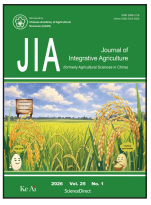
Journal of Integrative Agriculture 2026, 25(1): 326–338

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Intercropping grain crops with green manure under reduced chemical nitrogen improves the soil carbon stocks by optimizing aggregates in an oasis irrigation area

Xiaohui Xu[1, 2], Qiang Chai[2#], Falong Hu[1, 2], Wen Yin[1, 2], Zhilong Fan[1, 2], Hanting Li[1, 3], Zhipeng Liu[1, 2], Qiming Wang[1, 2]

[1] *State Key Laboratory of Aridland Crop Science, Lanzhou 730070, China*
[2] *College of Agronomy, Gansu Agricultural University, Lanzhou 730070, China*
[3] *College of Grassland Science, Gansu Agricultural University, Lanzhou 730070, China*

Highlights

- Intercropping green manure with reduced N improves the structure of aggregates.
- The impact of N reduction on SOC is compensated by intercropping green manure.
- The compensatory effect of intercropping increases with time.
- Intercropping with reduced N increases SOC by optimizing soil aggregates.

Abstract

Enhancing soil organic carbon (SOC) stocks is a key aspect of modern agriculture, but whether this can be achieved by incorporating legume green manure crops in cereal production to substitute synthetic N fertilizers is unknown. This study used a six-year (2017–2022) field study to explore the impacts of intercropping green manure with maize and reducing nitrogen fertilization on SOC stocks, while specifically focusing on the relationship between aggregate composition and carbon sequestration. Maize intercropped with common vetch (M/V), maize intercropped with rapeseed (M/R), and sole maize (M), were each tested at conventional (N2, 360 kg ha^{-1}) and reduced (N1, 270 kg ha^{-1}, 25% reduced) N application rates. Soil was sampled in 2020, 2021, and 2022. Compared with sole maize, intercropping with green manure (M/V and M/R) significantly increased SOC stocks which compensated for any negative effect due to the 25% reduction in N application. Based on 3-year averages, intercropping with M/V and M/R increased the SOC content compared to sole maize (M) by 12.1 and 9.1%, respectively, with intercropping further mitigating the negative impact of reduced nitrogen application. There was no significant difference between M/V and M/R. The SOC content at N1 was reduced by 9.3–10.5% compared to that at N2 in sole maize, but the differences in SOC stocks between N1 and N2 were not significant in the intercropping patterns (M/V and M/R). The intercropped M/V and M/R showed 20.9 and 16.3% higher SOC contents compared to sole maize at N1, with no differences at N2. Intercropping green manure led to a 5.3% greater SOC in the 0–20 cm depth soil in 2022 compared to that in 2020, due to the cumulative effect of two years of green manure intercropping. Intercropping green manure (M/V and M/R) increased the proportion of macroaggregates (>0.25 mm) and aggregate stability while reducing the proportion of microaggregates compared to sole maize under the N1 application. Structural equation modeling indicated that cropping patterns and nitrogen application levels mainly affect SOC indirectly by regulating the composition of macroaggregates and aggregate organic carbon (AOC). Correlation analysis further revealed that the composition of macroaggregates is significantly and positively correlated with the SOC content (R^2=0.64). In addition, intercropping green manure can maintain high crop yields by increasing SOC under reduced chemical nitrogen application. The results of this study show that intercropping green manure with grain crops can be a viable measure for increasing SOC sinks and maize productivity by optimizing the aggregate composition with reduced N application in the Hexi Oasis Irrigation Area.

Keywords: intercropping green manure, soil organic carbon, soil aggregate composition, maize

Received 13 December 2024; Received in revised form 12 February 2025; Accepted 24 March 2025; Available online 25 April 2025
Xiaohui Xu, E-mail: xuxiaohui0318@126.com; #Correspondence Qiang Chai, E-mail: chaiq@gsau.edu.cn

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2025.04.029

1. Introduction

Soil carbon (C) sequestration plays a significant role in global carbon cycling (Cotrufo *et al.* 2019) by removing atmospheric CO_2 to mitigate climate change (Bossio *et al.* 2020). Increasing organic matter inputs to the soil serves as a solution to enhance C sequestration (Chenu *et al.* 2019; Wang Y *et al.* 2023), which is an effective strategy to enhance soil health (Minasny *et al.* 2017; Zhang *et al.* 2023). Carbon storage in agricultural soil is sensitive to climatic factors (Hartley *et al.* 2021) and vulnerable to anthropogenic activities (Beillouin *et al.* 2023; Ren *et al.* 2024), so any changes in these two factors can lead to a C loss or increase (Lal *et al.* 2011; Amelung *et al.* 2020). Therefore, understanding the impact of agricultural practices on soil organic carbon (SOC) stocks and stability is important. However, with the spread of intensive large-scale monoculture cropping, arable land in many parts of the world has experienced serious C loss in the past few decades and this trend is continuing (Lal 2004a; Rockström *et al.* 2017; Wang *et al.* 2021).

Soil aggregates play a crucial role in protecting SOC from loss by reducing the contact between SOC and microorganisms, thus minimizing the losses caused by erosion and mineralization (Six *et al.* 2000). In fact, studies have shown that approximately 90% of the organic carbon in arable soils is stored in aggregates (Kabiri *et al.* 2015; Zhang *et al.* 2021). The hierarchical sequencing of aggregates usually results in a greater distribution of organic carbon with short-term storage in the larger aggregates (>0.25 mm) than in the smaller aggregates (Six *et al.* 2002; Zhang *et al.* 2023). Therefore, optimizing the soil aggregate hierarchies and C partitioning within aggregate classes is the key to increasing SOC stocks.

The adoption of sustainable soil management practices, such as diversifying cropping patterns (Nakamoto and Suzuki 2001; Chai *et al.* 2021), planting cover crops (Hu *et al.* 2023), applying an adequate amount of nitrogen fertilizer (Sun *et al.* 2023), implementing reduced or no tillage with crop residue retention (Zhang *et al.* 2022), and diversifying crop rotations (Yang X L *et al.* 2024), can enhance the persistence of SOC as these practices directly impact the formation and stability of soil aggregation. Numerous studies have shown that intercropping (i.e., two or more crops growing together spatially in the same plant community) increases plant biodiversity and crop yields, and lowers the environmental footprint while enhancing soil health (Chai *et al.* 2021; Yang X L *et al.* 2024). Intercropping can also increase SOC stocks by promoting the formation of aggregates for SOC storage compared to monocropping (Tian *et al.* 2019; Li X F *et al.* 2021). Rhizodeposit C and microbial necomass C were found to be the main drivers of increasing SOC stocks under intercropping (Zhao *et al.* 2023; Hu *et al.* 2024). Meanwhile, the impact of intercropping on the stability of SOC has shown that root inputs control SOC storage in cereal-legume intercropping systems, while soil nitrogen availability and microbial decomposition influence SOC stability (Wang W *et al.* 2024). In contrast, the stability of macroaggregates and the accumulation of soil organic matter (SOM) diminished when a single crop was cultivated continuously (Kabiri *et al.* 2015).

Furthermore, fertilizer application — a common practice to supplement soil nutrients to increase crop yields — is one way to promote C sequestration (Zhou *et al.* 2006; Wang *et al.* 2018). However, excessive inputs of N fertilizer may cause changes in the way microorganisms break down organic matter for energy, leading to accelerated microbial decomposition and mineralization of organic carbon in the soil (Wang *et al.* 2018; Yan *et al.* 2021; Ablimit *et al.* 2022). "Green manure" usually refers to plant material that is incorporated into the soil at a certain growth stage to improve soil texture and nutritional value. Green manure serves two distinct functions, as it contributes to the reduction of N_2O emissions (Zhang and Lassaletta 2022; Liu *et al.* 2024) and plays a significant role in promoting SOC accumulation (Zhang D B *et al.* 2018). Legume plants have a low C/N ratio, which can enhance soil microbial activities, thereby accelerating dry matter decomposition (Sainju *et al.* 2003; Xie *et al.* 2016; Hazra *et al.* 2019; Zhang *et al.* 2022). However, whether incorporating green manure into intercrop-based cropping systems can further optimize soil aggregate distribution and increase SOC stocks while reducing N application remains to be investigated.

Intercropping is practiced in the Hexi Corridor of northwestern China, such as intercropping maize with green manure (Chai *et al.* 2014; Gou *et al.* 2022). Studies have shown that the integration of grain crops with green manure under reduced N fertilization reduces CO_2 emissions and increases the C sequestration potential while maintaining crop yields (Li *et al.* 2023). However, the mechanism of the improved SOC content through intercropping with green manure is unclear. We hypothesized that intercropping green manure with grain crops can mitigate the adverse effect of N fertilizer reduction and increase the SOC content by optimizing soil aggregate composition. The objectives of this study were to: (1) assess the SOC stocks in different species of green manure intercropping under reduced N application; (2) determine the impacts of different cropping patterns and nitrogen application levels on the structure of aggregates and organic carbon distribution in different aggregate classes; and (3) determine the relationships among aggregate composition, soil carbon sequestration and crop productivity under different green manure and nitrogen fertilizer management practices.

2. Materials and methods

2.1. Experimental site

The experiment was conducted at the Oasis Experimental Station of Gansu Agricultural University, located in Huangyang County, Wuwei City, at the eastern end of the Hexi Corridor (37°31′N, 103°5′E). The soil is classified as Aridisol (FAO/UNESCO 1988). The soil profile (0–30 cm) properties at the initiation of the experiment were: soil organic carbon content 11.3 g kg^{-1}, pH 8.3, bulk density 1.53 g cm^{-3}, total nitrogen 0.67 g kg^{-1}, total phosphorus 1.42 g kg^{-1}, ammonium nitrogen 1.87 mg kg^{-1}, and nitrate nitrogen 12.51 mg kg^{-1}.

2.2. Experimental design

The experiment began in April 2017 with six treatments, each with three replications, resulting in a total of 18 plots. The

treatments were arranged in a split-plot design, with three planting patterns as the main plots: maize intercropped with common vetch (M/V), maize intercropped with rapeseed (M/R), and sole maize (M) (Fig. 1); and two N levels as the subplots: the local conventional N amount reduced by 25% (N1: 270 kg ha^{-1}), and the local traditional N amount (N2: 360 kg ha^{-1}). This study used sole maize and the local traditional N amount (MN2) as the control. The area of each plot was 6 m×9 m=54 m^2.

