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Nature-based nutrient management through returning agricultural organic waste enhances soil aggregate organic carbon stability

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ABSTRACT

Agricultural organic waste can enhance aggregate organic carbon stability, which is crucial for soil carbon sequestration in croplands. However, it is unclear how aggregate organic carbon stability changes with different nature-based nutrient management practices, especially with partial organic substitution. This study aimed to elucidate how different organic wastes (chicken manure, biochar, straw, and carbon-based materials from kitchen waste) influence aggregate organic carbon stability, including aggregate stability, the content of physically protected organic carbon, and the decomposability of aggregate carbon. The improvement of aggregate organic carbon stability was trialed in a 4-year field experiment with equivalent nitrogen and organic carbon input under nature-based nutrient management. The results showed that all nature-based nutrient management practices improved aggregate organic carbon stability compared to no nutrient addition. Biochar application dramatically improved aggregate organic carbon stability by 5.8–11.4 % in aggregate stability, 83.9–152.4 % in aggregate organic carbon, and 36.6–75.0 % in aggregate recalcitrant carbon content. By comparison, straw returning showed the lowest improvement in aggregate organic carbon stability, owing to substantial increases of microbial respiration and enzyme activities involved in carbon degradation. Organic carbon merely increased by 32.3 %, 33.6 %, and 29.5 % in large macroaggregates, small macroaggregates, and microaggregates, respectively. This study dissected the different efficiencies of nature-based nutrient management in improving aggregate organic carbon stability in vegetable fields. The findings highlight that appropriate nature-based nutrient management with organic waste could better implement the carbon neutrality in agroecosystems from the perspective of aggregate organic carbon stability.

1. Introduction

Optimizing nature-based nutrient management (NBS-NM) is crucial for enhancing soil organic carbon (SOC) storage other than the reduction of environmental pollution, which is a feasible pathway to implement the goal of ‘carbon neutrality’ in croplands. The carbon sequestration potential in soils is ~ 63 Pg carbon (Walker et al., 2022),

and cropland soils can serve as a viable carbon sink with appropriate agricultural practices (Amelung et al., 2020). However, the extensive removal of post-harvest crop residues has led to the gradual depletion of SOC, and therefore specific practices aimed at replenishing SOC are urgently needed (Bhattacharya et al., 2016). Agricultural organic waste includes unutilized or discarded straw, manure, and food residues (Khaleel et al., 1981; Obi et al., 2016). Partial substitution of these

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organic wastes for synthetic fertilizers in agroecosystems is expected to recycle nutrients and increase SOC (Tang et al., 2019). Nevertheless, different NBS–NM practices are likely to have varying impacts on soil carbon cycling (Beillouin et al., 2023; Bohoussou et al., 2023; Gross et al., 2021; Rocci et al., 2021). Therefore, optimizing NBS–NM practices to substantially enhance carbon sequestration is an important step towards carbon neutrality in croplands.

Soil aggregates play a key role in sequestering and stabilizing SOC in croplands. Three metrics have been proposed to assess the dynamics of aggregate organic carbon stability (AOCS), namely, aggregate stability, the content of physically protected SOC, and the decomposability of aggregate SOC (Wang et al., 2024). For example, particulate organic carbon can be encapsulated by aggregates, by which it is physically protected from exposure to microorganisms and extracellular enzymes, and the residence time of particulate organic carbon is prolonged (Lavallee et al., 2020). Aggregates also promote the formation and stabilization of mineral-associated organic carbon by facilitating the coagulation between SOC and the mineral matrix (Witzgall et al., 2021). As of now, the decomposability of protected carbon is perhaps the least understood aspect of AOCS under NBS–NM. The decomposability of protected carbon can vary greatly under different NBS–NM due to the responses of microbial activities (Bhattacharyya et al., 2022). For instance, less change in soil microorganisms was observed under NBS–NM with biochar compared with NBS–NM with straw (Huang et al., 2018), suggesting that the carbon decomposability may be lower under NBS–NM with biochar (Wu et al., 2021). In comparison, microbial activity may promote carbon decomposition under NBS–NM with manure (Liang and Zhu, 2021). Although soil microbial responses have received much attention (Liu et al., 2023), the extent to which the decomposability of protected carbon varies under different NBS–NM remains unclear (Basile-Doelsch et al., 2020). It is essential to determine how SOC decomposability varies with microbial characteristics across different NBS–NM practices.

Although the preliminary impacts of NBS–NM on AOCS have been shown in a meta-analysis (Wang et al., 2024), these results are subject to high uncertainty (Nakagawa et al., 2017). The different amount of organic carbon inputs and the heterogeneity of experiments (especially for the heterogeneous soil properties between experimental sites), may hinder the precise comparisons of AOCS improvement between different studies included in the meta-analysis (Nakagawa et al., 2017). For example, the increment of the physically protected carbon in macroaggregates varied significantly depending on the amount of organic fertilizers (Liu et al., 2013). The efficacy of AOCS changes is also difficult to discern when the quantity of organic carbon input is not controlled in field experiments (Bandyopadhyay et al., 2010; Bipfubusa et al., 2008; Sun et al., 2020). In addition, fertilizer application rates may impact the AOCS (Wang et al., 2024), and therefore the same nutrient application rate is also a prerequisite to accurately assess the effectiveness of AOCS changes across different NBS–NM. Furthermore, the responses of AOCS may vary under equal nutrient applications in soils with different physicochemical properties (Li et al., 2023; Murphy, 2015). For instance, a dramatic enhancement in AOCS occurs in soils with loamy textures (Ma et al., 2024). To precisely compare the efficacies of AOCS changes among different NBS–NM practices, experiments with equal organic carbon inputs and nutrient applications within the same soil type are required.

Given that biochar contains a substantial amount of recalcitrant carbon (Li et al., 2020), and possessed high cation exchange capacity and a large specific surface area (El-Naggar et al., 2018; Lian and Xing, 2017), it is hypothesized that NBS–NM with biochar may substantially enhance AOCS by improving soil structure and reducing the decomposability of SOC. Conversely, it is hypothesized that NBS–NM with straw will result in minor changes in AOCS due to increased microbial activity and higher carbon dioxide emissions (Joergensen and Wichern, 2018; Tomar and Baishya, 2020), and in tandem with its limited effectiveness in improving aggregate stability (Zhang et al., 2023). To test

these hypotheses, this study aimed to assess changes in AOCS within the top soil layer (0–20 cm) under NBS–NM treatments involving chicken manure, biochar, straw, and carbon-based materials derived from kitchen waste. A field experimental platform was used, with an equal amount of organic carbon input and nutrient application across treatments. The following key research questions were addressed: (1) how do AOCS metrics change with NBS–NM, particularly in terms of the decomposability of protected carbon? and (2) which NBS–NM treatment most effectively improves AOCS?

2. Materials and methods

2.1. Experiment site

A field experiment with NBS–NM was conducted from 2019 to 2023 at the National Purple Soil Fertility and Fertilizer Effectiveness Monitoring Station (29°48'45"N, 106°24'31"E) in Beibei District, Chongqing, China. The region is characterized by a humid subtropical monsoon climate with an annual precipitation of approximately 1161 mm and an average annual temperature of around 18.3°C. The experimental soil is classified as purple soil according to the Chinese soil classification (likely corresponding to Inceptisols), and consists of 88 % sand, 5 % silt, and 7 % clay. The cropping system follows a chili pepper–celery cabbage rotation, with chili peppers (*Capsicum annuum* L.) planted from April to August, and celery cabbages (*Brassica rapa* var. *glabra* Regel) planted from October to January of the following year. The initial physicochemical properties of the topsoil (0–20 cm) were as follows: 4.69 g kg^{−1} SOC, 0.37 g kg^{−1} total nitrogen, 14.1 mg kg^{−1} available phosphorus, 221 mg kg^{−1} available potassium, and a pH of 8.44.

