

Influence of straw, compost, and biochar on soil carbon and aggregates in Chernozem

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Abstract: Crop residue management is a major concern in agricultural ecosystems. These residues can be recycled into biochar and compost to efficiently promote soil organic carbon (SOC) storage in farmlands. However, the influences of straw and its derived materials on SOC (especially on humus fractions) in soil aggregates of varying sizes are largely unknown. To understand these effects, a nine-year field experiment was conducted on calcareous black soil, including five treatments: CK – no fertiliser; NPK – mineral nitrogen, phosphorus, and potassium fertiliser; NPKS – NPK + straw; NPKC – NPK + compost, and NPKB – NPK + biochar. Compared to CK and NPK, the NPKS and NPKC treatments resulted in a noticeable rise ($P < 0.05$) in the proportion of aggregates with > 0.25 mm size ($R_{0.25}$), as well as in the mean weight diameter and geometric mean diameter at 0–20 cm depth. The NPKS, NPKC, and NPKB treatments significantly ($P < 0.05$) increased the contents of large macroaggregates (> 2 mm), small macroaggregates (2–0.25 mm), microaggregates (0.25–0.053 mm), and non-aggregates in the 0–20 cm soil layer, as well as the levels of SOC, humic acid carbon (HAC) and humin carbon (HUC). These treatments also significantly ($P < 0.05$) enhanced organic carbon storage in the topsoil (0–20 cm). The effects were more pronounced after NPKB treatment relative to NPKS. Compared to CK, the application of mineral fertilisers alone and combined with organic materials significantly ($P < 0.05$) improved crop yields. The study's results indicate that the application of organic materials from corn significantly ($P < 0.05$) enhanced both soil quality and corn yield, with straw-derived biochar showing better effects on soil carbon sequestration.

Keywords: terrestrial ecosystem; soil stability; wet sieving; structural equation model

Rapid advances in various industries, accompanied by increased human activities, such as fossil fuel combustion, land-use change, and agricultural practices, have resulted in higher emission of greenhouse gases, which have further caused a rise in global surface temperature by 1.1 °C compared to

the pre-industrialisation (IPCC 2022). Among the carbon reservoirs in terrestrial ecosystems, soil is the largest and most active one. However, the soil is highly susceptible to environmental fluctuations and anthropogenic interferences, with small changes leading to a great impact on global climate (Yan et

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al. 2021). Soil can play a crucial role in stabilising the climate, with a great potential for carbon sequestration in agricultural soils (Amelung et al. 2020). Therefore, reducing greenhouse gas emissions and increasing carbon accumulation in agricultural lands are important strategies to ensure food security worldwide and cope with global climate change.

Aggregates are basic structural units of soil (Sun et al. 2020). Based on size and morphology, soil aggregates are broadly classified into microaggregates (< 0.25 mm) and macroaggregates (> 0.25 mm), with further categorisation into four groups with particle sizes of > 2 mm, $2-0.25$ mm, $0.25-0.053$ mm, and < 0.053 mm. There are close interactions between soil aggregates and soil organic carbon (SOC). SOC serves as a reinforcing substance during aggregates' formation and influences the structural stability and distribution of the particle sizes of aggregates (Six et al. 2000). On the other hand, aggregates protect SOC physically, chemically, and biologically, thereby improving the stability of SOC (Xu et al. 2020). Therefore, evaluating the SOC content in the aggregates of varying particle sizes can provide crucial insights into the physical protection mechanism of SOC (Liu et al. 2019).

The topsoil layer usually has a high humus content. It is a non-homogeneous macromolecular organic matter that exists in various natural environments (Ghabbour and Davies 2014). The key components of soil humus are humic acid (HA), fulvic acid (FA), and humin (HU). These components form the most stable fraction of SOC, highly susceptible to microbial degradation (Dou et al. 2020). Because of their stability, they play a crucial role in stabilising and sequestering carbon in soil (Loke et al. 2018). Humus also plays an important role in forming and stabilising soil aggregates (Tisdall and Oades 1982). Humus promotes the formation of porous, looser, and larger aggregates, allowing the soil to resist problems like soil compaction. It improves soil structure stability by influencing the interactions between the reinforcing substances and binding mineral particles to loose aggregates. Thus, humus promotes the formation of porous water-stable aggregates with stable structures. Therefore, comprehensive knowledge of the humus fraction of soil aggregates could be useful for developing strategies for effective carbon management and sustainable agriculture.