The maize intercropped with green manure was divided into a maize belt and a green manure belt. The maize and green manure planting belts were replaced the following year (Fig. 1). The green manure belt was not fertilized, while the maize belt received the same amount of fertilizer under both intercropping and monocropping treatments. The phosphorus fertilizer was applied as a basal fertilizer at 180 kg P_2O_5 ha^{-1} before sowing, and N fertilizer was applied in three parts, 30% as a basal fertilizer, 50% as a topdressing application during the pre-tasselling stage, and 20% in the filling stage, while no fertilizer was applied to the green manure strips. Irrigation of 405 mm was applied during the maize fertility period. All plots received 120 mm of irrigation in the previous fall before the soil froze.

Maize (cv. Xianyu 335) was planted at 82,500 plants ha^{-1} in April and harvested in the last week of September. The planting density was consistent for both sole maize and intercropped maize. The row spacing for both sole maize and intercropped maize was 40 cm, plant spacing for sole maize was 30 cm, and the plant spacing for intercropped maize was 18.5 cm (Fig. 1). Rape (cv. Qingza 5) was planted at 7.5 kg ha^{-1} in the last week of March. Common vetch (cv. Lanjian 2) was planted at 75 kg ha^{-1} in the first week of April. The green manure was mowed and mulched over the surface of the green manure belt at flowering time. The main purpose of planting the green manure crops was to generate organic matter and enhance soil fertility. The biomass of common vetch ranged from 3,676 to 3,857 kg ha^{-1}, and that of rapeseed ranged from 4,386 to 4,671 kg ha^{-1}.

2.3. Soil sample collection

Soil samples were collected using the “S” five-point sampling method after the maize harvests in 2020, 2021, and 2022. These sampling periods corresponded to the 4th, 5th, and 6th years of treatments after the experiment was initiated. In sole maize plots, soil was sampled between the maize rows, and in intercropping systems, soil samples were collected from the area between the maize and green manure rows. Two soil layers were sampled from each plot: 0–20 cm (surface layer) and 20–40 cm (subsurface layer). During sampling and transportation, care was taken to avoid extrusion or disturbance to prevent the destruction of soil aggregates and to preserve the soil structure. The soil samples were broken into soil blocks of approximately 5 cm in diameter, following the natural structure of the soil. Any debris, such as stones and plant roots, was removed, and the samples were then air-dried in the laboratory (Tian *et al.* 2019; Li X F *et al.* 2021).

2.4. Soil sample analysis

Air-dried soil samples were sieved with large top-down apertures of 2, 1, 0.5, and 0.25 mm, slowly moistened so that the topmost sieve of the set was completely immersed in water during oscillation, and shaken vertically. All levels of soil aggregates were washed into an aluminum box. Five aggregate size classes were obtained: (1) >2 mm, (2) 1–2 mm, (3) 0.5–1 mm, (4) 0.25–0.5 mm, and (5) <0.25 mm. All the aggregates with particle sizes of >0.25 mm (classes (1) through (4) above) are referred to as macroaggregates, and those <0.25 mm (class (5)) are microaggregates which include fine sand and silt particles. After drying and weighing, the mass percentage was calculated for each category of soil aggregates (Walkley and Black 1934).

The organic carbon content of the aggregates in each aggregate size and the organic carbon content of the 0–40 cm soil layer were determined by the dilution calorimetry method using potassium dichromate. A soil sample of 0.5000 g was weighed with a balance (accurate to 0.0001 g), K_2CrO_7-H_2SO_4 was added to oxidize the SOM, and then the remaining K_2CrO_7 was titrated with $FeSO_4$ (Heanes 1984).

2.5. Measurement indicators

Soil aggregate stability was evaluated using the mean weight diameter (MWD) and geometric mean diameter (GMD) of soil aggregates with the following equations (Udom *et al.* 2016; Wang Y L *et al.* 2023):

$$MWD=\sum_{i=1}^{n} W_j \times X_i / \sum_{i=1}^{n} W_j \quad (1)$$

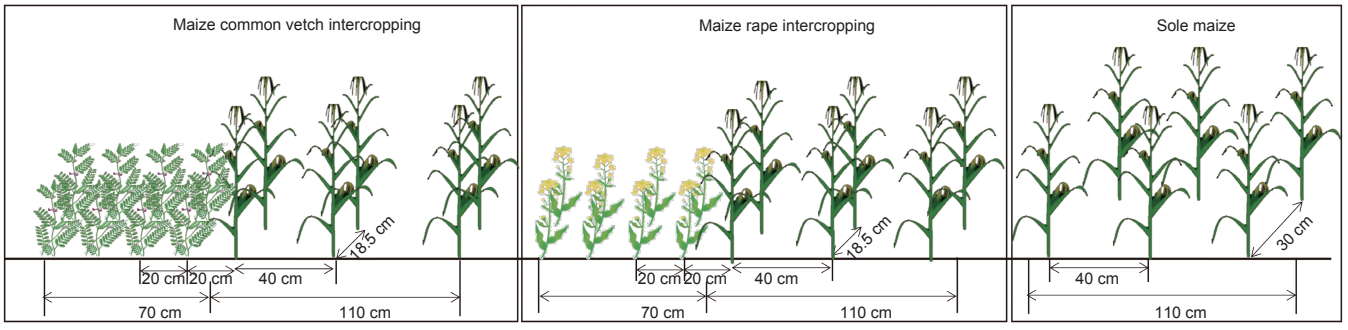


Fig. 1 The layout of maize common vetch intercropping, maize rapeseed intercropping, and sole maize in a field experiment carried out at the Oasis Experimental Station in Gansu, China.

$$GMD=\exp\left[\sum_{i=1}^{n}(W_j\times\ln X_i)/\sum_{i=1}^{n}W_j\right] \quad (2)$$

where W_j is the mass of the aggregates in each soil layer (g) and X_i is the average diameter of the corresponding aggregates in each layer (mm).

The following equation was used to calculate the contribution rates of aggregates of the different particle sizes to SOC in the soil layer (Wang Y L *et al.* 2023).

$$CR=SOC_i\times W_i/SOC_t \quad (3)$$

where CR is the contribution rate (%), SOC_i is the content of C in aggregates of a certain particle size (g kg^{-1}), W_i is the weight percentage of water-stable aggregates of each particle size (%), and SOC_t is the content of soil C in the soil layer (g kg^{-1}).

2.6. Crop productivity

All plots were harvested when the maize reached full maturity. The grain yield of maize was determined after air-drying, and the grain moisture content was adjusted to 14%. A portion of the grains and straw were dried at 60°C to determine the maize aboveground biomass. Root biomass of maize was estimated based on a previously determined straw-to-root ratio (Kong *et al.* 2005; Zhao *et al.* 2018).

2.7. Statistical analysis

All data were recorded and organized using Microsoft Excel 2016. Analysis of variance (ANOVA) was performed using SPSS 26 to compare the means of different treatment groups, with the significance level set at $P<0.05$. In cases where significant differences were detected by ANOVA, post hoc comparisons were conducted using the Tukey's honest significant difference (HSD) test to identify specific differences between treatment groups.

Graphs were created using Prism 9, and the relationships between variables were assessed using Pearson correlation analysis, performed in R with the "corrplot" package. Finally, structural equation modeling (SEM) was conducted using R to explore the interactions between variables.

3. Results

3.1. Organic carbon content of the soil profile for the different treatments

Intercropping green manure with grain crops contributed to increasing the SOC content at both the N1 and N2 application levels. The cropping system and N rate interactive effect on the SOC content was significant in the three years (Fig. 2). When averaged across sampling years, M/V and M/R increased the SOC contents by 12.1 and 9.1%, respectively, compared to sole maize. There was an average reduction of 9.3–10.5% in N1 compared to N2 for sole maize in the 0–40 cm soil layer. The differences between N1 and N2 were not significant in the intercropping patterns. The effect of intercropping on SOC accumulation became increasingly obvious with the increase in planting years. Specifically, M/V at the N1 level increased the SOC by 13.7% compared to the control (MN2) in 2022. However, the differences were not significant between M/V and M/R. This indicated that intercropping with green manure compensated for the negative effect of N fertilizer reduction on the SOC content.

3.2. Proportions of soil aggregate particles and stability

In the 0–40 cm soil layer, the cropping pattern, nitrogen application rate, and the interactive effect between them had significant effects on the proportions of macroaggregates and microaggregates. The inter-annual differences were only significant in the 0–20 cm soil layer (Table 1; Appendix A).

When averaged across sampling years, M/V and M/R showed increased proportions of macroaggregates (>0.25 mm) by 15.3 and 14.4% compared to sole maize in 0–40 cm soil. The proportion of macroaggregates for sole maize was 5.4% lower in N1 than in N2 in the 0–20 cm soil layer, however, the difference was not significant in the 20–40 cm soil layer. In the intercropping patterns, the differences between the two N application rates were not significant. Compared with MN2, the proportions of macroaggregates

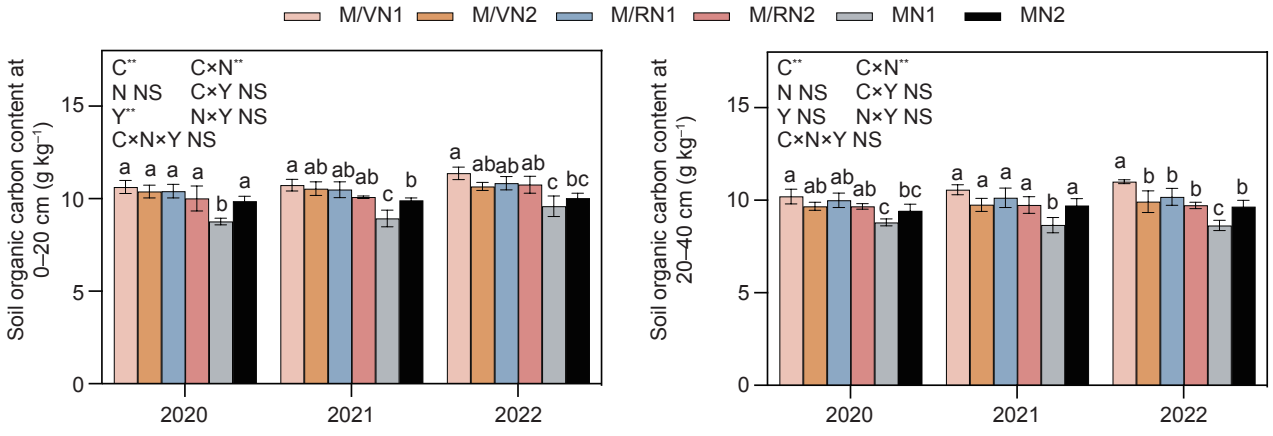


Fig. 2 The organic carbon contents of the 0–20 and 20–40 cm soil layers under different treatments in each experimental year (2020–2022). C represents the cropping pattern, N represents the nitrogen application level, and Y stands for the year. M/V, maize common vetch intercropping; M/R, maize rapeseed intercropping; M, sole maize. N1, the local conventional N amount reduced by 25% (270 kg ha^{-1}); N2, the local conventional N amount (360 kg ha^{-1}). Different lowercase letters indicate significant differences within one year ($P<0.05$). The error bars represent the standard error of the mean ($n=3$). Significant year-to-year variations in SOC content under different treatments are indicated. *, ** and ns indicate statistical significance of variance at $P<0.05$, $P<0.01$ and no significance.