2.2. Experimental design and soil sampling

The field experiment consisted of five treatments: (1) control, without nutrient addition (CK); (2) NBS–NM with biochar (BC); (3) NBS–NM with chicken manure (CM); (4) NBS–NM with carbon-based material derived from kitchen waste (KW); and (5) NBS–NM with straw (ST). Each treatment was replicated three times using a randomized block design, resulting in a total of 15 plots. The experimental area of each plot was 15.75 m² (4.5 m × 3.5 m), and the row spacing between plants was 40 cm × 40 cm. Total organic carbon, nitrogen, and phosphorus inputs for each NBS–NM practice were 1700 kg carbon ha^{−1}, 250 kg nitrogen ha^{−1}, and 160 kg diphosphorus pentoxide ha^{−1} (Table 1). Biochar, chicken manure, carbon-based material derived from kitchen waste, and straw provide organic carbon and some nitrogen and phosphorus. The nutrient content of biochar, chicken manure, carbon-based material derived from kitchen waste and straw is shown in Table S1. Chemical fertilizer was used to supplement the nitrogen and phosphorus to 250 kg nitrogen ha^{−1} and 160 kg diphosphorus pentoxide ha^{−1} in each NBS–NM treatment (Table 1). Fertilization was applied twice a year, before crop transplanting in each crop growing season.

Soil samples were collected using a soil auger (inner diameter of 5 cm) from five randomly selected locations within each plot, at a depth

Table 1
Fertilizer application for each treatment. CK: without nutrient addition. BC: NBS–NM with biochar. CM: NBS–NM with chicken manure. KW: NBS–NM with carbon-based material derived from kitchen waste. ST: NBS–NM with straw.

Treatment	Fertilizer application rate (kg ha ^{−1})							
	Organic fertilizer			Synthetic fertilizer		Total fertilizer		
	C	N	P ₂ O ₅	N	P ₂ O ₅	C	N	P ₂ O ₅
CK	0	0	0	0	0	0	0	0
BC	1700	51	21	199	139	1700	250	160
CM	1700	122	160	128	0	1700	250	160
KW	1700	92	35	158	125	1700	250	160
ST	1700	55	5	195	155	1700	250	160

of 0–20 cm, before planting chili peppers. Sampling avoided areas where fertilizers has been applied and plant root zones. Some of the soil samples were air-dried to measure their physical and chemical properties, while part of the soil sample was immediately stored at 4 °C for nutrient measurements.

2.3. Soil aggregate separation and the measurement of soil properties

Visible roots and stones were removed from the air-dried soil, and the aggregates were then wet-sieved following the method of Elliott (1986). The soil was classified into four particle size categories: large macroaggregate (> 2 mm); small macroaggregate (0.25–2 mm); microaggregate (0.053–0.25 mm), and silt+clay fraction (< 0.053 mm). The mass proportion of each particle size after drying was weighed. The stability index of soil aggregates, including water-stable aggregate, mean weight diameter, and geometric mean diameter, were calculated (Karami et al., 2012).

$$\text{Water Stable Aggregate} = \frac{M_{r>0.25}}{M_T}$$

$$\text{Mean Weight Diameter} = \sum_{i=1}^n X_i W_i$$

$$\text{Geometric Mean Diameter} = \exp\left(\frac{\sum_{i=1}^n W_i \ln W_i}{\sum_{i=1}^n W_i}\right)$$

Where, $M_r > 0.25$ represents the mass of aggregates with a particle size greater than 0.25 mm; M_T is the total mass of soil aggregates of different particle sizes. X_i is the mean diameter of aggregates for each particle size (mm); and W_i is the mass percentage of aggregates for each particle size.

Soil physical and chemical properties were measured as follows. The potassium dichromate oxidation method and continuous flow analyzer were used to quantify SOC and total nitrogen. Soil cores at a depth of 0–5 cm were collected using a metal cylinder (100 cm³). The soil samples were dried to constant weight. Bulk density was calculated by dividing soil dry mass contained in the metal cylinder by the cylinder's volume (Coulibaly et al., 2022).

Soil pH was measured using a pH meter (Mettler Toledo 2000). Soil microbial biomass carbon was determined by chloroform fumigation. Specifically, the soil sample (10 g) was placed in a desiccator, and a small beaker containing ethanol-free chloroform and zeolite was placed at the bottom of the desiccator. The pressure-tight desiccator was vacuumized to boil the chloroform for 5 min, after which the samples were tightly sealed in the dark at 25 °C for 2 days. Microbial biomass carbon was then calculated from the difference in carbon content between fumigated and unfumigated soil. The method of sieving and differentiating for particulate organic carbon and mineral-associated organic carbon followed Cotrufo et al. (2019), where a dispersant (sodium hexametaphosphate) was added to the soil samples, and then the mixtures were shaken for 18 hours and the SOC was separated into particulate organic carbon and mineral-associated organic carbon through a sieve of 53 µm. Infrared spectroscopy was utilized to determine the stability of carbon (Tucureanu et al., 2016) by calculating the ratio of the peak area near 1430 cm⁻¹ (aliphatic carbon) to the peak area near 1640 cm⁻¹ (aromatic carbon), while the higher ratio indicating a more stable carbon structure. The enzymatic activities involved in soil carbon and nitrogen cycling were measured, including α-glucosidase (AG), β-glucosidase (BG), and cellobiohydrolase (CBH), N-acetyl-d-(+)-glucosamine (NAG), and leucine arylamidase (LAP). Each soil sample was duplicated 4 times and then dropped into a 96-well microtiter reaction plate, after which soil samples were incubated in the dark for 4 h and then measured using a microplate reader (Infinite M200 PRO).

2.4. Soil incubation and the measurement of soil respiration rate

Carbon decomposability was reflected by the soil respiration rate, and a laboratory incubation of soil samples for each treatment was conducted to minimize the influence of complex external factors. Specifically, three undisturbed soil columns were collected from each experimental plot. Fresh soil was also collected near the sampling points to determine the soil moisture and bulk density. The soil columns were placed into plastic bottles and sealed with a rubber stopper and sealing film. The rubber stoppers were punched with 6 mm holes and fitted with triple valves and 10 cm of Teflon tubing. Soil incubation devices were placed in a constant temperature incubator at 30 °C. The gas was collected and stored in headspace bottles after 4-h incubation, and carbon dioxide was determined using a meteorological chromatograph (Agilent 7890A). The temperature sensitivity (Q_{10}) of the soil respiration rate was calculated as the ratio of the CO₂ production rate at 30 °C to the CO₂ production rate at 20 °C.

2.5. Statistical analyses

One-way ANOVA was used to compare the effects of different NBS-NM practices on soil variables, followed by the LSD test for pairwise comparisons between treatments. *T*-tests were used to compare differences in variables between two years. Violin plots were plotted using the geom violin function in the 'ggplot2' package; asymmetric correlation heatmaps were plotted using the 'pheatmap' package. Non-metric multidimensional scaling and entropy weighting methods were employed to explore the effect of NBS-NM on the AOCs. Non-metric multidimensional scaling analyses were performed using the 'vegan' package. Based on the entropy weighting method, the metrics for AOCs were aggregate stability indices (including water-stable aggregate, mean weight diameter, and geometric mean diameter), aggregate SOC, and the ratio of aggregate aliphatic/aromatic carbon. The data were initially normalized (Z-score normalization), and then the entropy p_i and weight (λ_i) were calculated (Xia et al., 2018).

$$\frac{x_{ij} - \min_i \{x_{ij}\}}{\max_i \{x_{ij}\} - \min_i \{x_{ij}\}} \quad (1)$$

where, x_{ij} is the observation value of the j -th indicator for the i -th sample, $i = 1, 2, \dots, m$, $j = 1, 2, \dots, n$, and normalized values are expressed as r_{ij} .

$$f_{ij} = \frac{r_{ij}}{\sum_{j=1}^n r_{ij}} \quad (2)$$

$$k = \frac{1}{\ln n} \quad (3)$$

$$p_i = -k \int_{j=1}^n f_{ij} \ln f_{ij} \quad (4)$$

$f_{ij} \ln f_{ij}$ is set to 0 when $f_{ij} = 0$.

$$\lambda_i = \frac{1 - p_i}{m - \sum_{i=1}^m p_i} \quad (5)$$

Weights range from 0 to 1, and their sum equals 1. All statistical analyses were performed with R (version 4.2.1).

