of biodegradable mulch film residues and associated microplastics pollution on plant-soil health. *J. Hazard. Mater.* 442, 130055.