Table 1 Proportions of soil aggregate particles at 0–20 and 20–40 cm depths

Year	Cropping pattern[1)]	N level[2)]	Proportion of soil aggregate particles (0–20 cm)					Proportion of soil aggregate particles (20–40 cm)				
			>2 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm	>2 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm
2020	M/V	N1	7.96 a	5.45 a	14.95 a	32.62 a	39.02 d	6.83 a	4.77 a	15.55 a	31.86 a	40.99 c
		N2	6.47 ab	4.50 abc	13.76 ab	31.64 ab	43.64 c	6.50 a	3.61 ab	13.89 ab	30.86 ab	45.14 b
	M/R	N1	7.81 a	5.40 a	14.94 a	32.39 a	39.46 d	6.85 a	3.86 ab	14.84 a	31.24 ab	43.22 bc
		N2	6.6 ab	4.9 ab	14.04 ab	31.83 ab	42.63 c	6.52 a	3.55 ab	14.38 ab	30.36 ab	45.19 b
	M	N1	3.62 c	3.77 c	11.89 c	30.15 b	50.57 a	4.29 b	3.06 b	11.27 c	29.64 b	51.74 a
		N2	5.81 b	3.82 bc	12.80 bc	30.25 b	47.31 b	4.82 b	3.13 b	12.12 bc	29.86 b	50.07 a
2021	M/V	N1	8.00 a	5.77 a	14.90 a	33.28 a	38.04 d	6.93 a	4.50 a	15.67 a	32.29 a	40.61 c
		N2	7.23 ab	5.06 a	13.70 abc	31.58 ab	42.41 c	6.79 a	3.93 a	13.84 ab	31.19 a	44.24 bc
	M/R	N1	7.93 a	5.56 a	15.07 a	33.09 a	38.36 d	6.87 a	4.03 a	14.83 ab	32.39 a	41.88 c
		N2	7.06 ab	5.09 a	14.31 ab	31.98 ab	41.56 c	6.68 a	3.35 a	13.4 abc	31.37 a	45.24 bc
	M	N1	4.79 c	3.63 b	12.59 c	30.2 b	48.78 a	4.15 b	3.19 a	11.29 c	30.03 a	51.33 a
		N2	6.10 b	3.83 b	13.22 bc	31.27 b	45.59 b	4.73 b	3.29 a	12.79 bc	30.34 a	48.86 ab
2022	M/V	N1	8.65 a	6.34 a	15.25 a	32.95 a	38.48 c	7.46 a	4.86 a	15.61 a	32.08 a	40.05 c
		N2	7.85 ab	5.31 b	13.83 a	31.61 ab	42.74 b	6.48 a	4.36 ab	13.87 ab	31.03 ab	44.26 b
	M/R	N1	8.31 a	5.98 ab	15.06 a	32.74 a	38.59 c	7.33 a	4.96 a	15.17 a	31.82 a	40.73 c
		N2	7.15 bc	5.33 b	14.21 a	31.91 ab	41.74 b	6.73 a	4.22 abc	13.87 ab	30.86 ab	44.32 b
	M	N1	4.87 d	3.70 c	12.00 b	30.18 c	49.91 a	4.22 b	3.41 c	11.75 b	29.72 b	50.05 a
		N2	6.29 c	3.83 c	12.04 b	30.76 bc	47.09 a	5.05 b	3.56 bc	12.65 b	30.21 b	49.37 a

[1)] M/V, maize common vetch intercropping; M/R, maize rapeseed intercropping; M, sole maize.
[2)] N1, the local conventional N amount reduced by 25% (270 kg ha^{-1}); N2, the local conventional amount of N (360 kg ha^{-1}).
Data are means of three repeated observations (n=3). Different lowercase letters indicate significant differences among treatments in a year (P<0.05).

under M/VN1 and M/RN1 were increased by 17.0 and 15.0% in 0–40 cm soil, respectively. The practice of intercropping green manure serves to offset the adverse consequences of diminished nitrogen inputs. Among the fractions, the main increases in the macroaggregate fractions were for those measuring >2 mm and 1–2 mm.

Conversely, the microaggregate (<0.25 mm) fraction occupied a large proportion of the aggregates in sole maize, while M/V and M/R reduced the proportions of microaggregates by 15.3 and 14.4%, respectively, compared to sole maize in the 0–40 cm soil layer. At the N1 level, M/V and M/R further reduced the proportions of microaggregates by averages of 24.2 and 21.9% compared to sole maize. At the N2 level, M/V and M/R reduced the proportions by 8.9 and 7.1% compared to sole maize. In other words, intercropping with green manure under the N reduction pattern facilitated the transfer of microaggregates to macroaggregates, and laid the foundation for a good soil aggregate structure.

The MWD and GMD are more responsive to soil structure, with higher values indicating better soil structure. Cropping patterns, nitrogen application rates, and their interactions had significant effects on aggregate stability (Fig. 3). MWD and GMD in the 0–20 cm depth were 3.8 and 2.4% greater in 2022 than in 2020, while the differences at 20–40 cm were not significant. In the 0–40 cm soil layer, M/V and M/R increased MWD and GMD by 15.2 and 14.2%, and by 11.3 and 10.5% compared to M, respectively. For M, MWD and GMD at N1 were 3.4–6.6% and 2.3–4.3% less than at N2. Under the intercropping pattern, the difference between N1 and N2 was not significant. The MWD and GMD were the highest in M/VN1, at 1.15 times and 1.86 times higher than those in MN2, respectively. This phenomenon suggests that the combination of reduced nitrogen application and intercropping with green manure is more conducive to increasing the stability of aggregates, laying the foundation for building a good and stable soil structure.

3.3. Organic carbon distribution in different particle size aggregates under intercropping with green manure and reduced nitrogen fertilizer application

The cropping patterns and the cropping pattern×N level interaction had significant effects on the organic carbon distributions in soil aggregates at both soil depths (Table 2; Appendix C). Nitrogen application levels affected organic carbon distribution in soil aggregates, except for the <0.25 mm aggregates in the 0–20 cm layer and 0.25–1.0 mm in the 20–40 cm soil layer.

For M, N1 reduced the organic carbon contents of macroaggregates by 12.0% and microaggregates by 5.0% in the 0–20 cm soil layer. The difference between N1 and N2 was not significant for the intercropping pattern. M/V enhanced the macroaggregate and microaggregate carbon by 19.3 and 19.8% compared to sole maize in the 0–40 cm soil layer. When comparing M/R and M, M/R enhanced the macroaggregate and microaggregate carbon significantly over M, but the differences between M/V and M/R were not significant. The organic carbon contents of all aggregate size classes in 2022 in the 0–20 cm soil layer were increased by 6.5, 5.8, 4.1, 5.3, and 6.2% compared to that in 2020, respectively. However, the effect of year was not significant for the 20–40 cm soil layer. These findings suggest that intercropping with green manure and reduced nitrogen application favor an increase in the organic carbon content in all grain-size aggregates, especially in the 0–20 cm soil layer.

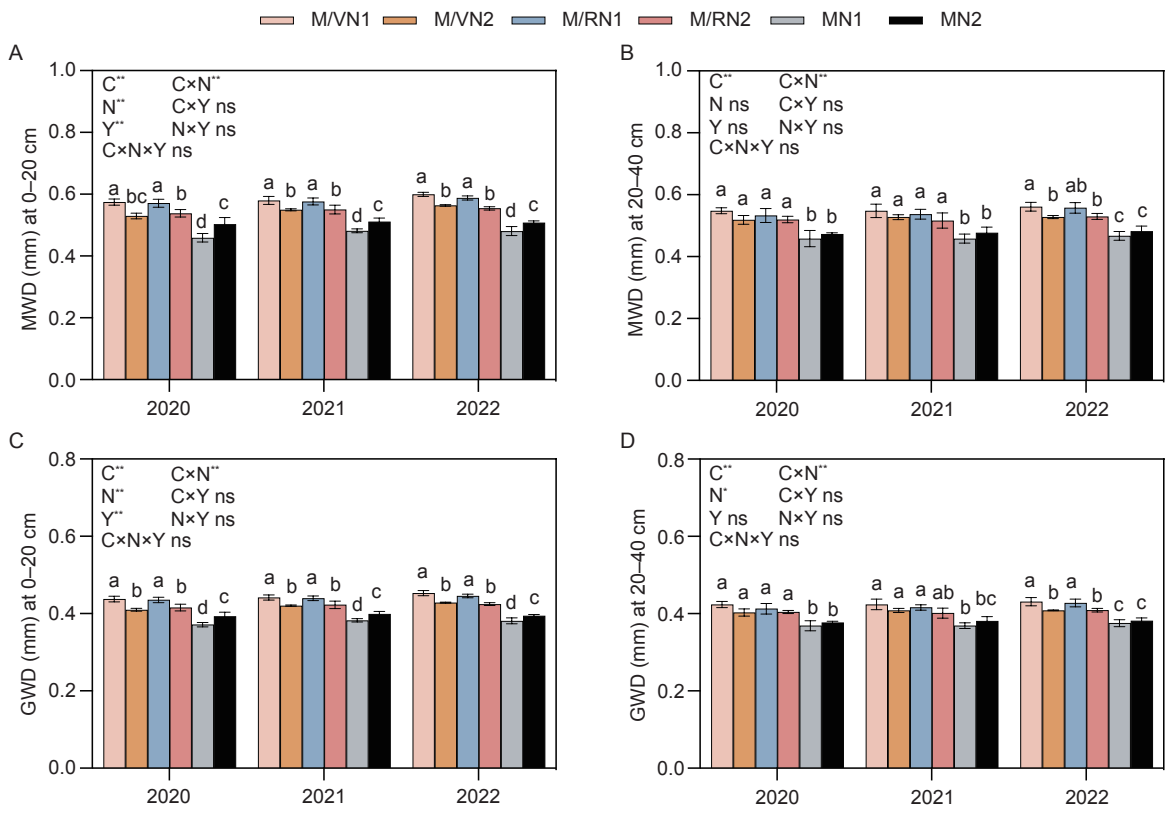


Fig. 3 The aggregates stability parameters from 0–20 and 20–40 cm soil layers under different treatments. C represents the cropping pattern, N represents the nitrogen application level, and Y represents the year. N1, the local conventional N amount reduced by 25% (270 kg ha^{-1}); N2, the local conventional N amount (360 kg ha^{-1}). M/V, maize common vetch intercropping; M/R, maize rapeseed intercropping; M, sole maize. MWD, mean weight diameter; GMD, geometric mean diameter. Different lowercase letters indicate significant differences within one year (P<0.05). The error bars represent the standard error of the mean (n=3). *, ** and ns indicate statistical significance of variance at P<0.05, P<0.01 and no significance.