3. Results

3.1. Nature-based nutrient management enhanced soil organic carbon, particulate organic carbon, and mineral-associated organic carbon

Different NBS–NM practices with equivalent organic carbon input significantly influenced soil properties and carbon content to varying degrees. Soil pH tended to neutralize from alkalinity and bulk density slightly decreased under all NBS–NM treatments (Table S2). The reduction in soil pH and bulk density was most pronounced under NBS–NM with straw (by 0.34 and 0.1, respectively), while NBS–NM with chicken manure had only a minimal effect (by 0.15 and 0.03, respectively). On average, the increase in SOC content was most pronounced in soils under NBS–NM with biochar (104.5 %), followed by NBS–NM with chicken manure (42.7 %) and carbon-based material derived from kitchen waste (41.5 %) (Fig. 1). The smallest SOC increase occurred

under NBS–NM with straw (31.8 %). In general, the increase in SOC was more pronounced after 4 years than that after 3 years with the same NBS–NM (Fig. 1, Table S3). Notably, NBS–NM with biochar further increased SOC content from 7.2 g kg^{−1} after 3 years to 10.3 g kg^{−1} after 4 years, while the smallest SOC increment always occurred under NBS–NM with straw (6.5 g kg^{−1} after 4 years).

NBS–NM with biochar significantly increased particulate organic carbon by an average of 234.7 %, surpassing other treatments (ranging from 30.3 % to 55.2 %) (Fig. 1). The increase in particulate organic carbon became more pronounced over time (Table S3), especially under NBS–NM with biochar. Mineral-associated organic carbon also increased across all treatments, with an average increase of 59.1 %. The mineral-associated organic carbon significantly increased under NBS–NM with biochar, chicken manure, and carbon-based material derived from kitchen waste (69.4 %, 45.3 %, and 96.8 %, respectively), while a smaller increase occurred under NBS–NM with straw (25.0 %).

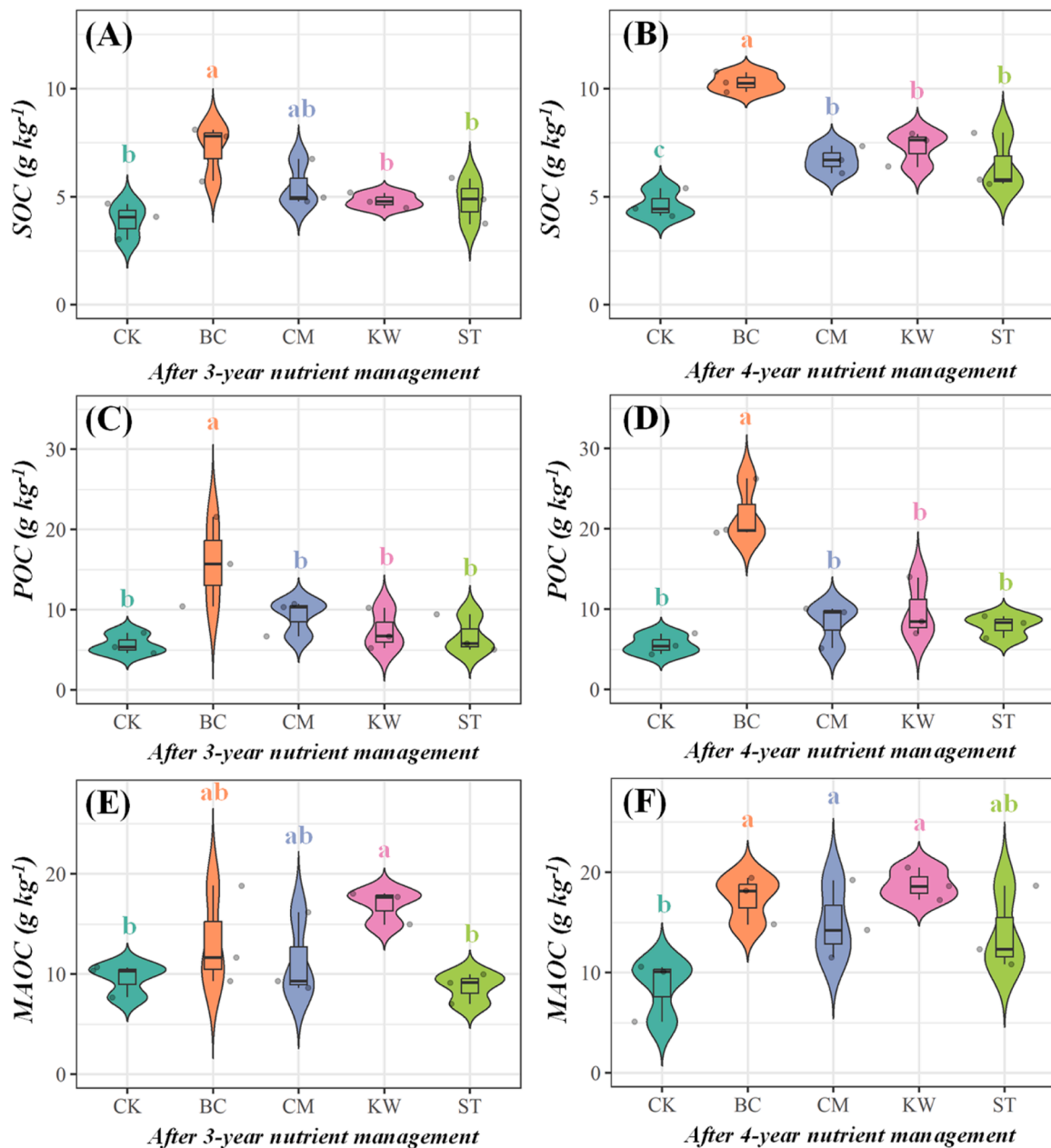


Fig. 1. Changes in SOC (A–B), POC (C–D), and MAOC (E–F) upon nutrient management. The different lowercase letters stand for significant differences between treatments. SOC: soil organic carbon, POC: particulate organic carbon, MAOC: mineral-associated organic carbon. CK: without nutrient addition. BC: NBS–NM with biochar. CM: NBS–NM with chicken manure. KW: NBS–NM with carbon-based material derived from kitchen waste. ST: NBS–NM with straw.

The mineral-associated organic carbon content was always the lowest under NBS-NM with straw (13.9 g kg^{-1}), although the change in mineral-associated organic carbon content ranged from -0.1% after 3 years to 62.5% after 4 years (Table S3).

3.2. Soil aggregate stability and large macroaggregate mass proportion enhanced upon nature-based nutrient management

NBS-NM improved the aggregate stability and altered the mass proportion of aggregates (Fig. 2). The application of NBS-NM with chicken manure resulted in the largest increase in water-stable aggregate content, with an average increase of 13.7% , while water-stable

aggregate content did not significantly change under NBS-NM with straw. Similar trends were observed for geometric mean diameter and mean weight diameter, where all NBS-NM except for NBS-NM with straw, enhanced mean weight diameter (e.g., a 17.0% increase under NBS-NM with chicken manure). The changes in aggregate stability indices were more pronounced after 4 years than after 3 years under all NBS-NM practices (Fig. 2D–F).