According to the National Bureau of Statistics (NBS) in China, corn stover is a major agricultural by-product in Northeast China, with an average yearly production

of about 98 billion kilograms. This easily accessible and highly abundant resource can be utilised as an organic fertiliser in this region, as returning crop straw to farmland soil is the most direct soil fertilisation method. As a fertiliser, straw can improve the soil structure, increase the levels of nutrients in the soil, and ultimately improve the quality of soil (Xu et al. 2019). In contrast, the extremely cold climate in Northeast China and less-than-optimal decomposition of straw returned to the field may often affect the emergence of new crops. Furthermore, it also aggravates the infestation of pests, diseases, and weeds in the field. To meet the growing demands of corn yield without compromising quality, various organic fertilisers have widely amended agricultural soils, including straw and its derivatives (Qian et al. 2024). Preparing compost from crop residues and using it as a soil fertiliser is an effective alternative to stover input (Gondek et al. 2018). The conditions during the thermophilic stage of straw mineralisation and humification can reduce the incidence of plant diseases (Wang et al. 2018). Additionally, the higher levels of humification may promote organic carbon accumulation at levels greater than those of corn straw (Diacono and Montemurro 2012). Studies over the past few decades have shown that the incorporation of biochar produced from pyrolysed crop residues in soil is more effective in SOC sequestration relative to their feedstock materials (Lu et al. 2021, Wang et al. 2023). Additionally, biochar can improve the physicochemical characteristics of soil, reduce soil erosion, and improve soil fertility, thereby increasing crop yield (Liu et al. 2020, Luo et al. 2020, Li et al. 2021). Due to their multifunctionality and great potential for environmental and agricultural applications, compost and biochar are used for carbon input in agricultural lands. However, the applicability of these materials is still uncertain in some specific areas due to climate conditions and farming practices (Stubbs et al. 2023). Therefore, this study aims to explore the effects of the application of corn stover, compost, and its biochar for six consecutive years on (1) the particle size distribution and water stability of soil aggregates and (2) SOC, stabilised SOC, and destabilised SOC contents in the water-stable soil aggregates of varying size. The study is based on the hypothesis that corn stover, compost, and biochar would significantly influence the stability of aggregates and the proportions of humus fraction. Those influences would vary depending on the composition of amendments.

MATERIAL AND METHODS

Field experiment and sample collection. A field experiment was established in April 2015 in Harahai, Jilin, China (44°33'21.8"N, 125°10'44.6"E). The region's climate is temperate continental monsoon. The area receives a mean yearly precipitation and temperature of 507.7 mm and 4.7 °C, respectively, with a no-frost period of 144 days and an effective cumulative temperature of 2 800 °C. According to the World Reference Base for Soil Resources (IUSS Working Group WRB 2015), the soil is classified as Chernozem.

Corn stover and pig manure were mixed at a 4:1 ratio (fresh weight), and this mixture was composted at 50–60 °C, maintaining a 40% moisture level (v/w). To prepare biochar, straw was slowly pyrolysed at 450 °C. Table 1 shows the characteristics of untreated soil (0–20 cm depth), straw, compost, and biochar.

Based on a randomised block design, the experiment was conducted by setting up five treatment groups, with three replicates (104 square meters per experimental plot): CK – no fertiliser; NPK – mineral nitrogen, phosphorus, and potassium fertiliser; NPKS – NPK + straw; NPKC – NPK + compost, and NPKB – NPK + biochar. N, P, and K fertilisers were applied to the soil at annual rates of 225.0 kg N/ha, 39.24 kg P/ha, and 74.7 kg K/ha, respectively. The organic fertilisers (i.e., straw, compost, and biochar) were incorporated into the soil at an equivalent carbon application rate of 3 200 kg C/ha (equivalent to

the carbon content of corn straw biomass per unit area). The application rates included a straw input of 7 511.74 kg/ha (with a nitrogen input of 55.2 kg/ha), a compost input of 10 884.35 kg/ha (with a nitrogen input of 181.02 kg/ha), and a biochar input of 5 513.54 kg/ha (with a nitrogen input of 71.68 kg/ha). Around 30% of N fertiliser was applied as basal fertiliser, while the rest was added to the soil as topdressing between early to mid-June. Mineral P and K fertilisers were used as basal fertilisers. The field had a monoculture cropping system of maize (*Zea mays* L., cv. Fumin 985), sown in late April at a density of 60 000 seeds per hectare. Corn was harvested in early October. After harvesting, crop residues were removed from all plots (except for NPKS plots). Straw and biochar were applied at a soil depth of 15 cm by rotary tillage.

After crop harvest, soil samples were collected in October 2023 from two soil depths: 0~10 cm and 10~20 cm. The five-point method was used for sampling soil from replicate plots. After collection, soil samples collected from the same plot were mixed. After sieved through a 10 mm sieve, fresh soil samples were air-dried. A portion of each sample was used to separate soil aggregates of different sizes, while another portion was sieved through a 2 mm sieve and used to analyse soil characteristics. While collecting soil samples, samples of undisturbed soil with a volume of 100 cm³ were taken in layers using a ring knife to measure the soil bulk density.