3.4. Contribution of aggregates to organic carbon under intercropped green manure and reduced nitrogen fertilizer application treatments

The contribution of soil aggregates to organic carbon was quantified annually in the different cropping systems at the two N levels (Table 3; Appendix D). The cropping pattern (except for the 20–40 cm microaggregates) and the interactive effect of cropping pattern and nitrogen application rate significantly affected the contributions of macroaggregates (>0.25 mm) and microaggregates (<0.25 mm) to organic carbon. Furthermore, the 0–20 cm soil layer exhibited greater susceptibility to the effect of cropping pattern with increasing years, although this effect was not significant in the 20–40 cm soil layer.

The intercropping with green manure mainly increased the organic carbon contribution of macroaggregates compared to M, with average increases of 14.5% for M/V and 16.7% for M/R. For sole maize in 2022, N1 reduced the contribution of organic carbon to macroaggregates by 12.5% compared with N2 in the 0–20 cm soil layer. The differences between N1 and N2 in the intercropping pattern were not significant in the 0–40 cm soil layer. M/V and M/R reduced the contributions of microaggregates by 12.0 and 11.3% compared to sole maize in the 0–20 cm soil layer. At the N1 level, M/V and M/R further reduced the contribution of microaggregates, with intercropped maize reducing the proportions of microaggregates by 23.3 and 21.9% in 2020 and by 23.9 and 20.1% in 2022, respectively, compared to sole maize. In other words, the intercropping pattern favored an increase in the organic carbon contributions from all grain-size aggregates in 0–20 cm, with the greatest contribution to the large aggregates.

3.5. Crop productivity under intercropping with green manure and reduced nitrogen fertilizer application

The analysis of variance (ANOVA) demonstrated that the cropping system and N application rate significantly impacted maize grain yield, and there was a significant interaction between them (Fig. 4-A). Averaged across study years, maize intercropped with either M/V or M/R increased the maize yield by 12.5 and 11.5% as compared to M, respectively. Sole maize showed reduced grain yield by 17.1% in N1 compared to N2. However, the yield differences between N1 and N2 were not significant in the intercropping patterns.

Similarly, the aboveground and root biomass levels were influenced by the cropping pattern, the level of nitrogen application, and their interactions (Fig. 4-B and C). Intercropping increased aboveground and root biomass compared to sole maize. Sole maize had lower aboveground and root biomass at N1 than at N2. In contrast, the maize

Table 2 Soil aggregate organic carbon (AOC) from 0–40 cm depths under different treatments

Year	Cropping pattern[1)]	N level[2)]	AOC (g kg^{-1}) (0–20 cm)					AOC (g kg^{-1}) (20–40 cm)				
			>2 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm	>2 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm
2020	M/V	N1	9.68 a	10.2 a	11.3 a	11.54 a	12.17 a	10.18 a	10.54 a	10.81 a	11.37 a	11.88 a
		N2	8.17 b	9.64 b	9.79 b	10.92 a	11.04 ab	7.91 b	9.37 bc	9.94 a	10.88 a	11.52 ab
	M/R	N1	8.99 a	10.48 a	10.83 a	11.37 a	11.52 bc	9.90 a	10.14 ab	10.55 a	10.79 a	12.01 a
		N2	7.79 bc	9.73 b	9.87 b	10.69 ab	11.07 bc	7.30 b	9.09 c	9.83 a	10.70 a	11.12 b
	M	N1	6.79 c	8.56 c	8.95 c	9.55 c	9.26 d	6.79 b	7.81 d	8.19 b	8.42 b	9.34 c
		N2	8.07 b	8.88 c	9.64 b	9.74 bc	10.33 c	7.23 b	8.19 d	8.53 b	9.15 b	9.96 c
2021	M/V	N1	9.55 a	11.75 a	10.75 a	12.16 a	12.44 a	9.91 a	10.86 a	11.02 a	11.02 a	12.19 a
		N2	8.62 b	10.68 ab	9.83 b	10.39 b	11.72 ab	7.24 c	9.87 ab	10.13 a	11.02 a	11.33 ab
	M/R	N1	9.7 a	11.26 a	11.06 a	11.09 ab	11.78 ab	10.06 a	10.19 ab	11.25 a	11.00 a	12.09 a
		N2	8.11 b	10.74 ab	9.79 b	10.69 bc	11.28 b	8.35 b	9.57 b	10.05 a	10.96 a	11.11 ab
	M	N1	7.25 b	8.12 c	9.04 c	8.66 d	10.11 c	7.99 b	7.65 c	8.38 b	8.47 b	9.51 c
		N2	7.59 b	9.63 b	9.55 bc	9.33 cd	11.28 b	8.37 bc	8.28 c	9.77 ab	9.07 b	10.21 bc
2022	M/V	N1	10.38 a	11.53 a	12.07 a	12.42 a	12.55 a	10.32 a	10.73 a	11.08 a	11.36 a	12.34 a
		N2	9.01 b	10.24 c	10.1 b	11.31 b	11.77 b	7.45 b	9.41 b	10.54 ab	11.06 ab	11.3 ab
	M/R	N1	9.67 a	11.26 b	10.49 b	12.20 a	12.42 a	10.34 a	9.76 ab	10.95 a	10.91 b	12.98 a
		N2	8.09 c	10.27 c	10.5 b	11.31 b	11.63 bc	7.83 b	9.2 b	10.02 b	10.74 b	11.79 a
	M	N1	6.78 e	8.26 e	9.35 b	9.66 d	9.69 d	7.24 b	7.55 c	8.42 d	8.27 d	9.66 b
		N2	7.87 d	9.23 d	10.31 c	10.27 c	11.37 c	8.35 b	8.6 bc	9.22 c	9.24 c	9.93 b

[1)] M/V, maize common vetch intercropping; M/R, maize rapeseed intercropping; M, sole maize.
[2)] N1, the local conventional N amount reduced by 25% (270 kg ha^{-1}); N2, the local conventional amount of N (360 kg ha^{-1}).
Different lowercase letters indicate significant differences among treatments in a year (P<0.05). The values are the means of three repeated observations (n=3).

Table 3 Contribution rates of aggregates of different particle sizes to soil organic carbon (CR) in the two soil layers

Year	Cropping pattern[1)]	N level[2)]	CR (%) (0–20 cm)					CR (%) (20–40 cm)				
			>2 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm	>2 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm
2020	M/V	N1	0.07 a	0.05 a	0.16 a	0.35 a	0.45 a	0.07 a	0.05 a	0.17 a	0.36 a	0.48 a
		N2	0.05 bc	0.04 bc	0.13 c	0.33 a	0.46 a	0.05 ab	0.04 ab	0.14 a	0.35 a	0.54 a
	M/R	N1	0.07 ab	0.05 a	0.16 ab	0.36 a	0.44 a	0.07 ab	0.04 ab	0.16 a	0.34 a	0.52 a
		N2	0.05 bc	0.05 ab	0.14 bc	0.34 a	0.48 a	0.05 ab	0.03 ab	0.15 ab	0.34 a	0.52 a
	M	N1	0.03 d	0.04 c	0.12 c	0.33 a	0.54 a	0.03 b	0.03 b	0.1 b	0.28 a	0.55 a
		N2	0.05 c	0.03 c	0.13 c	0.30 a	0.50 a	0.04 ab	0.03 ab	0.11 ab	0.29 a	0.53 a
2021	M/V	N1	0.07 a	0.06 a	0.15 ab	0.38 a	0.44 c	0.07 a	0.05 a	0.16 abc	0.34 a	0.47 a
		N2	0.06 ab	0.05 c	0.13 c	0.31 bc	0.47 bc	0.05 a	0.04 a	0.15 ab	0.37 a	0.53 ab
	M/R	N1	0.07 a	0.06 ab	0.16 a	0.35 ab	0.43 c	0.07 a	0.04 a	0.16 ab	0.35 a	0.5 a
		N2	0.06 ab	0.05 bc	0.14 bc	0.34 abc	0.47 bc	0.06 a	0.04 a	0.14 a	0.37 a	0.54 ab
	M	N1	0.04 b	0.03 d	0.13 c	0.29 c	0.55 a	0.04 a	0.03 a	0.11 cd	0.29 a	0.57 b
		N2	0.05 ab	0.04 d	0.13 c	0.30 c	0.52 ab	0.04 a	0.03 a	0.13 d	0.30 a	0.54 ab
2022	M/V	N1	0.08 a	0.06 a	0.16 a	0.36 a	0.41 d	0.07 a	0.05 a	0.16 a	0.34 a	0.46 a
		N2	0.07 ab	0.05 b	0.13 bcd	0.34 ab	0.46 bc	0.05 a	0.04 ab	0.15 ab	0.35 a	0.51 a
	M/R	N1	0.08 a	0.06 a	0.15 ab	0.37 a	0.44 cd	0.08 a	0.05 a	0.16 a	0.34 a	0.52 a
		N2	0.05 bc	0.05 b	0.14 bc	0.34 ab	0.45 cd	0.05 a	0.04 abc	0.14 abc	0.34 a	0.54 a
	M	N1	0.03 c	0.03 c	0.12 d	0.31 b	0.5 ab	0.04 b	0.03 c	0.12 c	0.29 b	0.56 a
		N2	0.05 bc	0.04 c	0.12 cd	0.32 b	0.53 a	0.05 b	0.03 bc	0.13 bc	0.30 b	0.53 a

[1)] M/V, maize common vetch intercropping; M/R, maize rapeseed intercropping; M, sole maize.
[2)] N1, the local conventional N amount reduced by 25% (270 kg ha^{-1}); N2, the local conventional amount of N (360 kg ha^{-1}).
Different lowercase letters indicate significant differences among treatments in a year (P<0.05). The values are the means of three repeated observations (n=3).

green manure intercropping pattern exhibited no discernible differences in the aboveground or root biomass between the N1 and N2 levels.