The effects of NBS-NM on aggregate mass proportions varied, particularly for large macroaggregates (Fig. 2G–H). NBS-NM with chicken manure and NBS-NM with biochar significantly increased the mass proportion of large macroaggregates (by 32.2% and 22.7% , respectively) and small macroaggregates (by 2.8% and 3.6% ,

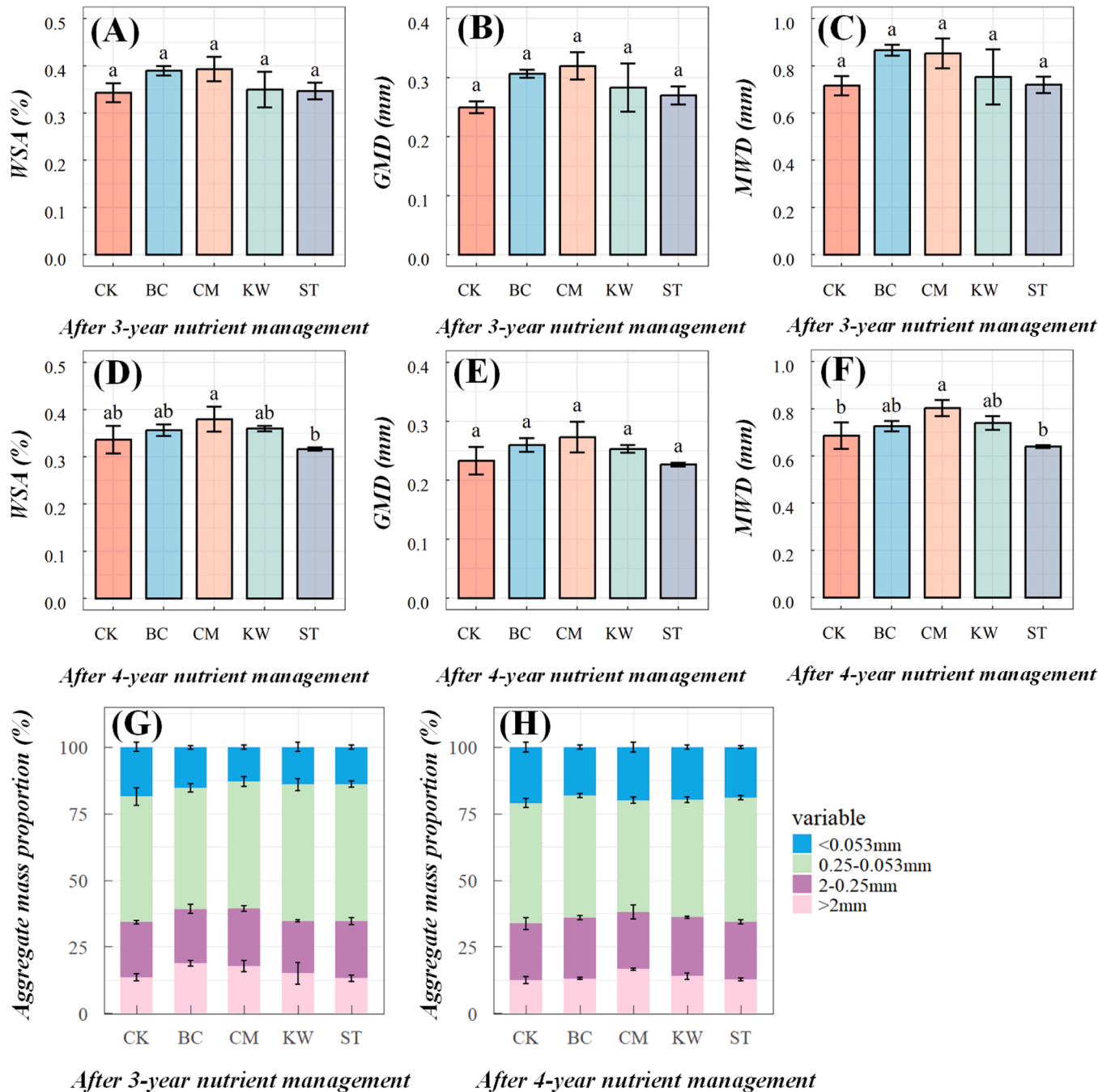


Fig. 2. The responses of aggregate stability (A–F) and aggregate mass proportion (G–H) to nutrient managements. The different lowercase letters stand for significant differences between treatments. WSA: water-stable aggregates, GMD: geometric mean diameter, MWD: mean weight diameter. CK: without nutrient addition. BC: NBS-NM with biochar. CM: NBS-NM with chicken manure. KW: NBS-NM with carbon-based material derived from kitchen waste. ST: NBS-NM with straw.

respectively). In contrast, NBS–NM with straw caused a decrease in large macroaggregates (–7.6 %) and a slight increase in small macroaggregates (1.4 %) (Fig. 2G–H). The proportion of microaggregates increased under NBS–NM with straw (7.6 %) and NBS–NM with carbon-based material derived from kitchen waste (3.1 %), but slightly decreased under the other NBS–NM practices. The proportion of macroaggregates generally increased under NBS–NM over time, except for NBS–NM with straw (Fig. 2).

3.3. Increment of microbial biomass carbon and enzyme activities accelerated carbon output

NBS–NM significantly increased soil microbial biomass carbon in bulk soils and aggregates (Fig. 3). The increases in microbial biomass carbon were 93.9 %, 34.5 %, 85.8 %, and 81.9 % in bulk soil, large macroaggregate, small macroaggregate, and microaggregate, respectively. The largest increase in microbial biomass carbon in bulk soil was under NBS–NM with straw (175.0 %), while the smallest increase occurred under NBS–NM with biochar (18.0 %) (Fig. 3A). At the aggregate level, NBS–NM with straw also resulted in the highest increases in microbial biomass carbon, particularly in small macroaggregates (226.7 %), whereas NBS–NM with biochar resulted in a modest increase in microbial biomass carbon (e.g., a 9.8 % increase in small macroaggregates) (Fig. 3B–D).

NBS–NM with the same amount of organic carbon input had varying effects on soil enzyme activities (Fig. 4A–J). NBS–NM with straw resulted in the greatest increases in enzyme activities, particularly for carbon-cycling enzymes such as α -glucosidase and β -glucosidase (by 173.5 % and 174.3 %, respectively). In comparison, NBS–NM with

biochar only increased α -glucosidase by 87.9 %, and kitchen-waste-derived carbon material increased β -glucosidase by 57.1 %. Although NBS–NM with kitchen-waste carbon material had a minor effect on carbon-cycling enzymes, it significantly increased nitrogen-cycling enzymes, such as leucine arylamidase (78.7 %). Despite slight declines in enzyme activity over time, the enzyme activities related to carbon degradation maintained relatively high after 4 years under NBS–NM with straw. For example, the increases in α -glucosidase and β -glucosidase were notable (by 25.6 % and 19.2 %). Soil respiration rates were changed to varying extents by different NBS–NM. Specifically, NBS–NM with straw increased soil respiration rate by 148.9 % at 30 °C, while NBS–NM with biochar only increased it by 33.6 % (Fig. 4K–L). Moreover, the highest Q_{10} was observed under NBS–NM with straw (1.82).

The changes in microbial biomass carbon and enzyme activities significantly impacted soil respiration rates under NBS–NM (Fig. 4M). In particular, soil respiration rate and Q_{10} showed a positive correlation with microbial biomass carbon in both bulk soil (slope = 0.8 and 0.62, respectively, both $p < 0.05$) and microaggregates (slope = 0.76 and 0.55, respectively; both $p < 0.05$). Enzyme activities involved in carbon degradation (α -glucosidase and β -glucosidase) were also positively correlated with microbial biomass carbon after 4 years under NBS–NM, particularly in microaggregates (slopes = 0.78 and 0.64, respectively; both $p < 0.05$). Notably, the neutralized soil pH may enhance enzyme activities and soil respiration rates. For instance, α -glucosidase, leucine arylamidase, and Q_{10} increased with decreasing soil pH (slope = – 0.56, – 0.51, and – 0.75 respectively; all $p < 0.05$).

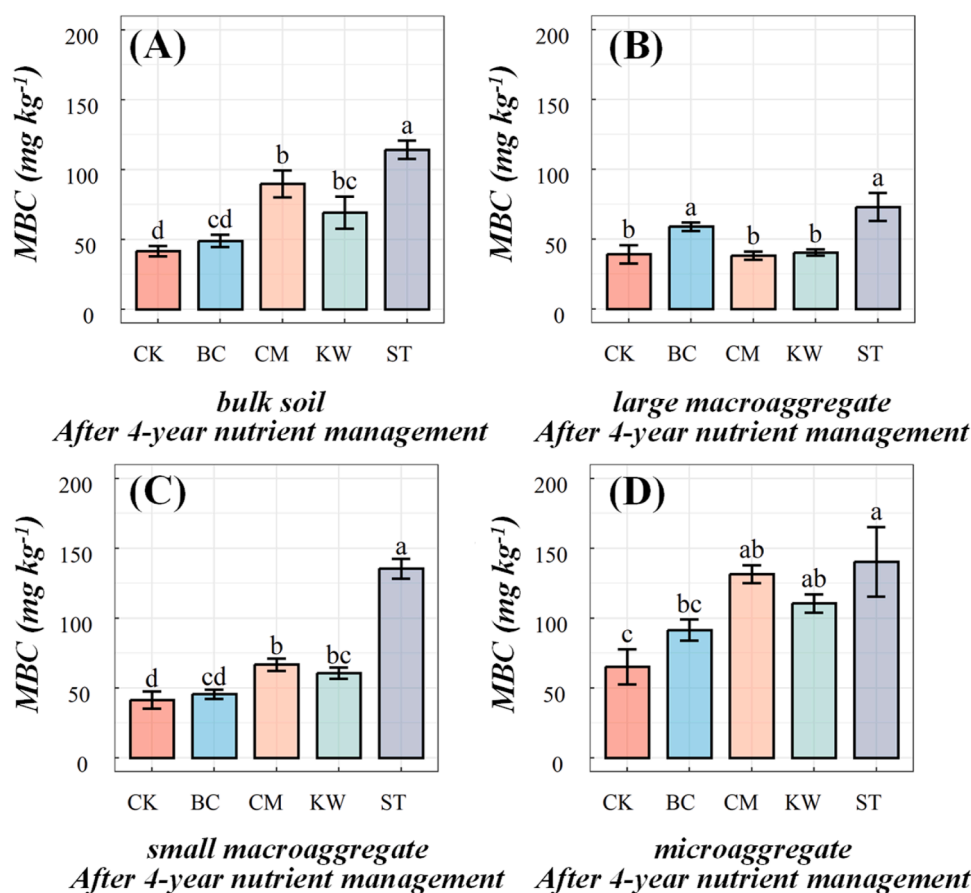


Fig. 3. Changes of microbial biomass carbon in bulk soil (A) and aggregates (B–D) after NBS–NM. The different lowercase letters stand for significant differences between treatments. MBC: microbial biomass carbon. CK: without nutrient addition. BC: NBS–NM with biochar. CM: NBS–NM with chicken manure. KW: NBS–NM with carbon-based material derived from kitchen waste. ST: NBS–NM with straw.