3.6. Relationships between soil aggregate stability index and soil aggregate carbon content and soil organic carbon content in the intercropping with green manure and reduced nitrogen application treatment

Positive correlations were found between structural indices, carbon concentration in aggregates, soil layer carbon content, and macroaggregates, but negative correlations were found between microaggregates and all studied parameters (Fig. 5-A). A highly significant positive correlation was noted between the carbon content of macroaggregates and the organic carbon content. The higher the proportion of macroaggregate carbon content (>0.25 mm), the more stable the soil aggregates and the greater the potential for carbon sequestration. Significant synergy was evident between SOC

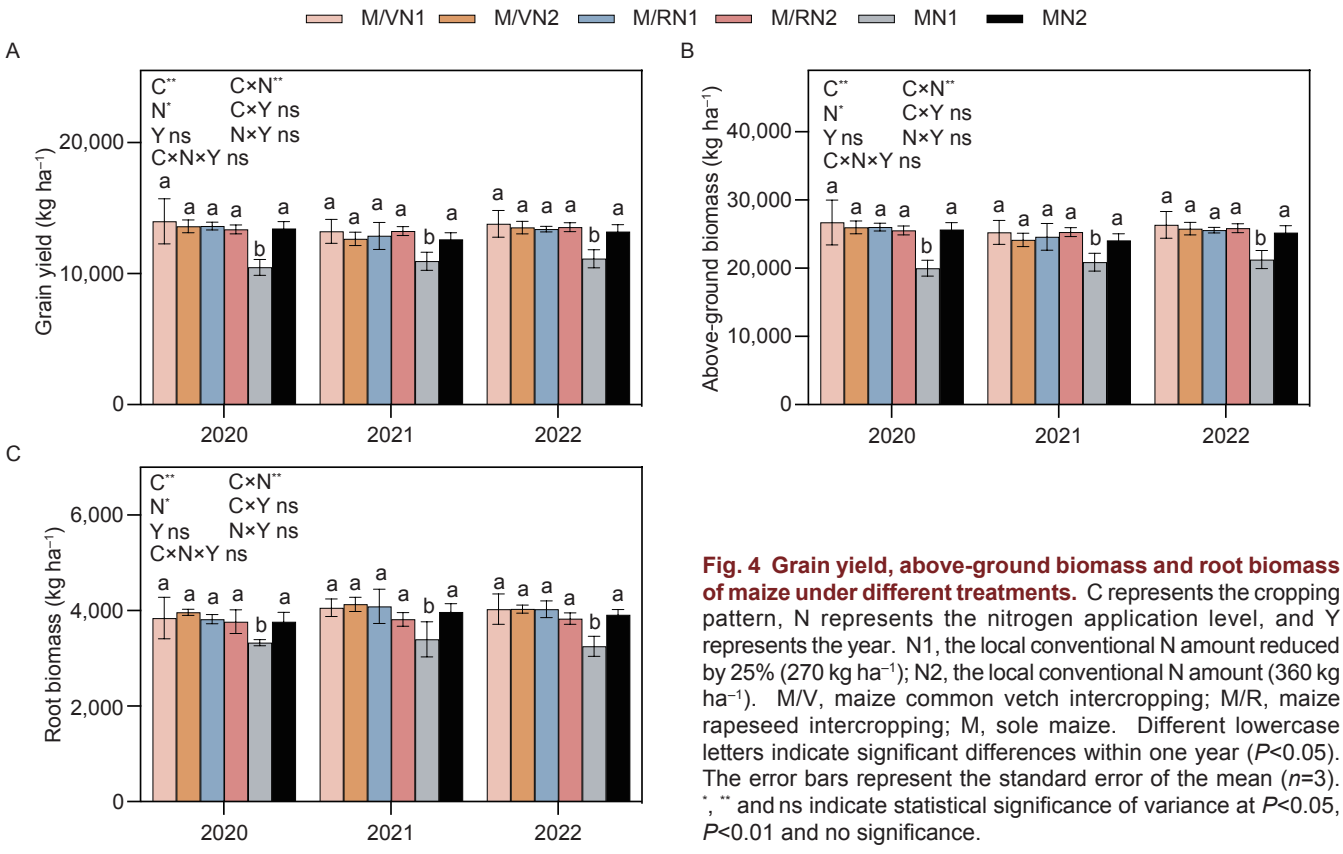


Fig. 4 Grain yield, above-ground biomass and root biomass of maize under different treatments. C represents the cropping pattern, N represents the nitrogen application level, and Y represents the year. N1, the local conventional N amount reduced by 25% (270 kg ha^{-1}); N2, the local conventional N amount (360 kg ha^{-1}). M/V, maize common vetch intercropping; M/R, maize rapeseed intercropping; M, sole maize. Different lowercase letters indicate significant differences within one year (P<0.05). The error bars represent the standard error of the mean (n=3). *, ** and ns indicate statistical significance of variance at P<0.05, P<0.01 and no significance.

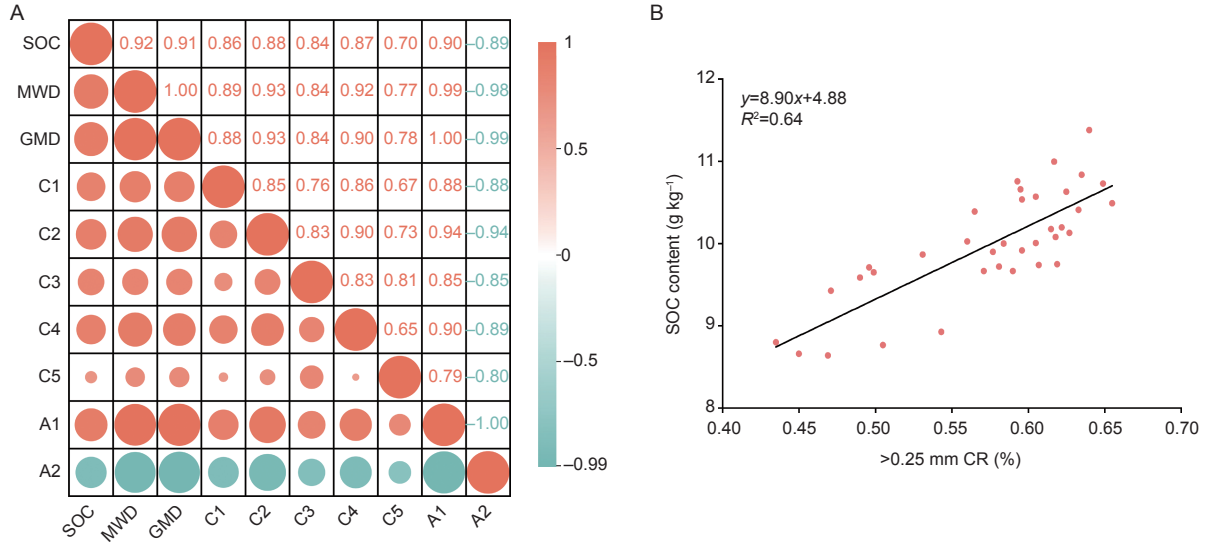


Fig. 5 Correlation analysis of soil organic carbon and the structural indices. A, correlation matrix of different soil structural indices, aggregate organic carbon, and soil layer organic carbon. SOC, soil organic carbon; MWD, mean weight diameter; GMD, geometric mean diameter; C1–C5, aggregate organic carbon >2 mm, 1–2 mm, 0.5–1 mm, 0.25–0.5 mm and <0.25 mm, respectively; A1, aggregate proportion of >0.25 mm; A2, aggregate proportion of <0.25 mm. B, regression relationship between SOC content and the contribution rate of all aggregates>0.25 mm to SOC (>0.25 mm CR).

and the contribution of macroaggregates (>0.25 mm) to SOC (Fig. 5-B).

The structural equation modeling results demonstrated that cropping patterns and nitrogen application rates can indirectly affect SOC, with the latter occurring through its influence on the composition of macroaggregates and aggregate organic carbon (AOC) (Fig. 6-A). Among these factors, the composition of macroaggregates and AOC were identified as playing a pivotal role in the total effect (Fig. 6-B). Furthermore, the findings indicated that SOC, cropping patterns and nitrogen application rates exert a notable direct impact on maize yield.

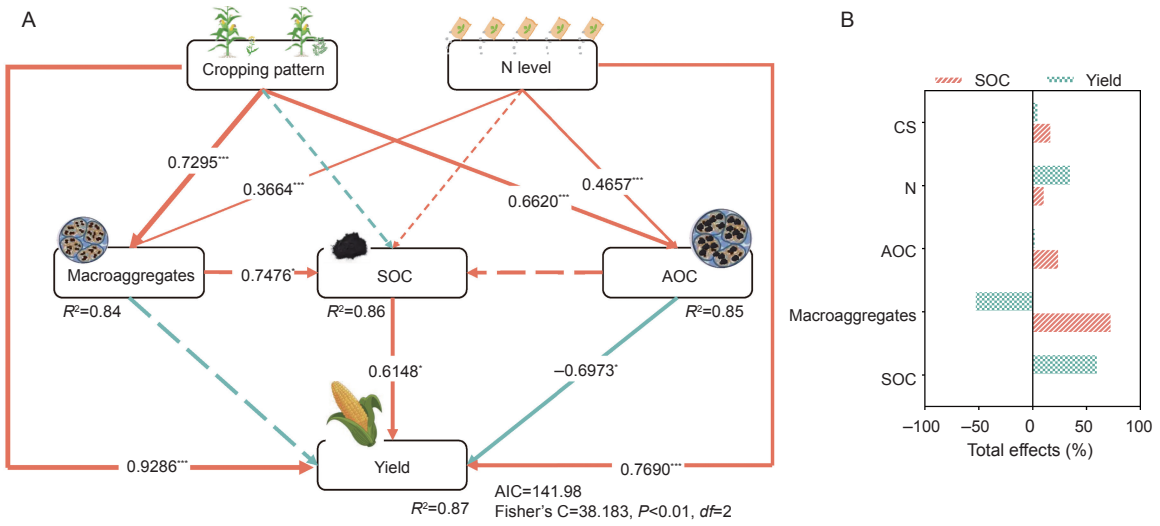


Fig. 6 Structural equation model (A) and total effects of all influencing factors on soil organic carbon (SOC) and maize yield (B). Asterisks stand for significant effects: *, $P<0.05$; ***, $P<0.001$. Green and red solid arrows indicate significant ($P<0.05$) negative and positive paths, respectively, the width of an arrow indicates the magnitude of that effect. Dashed lines indicate non-significant ($P>0.05$) paths. Numbers near the lines represent standardized path coefficients. Macroaggregates mean the aggregate proportion>0.25 mm. AOC means aggregates organic carbon.

4. Discussion

4.1. Changes in soil organic carbon content under intercropping with green manure and nitrogen reduction

Studies have shown that improved agronomic practices, such as cropping patterns, management of N fertilizer use, planting densities, and the use of cover crops, can increase SOC stocks in agricultural soils (Nakamoto and Suzuki 2001; Hu *et al.* 2023; Sun *et al.* 2023). The addition of N fertilizer can increase plant biomass and the SOC concentration, but N fertilization can also lead to the loss of SOC in some cases (He *et al.* 2015; Lu *et al.* 2021). Different experimental conditions and soil physicochemical properties may have contributed to these contradictory results. In this study, a reduction in N fertilizer application negatively affected SOC in sole maize soil. In intercropping systems, however, the negative effects of reduced N fertilizer application on SOC were compensated for as the number of years of cultivation increased (Fig. 3). The long-term continuous use of fertilizers in sole maize may inhibit aggregate-related microorganisms and the biochemical components, thereby stimulating SOM mineralization, reducing aggregate formation, and thus reducing the SOC content (Xu *et al.* 2016; Hazra *et al.* 2019; Sun *et al.* 2023). Our results indicate that intercropping green manure with grain crops (such as maize) is a way to increase C sequestration potential, compensating for the negative impacts of reduced N application (Li *et al.* 2023). On the one hand, intercropping leads to more light interception and more biomass production through photosynthesis, which increases C inputs (Martens 2000; Zhao *et al.* 2023). On the other hand, intercropping increases microbial residual carbon in the large particulate fraction of the surface soil, thereby increasing organic carbon stocks (Cong *et al.* 2014; Zhao *et al.* 2023). Specifically, green manure crops promote interactions between fungal residual C and mineral-associated organic matter, leading to the increase in soil organic C stocks (Xie *et al.* 2016; Kazmierczak *et al.* 2020; Hu *et al.* 2023). This is consistent with the finding that cover crops regulate soil multifunctionality by shaping specific microbial communities, which in turn drive the processes of organic matter decomposition and stabilization (Li *et al*. 2024).

The results of this study showed that the SOC content varied with soil depth. It was significantly higher in the 0–20 cm soil layer than in the 20–40 cm layer, suggesting that the addition of fresh plant residues to the soil surface layer benefits C pools and enhances C storage in the topsoil layers (Ansari *et al.* 2022). Most legume crops have root distribution in the 0–20 cm depth, and the six-year continuous organic C input can provide significantly more benefits to the topsoil C pools than the plant-derived C at the 20–40 cm depth (Ansari *et al.* 2022; Yan *et al.* 2023).

In this experiment, the effects of two green manure crops, common vetch and rapeseed, on the accumulation of SOC were investigated. Although the differences between the two in promoting SOC accumulation were not obvious, the results showed that common vetch slightly outperformed rapeseed in increasing the SOC content.

Some previous studies have shown that leguminous plants with a slow decomposition rate, such as common vetch, tend to accumulate more SOC over time (Li *et al.* 2023). Although the differences between the two intercrop plants were not obvious in this experiment, in terms of long-term trends and potential impacts, common vetch may have greater potential for the long-term accumulation and sequestration of SOC. With its fast decomposition rate, rapeseed releases more carbon in the short term and increases the SOC content to a certain extent, although its characteristic of rapid decomposition may lead to insufficient carbon sequestration sustainability in the long run (Han *et al.* 2023).

At the initiation of this experiment, the SOC content was 11.3 g kg^{-1} in both the 0–20 and 20–40 cm layers. The

SOC contents in most of the treatments remained close to or slightly lower than that baseline value, particularly in the deeper soil layers (20–40 cm). First, the soil properties and climatic conditions of the study area may limit SOC accumulation due to the high soil pH (8.2), low nutrient content, and low soil moisture (Donhauser *et al.* 2020; Li H *et al*. 2021). These conditions could limit microbial activity and organic matter stabilization, which are key processes in SOC sequestration (Patoine *et al.* 2022). In addition, although intercropping systems do promote SOC sequestration, the accumulation process is typically slow, and a short period may not be sufficient to observe a significant increase in the SOC content above baseline levels (Lal 2004b). Meanwhile, the decomposition rate of green manure residues, particularly rapeseed residues, may offset some of the SOC increase in the short term, especially under reduced nitrogen fertilization (Wang W *et al.* 2023).

4.2. Effects of intercropping with green manure and nitrogen reduction treatments on the distribution and stability of soil aggregates

Soil aggregates are the basis for good soil structure (Six *et al.* 2000). The stability of aggregates is a determinant of soil productivity, soil quality, and erosion resistance (Ablimit *et al.* 2022). The physical protection of aggregates promotes C sequestration, as evidenced by the positive association between aggregate structure and SOC stocks. Studies have shown that the under- or over-application of chemical fertilizers can lead to crop nutrient imbalance, the destruction of soil structure, and the reduction of SOC confined in aggregates (Chen *et al.* 2010; Wang *et al.* 2021). Higher N applications lead to soil acidification with increases in H^+ and NH_4^+ and a reduction in microbial activity associated with carbon decomposition (Yan *et al.* 2021; Wang J *et al.* 2024). Mycelial networks of fungi and mycorrhizae are essential for the formation and stabilization of macroaggregates (>0.25 mm), but there are fewer macroaggregates at higher N fertilizer application rates (Bauçà *et al.* 2019). This suggests a threshold effect between the amount of N applied and the level of aggregates and could explain the lower proportion of macroaggregates and poorer aggregate stability with reduced N fertilization in the sole maize soil in this study.

Microorganisms in root hairs, fungal mycelial entanglement in the hydrosphere, and the secretion of polysaccharides by microbes facilitate the binding of soil aggregates, mycelium, and minerals, and the formation of macroaggregates (Zhang Y C *et al.* 2018; Hazra *et al.* 2019; Agnihotri *et al.* 2022). Studies have shown that microorganisms in green manure-based intercropping patterns produce secretions that are important binders for the aggregation of soil particles and the stability of aggregates (Brooker *et al.* 2014; Li X F *et al.* 2021). Green manure mulched and returned to the field increases the input of organic materials, reduces the over-compaction and crusting on the soil surface, and increases the gelling substances, which adsorb and fix the microaggregates to be combined into macroaggregates (Franke *et al.* 2008; Liu *et al.* 2019; Liu *et al.* 2022). The formation and destruction of large aggregates are more sensitive to N fertilization than smaller aggregates. Coarse root residues that usually accumulate in large aggregates are more attractive to fungi than to bacteria (Ding and Han 2014). The correlation analysis in this study showed that the proportion of large aggregates and aggregate stability were significantly and positively correlated with SOC (Fig. 5).

4.3. Changes in aggregate associated carbon under intercropping with green manure and nitrogen reduction

Studies have shown that the carbon content in the macroaggregate fraction has a significant effect on the total organic carbon (Wang Y *et al.* 2023), and there was a remarkable synergy between SOC and macroaggregate C content in our results. Intercropping grain crops with green manure increased the SOC content in the macroaggregate fraction under reduced N application as compared to traditional maize monoculture (Table 2). In addition, the benefits of the intercropping pattern were more pronounced under the 270 kg ha^{-1} N application rate, as the formation of soil aggregates played a crucial role in protecting SOC. Soil aggregation can protect SOC, as this physical protection significantly reduces microbial access and slows down carbon mineralization (Six *et al.* 2000). The combined application of a moderate amount of chemical nitrogen fertilizer and crop residues produced by the intercropping system enhances this effect, contributing to improved carbon sequestration (Wang *et al.* 2018; Han *et al.* 2023).

On the one hand, the traditional application of nitrogen might affect microbial activities and destroy soil structure, thus accelerating the decomposition and mineralization of organic matter and especially reducing the content of organic carbon in large aggregates (Udom *et al.* 2016). On the other hand, under the intercropping pattern of legumes with cereals, legumes are less competitive for soil nitrogen than non-legumes, and additional nitrogen is obtained from the nitrogen released into the soil by the legumes (Hauggaard Nielsen and Jensen 2005; Brooker *et al.* 2014). Legume manure penetrates aggregates with a low root length density, which is favorable for combining crop residues with soil particles to form large aggregates and increase the organic carbon contents at all grain levels, especially in later crops (Chen *et al.* 2009; Ansari *et al.* 2022). In addition, appropriate C/N ratios in leguminous green manure can promote faster SOC formation, and these organics can act as binders for microaggregates to promote the formation of macroaggregates (Ansari *et al.* 2022; Hu *et al.* 2023). This mechanism could also explain why both M/VN1 and M/RN1 were dominated by macroaggregates.

The magnitude of the contribution of aggregates to SOC is mainly affected by the proportion of aggregates, so all the factors affecting the composition of aggregates affect the contribution of SOC. The transformation of microaggregates into macroaggregates will shift the soil aggregate organic carbon at the same time, thus changing the contribution of aggregate organic carbon (Chen *et al.* 2019; Wang Y *et al.* 2023). Therefore, intercropping with green manure and reducing nitrogen application are strategies that can increase

soil organic C stocks.

4.4. Changes in crop productivity under intercropping with green manure and nitrogen reduction

Many studies have demonstrated that intercropping can enhance field biodiversity and the combined yields of the two or more intercropped species (Chai *et al.* 2021; Li X F *et al.* 2021). This study added a new value that intercropping with green manure increased grain yield by an average of 12.0% compared to sole maize, which compensated for the negative effects of reducing the N fertilizer rate. The compensatory effect of intercropping with green manure in time and space after the green manure clipping increased the dry matter accumulation of maize thereby leading to higher yields (Li *et al.* 2023).

We found that the sustained or even increased crop yields under a reduced N rate reflected the potential N transfer from the intercropped green manure to the maize. Intercropped green manure crops provide abundant carbon and nitrogen sources for microorganisms in the soil (De Medeiros *et al.* 2018), which stimulates soil microbial activity, thereby facilitating organic N mineralization and enhancing the content of SOM (Elfstrand *et al.* 2007; De Medeiros *et al.* 2018).

The intercropping of green manure, therefore, has the potential to effectively improve SOC, while providing favorable conditions for increased crop yield, due to enhanced nutrient availability in the soil (Li X F *et al.* 2021; Ablimit *et al.* 2022; Zhao *et al.* 2023; Yang C Q *et al*. 2024). Moreover, the incorporation of straw and root exudates increased the correlation with enhanced crop yield, thus reinforcing the improvement in soil fertility (Wang W *et al.* 2023). Although chemical N can indirectly increase SOM by increasing plant biomass and returning carbon to the soil, it also promotes SOC decomposition by increasing microbial biomass and DOC and N availability (Qiu *et al.* 2016). Thus, intercropping with green manure combined with N reduction practices will be better for the soil structure, carbon sequestration, and crop productivity in the Hexi Oasis irrigation area.