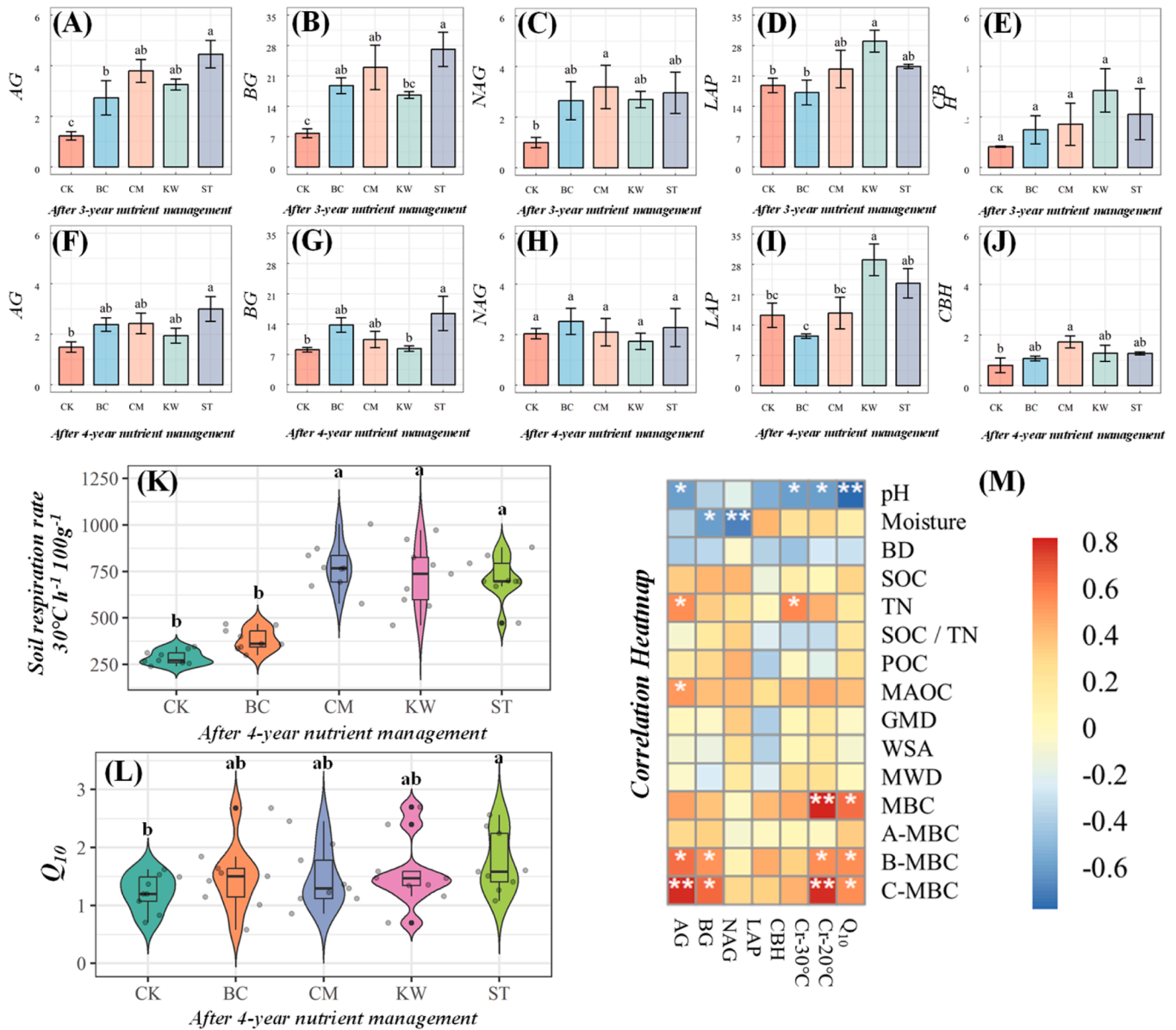


Fig. 4. Changes in soil enzyme activities after nutrient management (A–J), the comparisons of soil respiration rate, temperature sensitivity of soil respiration rate between different nutrients (K–L), and the correlations heatmap of carbon output with soil properties (M). The different lowercase letters stand for significant differences between treatments. The * in heatmap represents $p < 0.05$, and ** represents $p < 0.01$. AG: α -glucosidase, BG: β -glucosidase, NAG: N-acetyl-d-(+)-glucosamine, LAP: leucine arylamidase, CBH: cellobiohydrolase. BD: bulk density, SOC: soil organic carbon, TN: total nitrogen, SOC/TN: the ratio of soil organic carbon to total nitrogen, POC: particulate organic carbon, MAOC: mineral-associated organic carbon, GMD: geometric mean diameter, WSA: water-stable aggregates, MWD: mean weight diameter, MBC: microbial biomass carbon. Abbreviation Cr, CO_2 production rate. Q_{10} , temperature sensitivity of CO_2 production rate. A-MBC, B-MBC, and C-MBC represent microbial biomass carbon in large macroaggregate, small macroaggregate, and microaggregate, respectively. CK: without nutrient addition. BC: NBS-NM with biochar. CM: NBS-NM with chicken manure. KW: NBS-NM with carbon-based material derived from kitchen waste. ST: NBS-NM with straw.

3.4. Nature-based nutrient management with straw improves aggregate soil organic carbon and aggregate organic carbon stability to a minor extent

NBS-NM increased aggregate SOC content to varying extents (Fig. 5). NBS-NM with biochar resulted in the largest increase in aggregate SOC by 90.3 %, 116.0 %, and 48.3 % for large macroaggregate, small macroaggregate, and microaggregate, respectively. NBS-NM with chicken manure and carbon-based material derived from kitchen waste also boosted SOC in large macroaggregates by 54.1 % and 55.6 %, respectively. Over time, NBS-NM with biochar showed the greatest improvement, significantly increasing SOC from 1.9 g kg^{-1} to 4.2 g kg^{-1} in large macroaggregates, 3.1 g kg^{-1} to 5.4 g kg^{-1} in small

macroaggregates, and 3.8 g kg^{-1} to 6.2 g kg^{-1} in microaggregates after 4 years compared with that after 3 years. In comparison, SOC changes under NBS-NM with straw were minimal after 4 years, with only slight alterations of 0.2, 0.2, and -0.4 g kg^{-1} in large macroaggregates, small macroaggregates, and microaggregates, respectively.

The stability of aggregate SOC (aliphatic/aromatic-C) increased with larger aggregate sizes (Fig. 5G–I). The stability of SOC in large macroaggregates was more pronounced under NBS-NM with biochar (ratio of 0.63), while NBS-NM with chicken manure enhanced SOC stability in small macroaggregates (ratio of 0.51), and NBS-NM with carbon-based material derived from kitchen waste increased SOC stability in microaggregates (ratio of 0.23). NBS-NM with straw resulted in a relatively minor increase in SOC stability in large macroaggregates

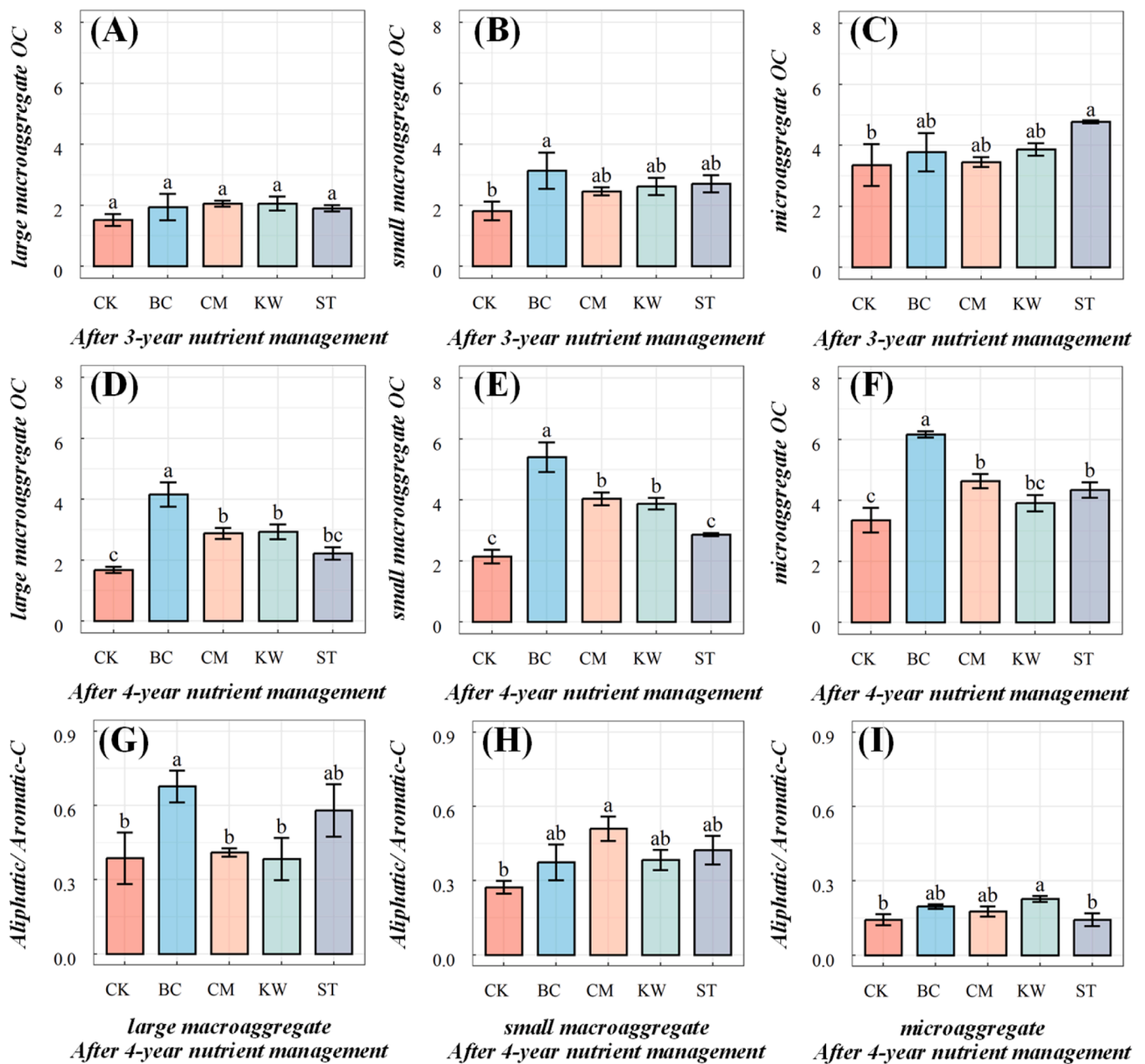


Fig. 5. Changes of organic carbon (A–F) and aliphatic carbon:aromatic carbon ratio (G–I) in aggregate after nutrient management. The different lowercase letters stand for significant differences between treatments. CK: without nutrient addition. BC: NBS–NM with biochar. CM: NBS–NM with chicken manure. KW: NBS–NM with carbon-based material derived from kitchen waste. ST: NBS–NM with straw.

(50.0 %) and small macroaggregates (54.9 %), with little effect on SOC stability in microaggregates.

The enhancement of AOCS under NBS–NM with straw was relatively limited (Fig. 6). Non-metric multidimensional scaling revealed that the differences in AOCS were primarily determined by the magnitude of soil respiration (Fig. 6A). Moreover, the increase in AOCS under NBS–NM with straw was the smallest among the NBS–NM practices (Fig. 6B). The AOCS index was only 19.2 under NBS–NM with straw, while the AOCS index under NBS–NM with biochar reached 60 (about 211.6 % higher than that under NBS–NM with straw). The application of NBS–NM with straw did not enhance the stability of soil aggregates, since it led to a substantial increase in microbial biomass carbon and enzyme activities involved in carbon degradation. Consequently, the NBS–NM with straw only slightly increased AOCS (Fig. 6C).

4. Discussion

Comparing NBS–NM practices with equal organic carbon input provides a valuable insight into understanding the differential improvements in AOCS in croplands. Given the heterogeneity of soil conditions across experimental sites, meta-analyses fall short in discerning the fine distinctions in AOCS improvement (Nakagawa et al., 2017). Although some field experiments have compared the effects of nutrient management on individual AOCS characteristic (e.g., aggregate carbon content), differences in organic carbon input have hindered accurate comparisons of AOCS (Bandyopadhyay et al., 2010; Bipfubusa et al., 2008; Sun et al., 2020). This study investigated the differential responses of AOCS characteristics to NBS–NM with equal organic carbon input. The appropriate NBS–NM practices can enhance carbon sequestration at the aggregate scale, which is of great importance for achieving ‘carbon

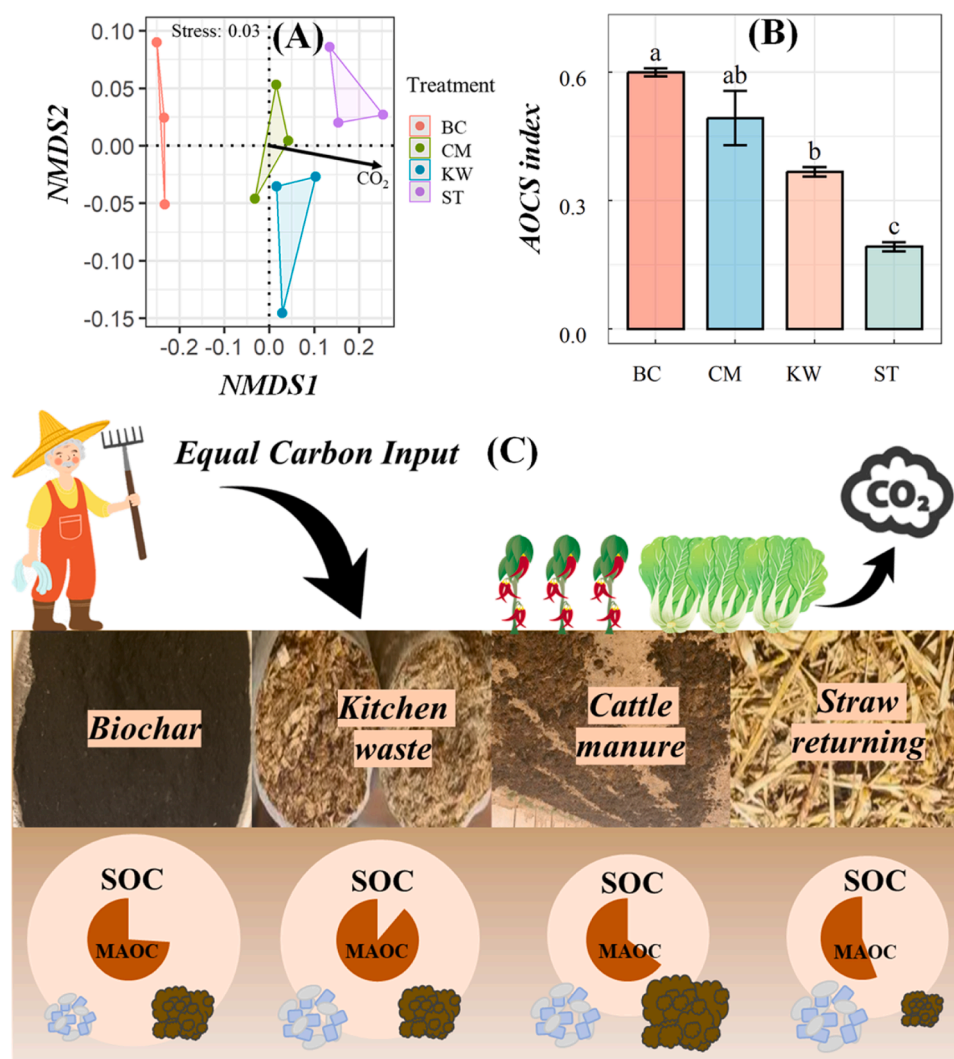


Fig. 6. Comparison of aggregate organic carbon stability under different nutrient management, using non-metric multidimensional scaling (A), entropy weighting index (B), and a schematic diagram for AOCs under different nutrient management (C). AOCs: aggregate organic carbon stability, SOC: soil organic carbon, MAOC: mineral-associated organic carbon. CK: without nutrient addition. BC: NBS–NM with biochar. CM: NBS–NM with chicken manure. KW: NBS–NM with carbon-based material derived from kitchen waste. ST: NBS–NM with straw.

neutrality' in croplands.

4.1. The different changes in aggregate organic carbon stability upon nature-based nutrient management in croplands

Different NBS–NM practices change aggregate stability in croplands to a varying extent, in which NBS–NM with chicken manure maximally improved aggregate stability after 4 years, followed by NBS–NM with biochar, while NBS–NM with straw minimally improved aggregate stability. The greatest increase in aggregate stability after 4-years under NBS–NM with chicken manure aligns with findings in orchards (Peng et al., 2016). The polysaccharides generated during the decomposition of chicken manure can enhance the adhesion between soil particles (Costa et al., 2018), thereby facilitating the formation of macroaggregates (Blanco-Canqui and Lal, 2004). Furthermore, biochar can enhance aggregate stability by facilitating the formation of macroaggregates (Ghorbani and Amirahmadi, 2024). The hydroxyl and carboxyl groups on biochar can interact with soil particles and clay minerals, leading to the formation of larger aggregates (Situ et al., 2022). However, the high pH of biochar may induce electrostatic repulsion between colloidal iron oxides involved in aggregation (Kosmulski, 2006), which could explain the smaller improvement in soil aggregation under NBS–NM with

biochar compared with that observed under NBS–NM with chicken manure. In comparison, the improvement in aggregate stability was the least pronounced under NBS–NM with straw, and the proportion of water-stable aggregates under NBS–NM with straw was at the same level as that observed in the absence of nutrient addition (Fig. 2). A reduction in soil aggregates stability occurred when the straw was mechanically incorporated into the soil (Hartmann and Six, 2023). Moreover, the different changes in microbial activities under NBS–NM may also impact aggregate stability. The addition of chicken manure containing low-molecular-weight organic nitrogen and ammonium (Zhou et al., 2018), can increase the activity of arbuscular mycorrhizal (Gryndler et al., 2006). The mycelium and/or glomalin proteins promote the coagulation of soil particles (Hammer et al., 2011; Wang et al., 2022). By contrast, straw with a high C:N ratio may inhibit the functioning of some microbes (Cui et al., 2022), which in turn weakens soil aggregation.

NBS–NM with straw exhibited the greatest impact on microbial traits while NBS–NM with biochar little affected these traits. The NBS–NM with straw significantly increased enzymatic activities involved in carbon degradation, including α -glucosidase (by 100.9 %) and β -glucosidase (by 103.3 %). This is likely due to the high cellulose and hemicellulose content of straw (Liu et al., 2022), and the soluble organic

matter released during straw degradation effectively promotes the microbial growth (Jin et al., 2020). NBS–NM with biochar only slightly impacted the microbial traits (Figs. 3 and 4A). The reduced soil bulk density (Table S2) may provide an aerobic environment for microbial growth (Gul et al., 2015), but the recalcitrant carbon in biochar (Li et al., 2020; Luo et al., 2023) is less utilized by microorganisms. Additionally, changes in soil pH under NBS–NM with straw can also influence microbial biomass and enzymatic activities (Malik et al., 2018). The significant neutralization in soil pH under NBS–NM with straw (Table S2), coupled with the increased availability of phosphorus, facilitates the microbial survival (Malik et al., 2018). In contrast, other NBS–NM practices little impacted soil pH. Changes in microbial biomass carbon in aggregates under NBS–NM with chicken manure, carbon-based material derived from kitchen waste, and straw led to substantial increases in soil respiration rates, whereas NBS–NM with biochar exhibited relatively low rates of soil respiration (Fig. 4K–L). This may be due to the easily decomposable glucose and sucrose in the solid waste of NMS–NM, whereas organic compounds with a more complex structure are rich in biochar (Tian et al., 2019).