5. Conclusion

Intercropping grain crops such as maize with green manure increased the soil organic carbon stocks as compared with conventional sole maize regardless of the N fertilization level. Specifically, intercropping combined with reduced N fertilization (N1, 270 kg ha^{-1}) resulted in a 5.9–10.1% increase in the SOC content over three years compared to sole maize, irrespective of N levels. This suggests that intercropping with legume green manure can help offset the negative impact of reduced N application on SOC accumulation. The differences between M/V and M/R were not significant. Notably, M/V at the N1 level increased SOC by 13.7% compared to MN2 in 2022. Intercropping with green manure increased the proportion of macroaggregates (>0.25 mm) and aggregate stability but reduced the proportion of microaggregates (<0.25 mm) compared to sole maize under reduced N application. Meanwhile, intercropping with green manure increased macroaggregate C and its contribution rate to SOC stocks, while sole maize increased microaggregate C and its contribution rate to SOC stocks. In addition, intercropping with green manure can maintain high crop yields by increasing SOC under reduced nitrogen application. We conclude that green manure as an intercropping component in the maize cropping system, along with reduced chemical N inputs, can increase soil organic carbon stocks and maize productivity in the Hexi Oasis irrigation area by optimizing the composition of soil aggregates.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2021YFD1700204), the National Natural Science Foundation of China (U21A20218 and 32372238), the Modern Agro-Industry Technology Research System of China (CARS-22-G-12) and the “Innovation Star” Program of Graduate Students in 2025 of Gansu Province, China (2025CXZX-749).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.04.029

References

Ablimit R, Li W K, Zhang J D, Gao H N, Zhao Y M, Cheng M M, Meng X Q, An L Z, Chen Y. 2022. Altering microbial community for improving soil properties and agricultural sustainability during a 10-year maize–green manure intercropping in Northwest China. *Journal of Environmental Management*, **321**, 115859–115871.

Agnihotri R, Sharma M P, Prakash A, Ramesh A, Bhattacharjya S, Patra A K, Manna M C, Kurganova I, Kuzyakov Y. 2022. Glycoproteins of arbuscular mycorrhiza for soil carbon sequestration: Review of mechanisms and controls. *Science of the Total Environment*, **806**, 150571–150581.

Amelung W, Bossio D, de Vries W, Kögel Knabner I, Lehmann J, Amundson R, Bol R, Collins C, Lal R, Leifeld J, Minasny B, Pan G, Paustian K, Rumpel C, Sanderman J, van Groenigen J W, Mooney S, van Wesemael B, Wander M, Chabbi A. 2020. Towards a global-scale soil climate mitigation strategy. *Nature Communications*, **11**, 5427.

Ansari M A, Choudhury B U, Layek J, Das A, Lal R, Mishra V K. 2022. Green manuring and crop residue management: Effect on soil organic carbon stock, aggregation, and system productivity in the foothills of Eastern Himalaya (India). *Soil & Tillage Research*, **218**, 105318.

Bauçà S C, Marqués A, Vidal N L, Bota J, Baraza E. 2019. Long-term establishment of natural green cover provides agroecosystem services by improving soil quality in a Mediterranean vineyard. *Ecological Engineering*, **127**, 285–291.

Beillouin D, Corbeels M, Demenois J, Berre D, Boyer A, Fallot A, Feder F, Cardinael R. 2023. A global meta-analysis of soil organic carbon in the Anthropocene. *Nature Sustainability*, **14**, 3700.

Bossio D, Cook Patton S, Ellis P, Fargione J, Sanderman J, Smith P, Wood S, Zomer R, Von Unger M, Emmer I. 2020. The role of soil carbon in natural climate solutions. *Nature Sustainability*, **3**, 391–398.

Brooker R W, Bennett A E, Cong W F, Daniell T J, George T S, Hallett P D, Hawes C, Iannetta P P M, Jones H G, Karley A J. 2014. Improving

intercropping: A synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, **206**, 107–117.
Chai Q, Nemecek T, Liang C, Zhao C, Yu A Z, Coulter J A, Wang Y F, Hu F L, Wang L, Siddique K H. 2021. Integrated farming with intercropping increases food production while reducing environmental footprint. *Proceedings of the National Academy of Sciences of the United States of America*, **118**, e2106382118.
Chai Q, Qin A Z, Gan Y T, Yu A Z. 2014. Higher yield and lower carbon emission by intercropping maize with rape, pea, and wheat in arid irrigation areas. *Agronomy for Sustainable Development*, **34**, 535–543.
Chen H Q, Hou R X, Gong Y X, Li H W, Fan M S, Kuzyakov Y. 2009. Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in Loess Plateau of China. *Soil & Tillage Research*, **106**, 85–94.
Chen H Q, Liang Q, Gong Y S, Kuzyakov Y, Fan M S, Plante A F. 2019. Reduced tillage and increased residue retention increase enzyme activity and carbon and nitrogen concentrations in soil particle size fractions in a long-term field experiment on Loess Plateau in China. *Soil & Tillage Research*, **194**, 104296–104303.
Chen Y, Zhang X D, He H B, Xie H T, Yan Y, Zhu P, Ren J, Wang L C. 2010. Carbon and nitrogen pools in different aggregates of a Chinese Mollisol as influenced by long-term fertilization. *Journal of Soils & Sediments*, **10**, 1018–1026.
Chenu C, Angers D A, Barré P, Derrien D, Arrouays D, Balesdent J. 2019. Increasing organic stocks in agricultural soils: Knowledge gaps and potential innovations. *Soil & Tillage Research*, **188**, 41–52.
Cong W F, Ruijven J V, Mommer L, Deyn G B D, Hoffland E. 2014. Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *Journal of Ecology*, **102**, 1163–1170.
Cotrufo M F, Ranalli M G, Haddix M L, Six J, Lugato E. 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience*, **12**, 989–994.
Ding X L, Han X Z. 2014. Effects of long-term fertilization on contents and distribution of microbial residues within aggregate structures of a clay soil. *Biology and Fertility of Soils*, **50**, 549–554.
Donhauser J, Qi W, Pinto B B, Frey B. 2020. High temperatures enhance the microbial genetic potential to recycle C and N from necromass in high-mountain soils. *Global Change Biology*, **27**, 1365–1386.
Elfstrand S, Hedlund K, Mårtensson A. 2007. Soil enzyme activities, microbial community composition and function after 47 years of continuous green manuring. *Applied Soil Ecology*, **35**, 610–621.
FAO/UNESCO (Food and Agriculture Organization of the United Nations). 1988. Soil Map of the World: Revised Legend. FAO/UNESCO. [2025-2-19]. http://www.fao.org/climatechange
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. *Nutrient Cycling in Agroecosystems*, **82**, 117–135.
Gou Z W, Yin W, Asibi A E, Fan Z L, Chai Q, Cao W D. 2022. Improving the sustainability of cropping systems via diversified planting in arid irrigation areas. *Agronomy for Sustainable Development*, **42**, 1–16.
Han F, Guo R, Hussain S, Guo S, Cai T, Zhang P, Jia Z, Naseer M A, Saqib M, Chen X. 2023. Rotation of planting strips and reduction in nitrogen fertilizer application can reduce nitrogen loss and optimize its balance in maize–peanut intercropping. *European Journal of Agronomy*, **143**, 126707–126720.
Hartley I P, Hill T C, Chadburn S E, Hugelius G. 2021. Temperature effects on carbon storage are controlled by soil stabilisation capacities. *Nature Communications*, **12**, 6713.
Hauggaard Nielsen H, Jensen E S. 2005. Facilitative root interactions in intercrops. *Plant and Soil*, **274**, 237–250.
Hazra K K, Nath C P, Singh U, Praharaj C S, Kumar N, Singh S S, Singh N P. 2019. Diversification of maize-wheat cropping system with legumes and integrated nutrient management increases soil aggregation and carbon sequestration. *Geoderma*, **353**, 308–319.
He Y T, Zhang W J, Xu M G, Tong X G, Sun F X, Wang J Z, Huang S M, Zhu P, He X H. 2015. Long-term combined chemical and manure fertilizations increase soil organic carbon and total nitrogen in aggregate fractions at three typical cropland soils in China. *Science of the Total Environment*, **532**, 635–644.
Heanes D L. 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. *Communications in Soil Science and Plant Analysis*, **15**, 1191–1213.
Hu Q J, Jiang T, Thomas B W, Chen J, Xie J, Hu Y X, Kong F i, Yang Y, Chen X P, Zhang Y T, Shi X J. 2023. Legume cover crops enhance soil organic carbon via microbial necromass in orchard alleyways. *Soil & Tillage Research*, **234**, 105858.
Hu Q J, Zhang Y T, Cao W D, Yang Y Y, Hu Y X, He T G, Li Z Y, Wang P, Chen X P, Chen J, Shi X J. 2024. Legume cover crops sequester more soil organic carbon than non-legume cover crops by stimulating microbial transformations. *Geoderma*, **450**, 117024.
Kabiri V, Raiesi F, Ghazavi M A. 2015. Six years of different tillage systems affected aggregate-associated SOM in a semi-arid loam soil from Central Iran. *Soil & Tillage Research*, **154**, 114–125.
Kazmierczak T, Yang L, Boncompagni É, Meilhoc E, Brouquisse R. 2020. Legume nodule senescence: A coordinated death mechanism between bacteria and plant cells. *Advances in Botanical Research*, **94**, 181–212.
Kong A Y Y, Six J, Bryant D C, Denison R F, van Kessel C. 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. *Soil Science Society of America Journal*, **69**, 1078–1085.
Lal R. 2004a. Offsetting China's CO_2 emissions by soil carbon sequestration. *Climatic Change*, **65**, 263–275.
Lal R. 2004b. Soil carbon sequestration to mitigate climate change. *Geoderma*, **123**, 1–22.
Lal R, Delgado J A, Groffman P M, Millar N, Dell C, Rotz A. 2011. Management to mitigate and adapt to climate change. *Journal of Soil and Water Conservation*, **66**, 276–285.
Li G L, Chen X F, Qin W J, Chen J R, Leng K, Sun L Y, Liu M, Wu M, Fan J B, Xu C X, Liu J. 2024. Characteristics of the microbial communities regulate soil multi-functionality under different cover crop amendments in Ultisol. *Journal of Integrative Agriculture*, **23**, 2099–2111.
Li H, Yang S, Semenov M V, Yao F, Ye J, Bu R C, Ma R A, Lin J, Kurganova I, Wang X G, Deng Y, Kravchenko I, Jiang Y, Kuzyakov Y. 2021. Temperature sensitivity of SOM decomposition is linked with a K-selected microbial community. *Global Change Biology*, **27**, 2763–2779.
Li H T, Fan Z L, Wang Q M, Wang G C, Yin W, Zhao C, Yu A Z, Cao W D, Chai Q, Hu F L. 2023. Green manure and maize intercropping with reduced chemical N enhances productivity and carbon mitigation of farmland in arid areas. *European Journal of Agronomy*, **145**, 126788.
Li X F, Wang Z G, Bao X G, Sun J H, Yang S C, Wang P, Wang C B, Wu J P, Liu X R, Tian X L. 2021. Long-term increased grain yield and soil fertility from intercropping. *Nature Sustainability*, **4**, 943–950.
Liu K L, Han T F, Huang J, Huang Q H, Li D M, Hu Z H, Yu X C, Muhammad Q, Ahmed W, Hu H W. 2019. Response of soil aggregate-associated potassium to long-term fertilization in red soil. *Geoderma*, **352**, 160–170.
Liu R, Jiang P, Zhou G P, Chang D N, Liang H, Chai Q, Cao W D. 2024. Co-incorporation of wheat straw and hairy vetch reduced soil N_2O emission via regulating nitrifier and denitrifier structure on the Qinghai Plateau. *Applied Soil Ecology*, **202**, 105574.
Liu W X, Wei Y X, Li R C, Chen Z, Wang H D, Virk A L, Lal R, Zhao X, Zhang H L. 2022. Improving soil aggregates stability and soil organic carbon sequestration by no-till and legume-based crop rotations in the North China Plain. *Science of the Total Environment*, **847**, 157518.
Lu X F, Gilliam F S, Guo J Y, Hou E Q, Kuang Y W. 2021. Decrease of soil acidity has greater effects than increase of aboveground carbon inputs on soil organic carbon in terrestrial ecosystems of China under nitrogen enrichment. *Journal of Applied Ecology*, **59**, 768–778.
Martens D A. 2000. Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. *Soil Biology and Biochemistry*, **32**, 361–369.
De Medeiros E V, Silva A O, Duda G P, Dos Santos U J, Junior A J D S. 2018. The combination of *Arachis pintoi* green manure and natural phosphate improves maize growth, soil microbial community structure and enzymatic activities. *Plant and Soil*, **435**, 175.170–185.170.
Minasny B, Malone B P, Mcbratney A B, Angers D A, Winowiecki L A. 2017. Soil carbon 4 per mille. *Geoderma*, **292**, 59–86.
Nakamoto T, Suzuki K. 2001. Influence of soybean and maize roots on the seasonal change in soil aggregate size and stability. *Plant*