The smaller carbon increment in macroaggregates under NBS–NM with straw can primarily be attributed to the faster decomposition rate of straw (Fig. 4) (Huang et al., 2018). The accelerated decomposition rate reduces SOC storage (Ekschmitt et al., 2005). In addition, the decline in water-stable aggregates under NBS–NM with straw (Fig. 2F) hinders SOC storage at the aggregate levels, given the positive relationship between SOC and aggregate stability (Mustafa et al., 2020). The accrual of SOC under NBS–NM is predominantly observed in microaggregates (Fig. 5C and F), indicating that microaggregates are crucial for SOC storage (Fig. 3) (Yao et al., 2024). Microaggregates exhibit higher resistance to disturbances (Thomaz et al., 2022) due to the stronger internal interactions (Totsche et al., 2018), facilitating the long-term reserve of SOC. The relatively minor change in SOC observed in microaggregates, coupled with the higher decomposability of aggregate SOC (Fig. 5), suggests that the SOC sequestration capacity in microaggregates is limited under NBS–NM with straw.

4.2. Discernable differences in carbon increment in bulk soil upon nature-based nutrient management in croplands

This study revealed the changes in SOC in response to different NBS–NM practices in croplands (Fig. 1). The NBS–NM has been proven to increase SOC in topsoil (Wang et al., 2024), with an average increase of 2–3.5 g carbon kg⁻¹ across croplands. However, the effectiveness of SOC improvement varies depending on the specific nutrient management practice employed (Han et al., 2016; Wang et al., 2024). NBS–NM with biochar resulted in a 5.65 g carbon kg⁻¹ increase in SOC, while the SOC increment ranged from 1.81 to 2.65 g carbon kg⁻¹ with the addition of other organic wastes (Fig. 1). NBS–NM with biochar improved SOC by 9.6–23.1 % more than NBS–NM with chicken manure (Chen et al., 2023). In contrast, the increase in SOC was lower under the NBS–NM with chicken manure and carbon-based material derived from kitchen waste (Fig. 1A–B).

The disparity in SOC improvement across the four NBS–NM practices, with the most pronounced improvement observed in NBS–NM with biochar and least pronounced in NBS–NM with straw (Fig. 1), can be attributed to several reasons. The carbon-based materials presented in different NBS–NM exhibit varying degrees of decomposability (Baldock et al., 1997). Biochar, containing high levels of recalcitrant carbon such as polycyclic aromatic structures (Li et al., 2020; Luo et al., 2023), can remarkably accrue SOC due to its lower decomposability (Liu et al., 2021; Rasul et al., 2022). Isotopic labeling experiments have shown that biochar could preserve residues of exogenous ¹³C-glucose in soils, thereby effectively decreasing the mineralization rate of SOC (Kalu et al., 2024). In contrast, straw contains abundant soluble substances with relatively high decomposability, such as polysaccharides and amino acids, making the carbon in straw more susceptible to rapid

decomposition or loss through leaching (Li et al., 2024). Additionally, differences in adsorption between the mineral matrix and organic materials also affect the effectiveness of SOC improvement across the NBS–NM practices (Kleber et al., 2021). Biochar can rapidly interact with the soil mineral matrix due to electrostatic attraction, which is apt to form an organometallic iron-organic carbon complex (Yang et al., 2016). Similarly, the organic carbon in chicken manure and carbon-based material derived from kitchen waste can also be bound by the soil mineral matrix to form organic-inorganic complexes (Yu et al., 2021), thereby promoting soil carbon sequestration. However, straw enrich with cellulose is not easily bound by soil minerals (Ruwoldt et al., 2023).

4.3. Implications and limitations

The enhancement of AOCS and SOC accrual varied among NBS–NM practices with equal organic carbon input, highlighting the fact that suitable NBS–NM can promote carbon sequestration efficiency. The AOCS framework serves as a crucial tool for selecting appropriate nutrient management practices in croplands. For instance, although NBS–NM with straw has the potential to enhance agricultural sustainability (Turmel et al., 2015), further research is needed to explore effective application techniques, such as deep-buried straw (Chen et al., 2017), to improve AOCS.

As with all research, this study has its limitations. The available evidence was insufficient to elucidate the role of nutrient forms in AOCS improvement across different aggregate sizes (e.g., macro-aggregate and micro-aggregate). Infrared spectral analysis revealed distinct effects of NBS–NM on soil carbon stability (Peltre et al., 2017), thus further investigation into how different nutrient forms influence AOCS improvement, particularly with respect to carbon structure analysis in aggregates, is warranted. Furthermore, soil respiration rate was only measured under laboratory incubation using undistributed soil column. While laboratory incubation is valuable for accurately measuring soil respiration rate (Bond-Lamberty et al., 2024), in situ measurements would more actually reflect the carbon output (Heimlich et al., 2024). Additionally, the microbial mechanisms underlying changes in AOCS remain unclear. Future research is needed to elucidate how the dynamics of microbial populations influence AOCS improvements, as soil microbial community structure, activity, and assembly mechanisms can significantly affect soil carbon cycling (Wu et al., 2024).

5. Conclusions

This study assessed the impact of four NBS–NM practices on AOCS, with an equal organic carbon input and nutrient application in a vegetable cropland. NBS–NM with straw mildly improved AOCS, with a slight decrease in aggregate stability. The NBS–NM with straw resulted in an increase in microbial biomass and enzyme activities in aggregates, likely due to the soluble organic matter released during straw degradation, which effectively promotes the microbial growth. Consequently, NBS–NM with straw induced a significant increase in soil respiration rates (by 80.0–148.9 %), leaving minor accrual of aggregate SOC. The relatively small change in SOC observed in microaggregates, given that the microaggregates can facilitate the long-term reserve of SOC, suggests that SOC sequestration capacity is limited under NBS–NM with straw. Conversely, NBS–NM with biochar led to a significant enhancement in AOCS, which can be attributed to the high levels of recalcitrant carbon in biochar, such as polycyclic aromatic structures. Therefore, NBS–NM with biochar comparatively facilitates the long-term SOC sequestration. The disparities in the effectiveness of aggregate SOC sequestration under different NBS–NM highlight the importance of selecting appropriate NBS–NM practices to attain 'carbon neutrality' in croplands.

CRediT authorship contribution statement

simon willcock: Writing – review & editing. **jonathan storkey:** Writing – review & editing. **xunzhuo dong:** Validation, Supervision, Data curation. **yunyao zhong:** Validation, Supervision, Data curation. **xiaozhong wang:** Conceptualization. **yan deng:** Conceptualization. **wei zhang:** Conceptualization. **qirui li:** Writing – review & editing. **xinping chen:** Conceptualization. **yini wang:** Writing – original draft, Visualization, Project administration, Investigation, Formal analysis, Data curation. **Zhaolei Li:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **yanzhong yao:** Validation, Supervision, Formal analysis, Data curation. **bingbing han:** Validation, Supervision, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflict of interest

The authors declare that there is no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2024.109467](https://doi.org/10.1016/j.agee.2024.109467).

Data availability

Data will be made available on request.

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