Production Science, **4**, 317–319.
Patoine G, Eisenhauer N, Cesarz S, Phillips H R P, Xu X, Zhang L, Guerra C A. 2022. Drivers and trends of global soil microbial carbon over two decades. *Nature Communications*, **13**, 4195.
Qiu Q Y, Wu L F, yang Z O, Li B B, Xu Y Y, Wu S S, Gregorich E G. 2016. Priming effect of maize residue and urea N on soil organic matter changes with time. *Applied Soil Ecology*, **100**, 65.60–74.60.
Ren S, Terrer C, Li J, Cao Y F, Yang S S, Liu D. 2024. Historical impacts of grazing on carbon stocks and climate mitigation opportunities. *Nature Climate Change*, **14**, 1–7.
Rockström J, Williams J, Daily G, Noble A, Matthews N, Gordon L, Wetterstrand H, DeClerck F, Shah M, Steduto P, de Fraiture C, Hatibu N, Unver O, Bird J, Sibanda L, Smith J. 2017. Sustainable intensification of agriculture for human prosperity and global sustainability. *AMBIO*, **46**, 4–17.
Sainju U, Whitehead W, Singh B. 2003. Cover crops and nitrogen fertilization effects on soil aggregation and carbon and nitrogen pools. *Canadian Journal of Soil Science*, **83**, 155–165.
Six J, Conant R T, Paul E A, Paustian K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil*, **241**, 155–176.
Six J, Elliott E T, Paustian K. 2000. Soil structure and soil organic matter: II. A normalized stability index and the effect of mineralogy. *Soil Science Society of America Journal*, **64**, 1042–1049.
Sun T, Mao X L, Han K F, Wang X J, Cheng Q, Liu X, Zhou J J, Ma Q X, Ni Z H, Wu L H. 2023. Nitrogen addition increased soil particulate organic carbon via plant carbon input whereas reduced mineral−associated organic carbon through attenuating mineral protection in agroecosystem. *Science of the Total Environment*, **899**, 165705.
Tian X L, Wang C B, Bao X G, Wang P, Li X F, Yang S C, Ding G C, Christie P, Li L. 2019. Crop diversity facilitates soil aggregation in relation to soil microbial community composition driven by intercropping. *Plant and Soil*, **436**, 173–192.
Udom B, Nuga B, Adesodun J. 2016. Water-stable aggregates and aggregate-associated organic carbon and nitrogen after three annual applications of poultry manure and spent mushroom wastes. *Applied Soil Ecology*, **101**, 5–10.
Walkley A, Black I A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, **37**, 29–38.
Wang J, Sun X, Du L, Sun W, Wang X, Gaafar A R Z, Zhang P, Cai T, Liu T, Jia Z, Chen X, Ren X. 2024. Appropriate fertilization increases carbon and nitrogen sequestration and economic benefit for straw-incorporated upland farming. *Geoderma*, **441**, 116743.
Wang W, Chen G, Li M Y, Chen Y, Wang Y, Tao H, Hou H, Rehman M M U, Ashraf M, Song Y, Kavagi L, Wang B Z, Xiong Y. 2024. Long-term cereal-legume intercropping accelerates soil organic carbon loss in subsoil of dryland. *Resources Conservation and Recycling*, **211**, 107898.
Wang W, Li M, Zhu S, Khan A, Tao X, Liu H, Zhang W, Tao H, Gong D, Song C, Xiong Y. 2023. Plant facilitation improves carbon production efficiency while reducing nitrogen input in semiarid agroecosystem. *Catena*, **230**, 107247.
Wang X, Chen Y, Yang K, Duan F, Liu P, Wang Z, Wang J. 2021. Effects of legume intercropping and nitrogen input on net greenhouse gas balances, intensity, carbon footprint and crop productivity in sweet maize cropland in South China. *Journal of Cleaner Production*, **314**, 127997–128006.
Wang Y, Pang J, Zhang M, Tian Z, Wei T, Jia Z, Ren X, Zhang P. 2023. Is adding biochar be better than crop straw for improving soil aggregates stability and organic carbon contents in film mulched fields in semiarid regions? Evidence of 5-year field experiment. *Journal of Environmental Management*, **338**, 117711.
Wang Y D, Wang Z L, Zhang Q Z, Hu N, Li Z F, Lou Y L, Li Y, Xue D M, Chen Y, Wu C Y. 2018. Long-term effects of nitrogen fertilization on aggregation and localization of carbon, nitrogen and microbial activities in soil. *Science of the Total Environment*, **624**, 1131–1139.
Wang Y L, Wu P N, Qiao Y B, Li Y M, Liu S M, Gao C K, Liu C S, Shao J, Yu H L, Zhao Z H, Guan X K, Wen P F, Wang T C. 2023. The potential for soil C sequestration and N fixation under different planting patterns depends on the carbon and nitrogen content and stability of soil aggregates. *Science of the Total Environment*, **897**, 165430.
Xie Z, Tu S, Shah F, Xu C, Chen J, Han D, Liu G, Li H, Muhammad I, Cao W. 2016. Substitution of fertilizer-N by green manure improves the sustainability of yield in double-rice cropping system in south China. *Field Crops Research*, **188**, 142–149.
Xu X R, Zhang W J, Xu M G, Li S Y, An T T, Pei J B, Xiao J, Xie H T, Wang J K. 2016. Characteristics of differently stabilised soil organic carbon fractions in relation to long-term fertilisation in Brown Earth of Northeast China. *Science of the Total Environment*, **572**, 1101–1110.
Yan Z J, Zhou J, Liu C Y, Jia R, Mganga K Z, Yang L, Yang Y D, Peixoto L, Zang H D, Zeng Z H. 2023. Legume-based crop diversification reinforces soil health and carbon storage driven by microbial biomass and aggregates. *Soil & Tillage Research*, **234**, 105848.
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? *Environmental Science and Pollution Research International*, **28**, 65862–65871.
Yang C Q, Wang X J, Li J N, Zhang G W, Shu H M, Hu W, Han H Y, Liu R X, Guo Z C. 2024. Straw return increases crop production by improving soil organic carbon sequestration and soil aggregation in a long-term wheat–cotton cropping system. *Journal of Integrative Agriculture*, **23**, 669–679.
Yang X L, Xiong J R, Du T S, Ju X T, Gan Y T, Li S, Xia L L, Shen Y J, Pacenka S, Steenhuis T S, Siddique K H M, Kang S Z, Butterbach-Bahl K. 2024. Diversifying crop rotation increases food production, reduces net greenhouse gas emissions and improves soil health. *Nature Communications*, **15**, 198.
Zhang D B, Yao P W, Zhao N, Cao W D, Zhang S Q, Li Y Y, Huang D L, Zhai B N, Wang Z H, Gao Y J. 2018. Building up the soil carbon pool via the cultivation of green manure crops in the Loess Plateau of China. *Geoderma*, **337**, 425–433.
Zhang W J, Munkholm L J, Liu X, An T T, Xu Y D, Ge Z, Xie N H, Li A M, Dong Y Q, Peng C, Li S Y, Wang J K. 2023. Soil aggregate microstructure and microbial community structure mediate soil organic carbon accumulation: Evidence from one-year field experiment. *Geoderma*, **430**, 116324.
Zhang X, Lassaletta L. 2022. Manure management benefits climate with limits. *Nature Food*, **3**, 312–313.
Zhang X X, Gregory A S, Whalley W R, Coleman K, Illangasekare T H. 2021. Relationship between soil carbon sequestration and the ability of soil aggregates to transport dissolved oxygen. *Geoderma*, **403**, 115370.
Zhang Y C, Zuo Q Y, Du B B, Chen W L, Wei D, Huang Q Y. 2018. Contrasting responses of bacterial and fungal communities to aggregate-size fractions and long-term fertilizations in soils of northeastern China. *Science of the Total Environment*, **635**, 784–792.
Zhang Z H, Jun N, Liang H, Wei C L, Yun W, Liao Y L, Lu Y H, Zhou G G, Gao S J, Cao W D. 2022. The effects of co-utilizing green manure and rice straw on soil aggregates and soil carbon stability in a paddy soil in South China. *Journal of Integrative Agriculture*, **22**, 1529–1545.
Zhao H L, Shar A G, Li S, Chen Y L, Shi J L, Zhang X Y, Tian X H. 2018. Effect of straw return mode on soil aggregation and aggregate carbon content in an annual maize–wheat double cropping system. *Soil & Tillage Research*, **175**, 178–186.
Zhao X P, Hao C K, Zhang R Q, Jiao N Y, Tian J, Lambers H, Liang C, Cong W F, Zhang F S. 2023. Intercropping increases soil macroaggregate carbon through root traits induced microbial necromass accumulation. *Soil Biology and Biochemistry*, **185**, 109146.
Zhou P, Zhang X X, Pan G X. 2006. Effect of long-term fertilization on content of total and particulate organic carbon and their depth distribution of a paddy soil: An example of huangnitu from the Tai Lake region, China. *Plant Nutrition & Fertilizer Science*, **12**, 765–771. (in Chinese)

Executive Editor-in-Chief Jinshui Wu
Managing Editor Lujuan Sun