Table 1. Soil, maize straw (MS), maize straw and pig manure co-compost (MSC), and maize straw biochar (MSB) properties in Hala Hai, Jilin Province, China, in 2015

	Soil	MS	MSC	MSB
pH	8.10 ± 0.36	6.06 ± 0.10	8.22 ± 0.15	9.41 ± 0.22
SOC (g/kg)	13.0 ± 0.70	nd	nd	nd
TC (g/kg)	nd	426.01 ± 5.45	294.01 ± 6.04	580.41 ± 15.87
TN (g/kg)	1.22 ± 0.13	7.42 ± 0.19	16.61 ± 0.57	13.53 ± 0.44
TP (g/kg)	0.56 ± 0.03	0.42 ± 0.03	5.06 ± 0.32	2.81 ± 0.22
TK (g/kg)	20.0 ± 0.15	2.63 ± 0.22	26.63 ± 1.04	21.2 ± 0.71
AN (mg/kg)	103.84 ± 5.07	nd	nd	nd
AP (mg/kg)	12.90 ± 0.90	nd	nd	nd
AK (mg/kg)	129.25 ± 2.39	nd	nd	nd
CEC (cmol/kg)	13.72 ± 0.41	nd	nd	nd
CO ₃ ²⁻ (cmol/kg)	0.06 ± 0.01	nd	nd	nd

SOC – soil organic carbon; TC – total carbon; TN – total nitrogen; TP – total phosphorus; TK – total potassium; AN – available nitrogen; AP – available phosphorus; AK – available potassium; CEC – cation exchange capacity; CO₃²⁻ – carbonate; nd – not determined

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During 2015–2023, the grain yield of maize was determined to have a 14% water content, as shown by the manual harvesting of each plot with an area of 13 m².

Soil analysis

Separation of soil aggregates based on their sizes. Aggregates of varying sizes, including large macroaggregate (> 2 mm), small macroaggregate (2~0.25 mm), microaggregate (0.25~0.053 mm), and silt + clay (< 0.053 mm), were obtained by employing the wet sieving method (Cambardella and Elliott 1993).

Extraction of humus fractions of soil. The procedures described in the former studies (Zhang et al. 2010, Wang et al. 2016) were followed to extract the fractions of HA, FA, and HU. Whole soil, as well as aggregated soil, was weighed (5 g) and added to a centrifuge tube. Subsequently, distilled water was added to the soil. After shaking it 1 h, the mixture was centrifuged to transfer the water-soluble substances. A mixture of 0.1 mol/L NaOH and Na₄P₂O₇ (pH = 13) was used to extract HA. To separate HA and FA using 6 mol/L HCl. The remaining residue in the centrifuge tube was HU.

Determination of organic and humic C fractions. Organic and humic C fractions were quantified using potassium dichromate through oxidative external heating (Clapp et al. 2003). A 0.5 g sample of soil agglomerates and whole soil were taken in a triangular flask (150 mL), and then potassium dichromate (0.4 mol/L) was added into the flask. The mixture was heated on a hot plate for 5 min at 180 °C. Afterwards, the mixture was cooled down and diluted using distilled water. The diluted solution was titrated with 0.1 mol/L ferrous sulfate to measure the contents, using O-phenanthroline as an indicator.

Calculation and statistical analysis

External carbon input. The external plant carbon in the soil is primarily derived from straw, root residues, and rhizosphere deposition. The root residue biomass has been calculated to form 23% of the straw biomass (dry weight) and the carbon mass fraction in the straw and root residues was estimated at 40% (Kong et al. 2005). The total amount of carbon derived from rhizosphere deposition equals the carbon contents of mature root residues (Bolinder et al. 1999).

Calculation of SOC storage and sequestration. SOC stock, the amount of sequestered carbon

(ΔSOC stock) and the annual rate of SOC sequestered (SOC_{SR}) was calculated as follows:

$$\text{SOC}_{\text{stock}} = \sum_{i=1}^n (\text{SOC}_i \cdot \text{BD}_i \cdot H_i) \times 10 \quad (1)$$

$$\Delta \text{SOC}_{\text{stock}} = \text{SOC}_{\text{stock-treatment}} - \text{SOC}_{\text{stock-initial}} \quad (2)$$

$$\text{SOC}_{\text{SR}} = \Delta \text{SOC}_{\text{stock}} / \text{year} \quad (3)$$

where: SOC_{stock} – SOC stocks (t/ha); SOC_i – carbon concentration (g/kg); BD_i – bulk density (g/cm³) at each depth; H_i – soil depth (cm); SOC_{stock-treatment} – SOC stock under each treatment in 2023; SOC_{stock-initial} – SOC stock in 2015.

The stability of soil aggregate is denoted by R_{0.25} (for aggregates > 0.25 mm), mean weight diameter (MWD, mm), and geometric mean diameter (GMD, mm). These parameters were calculated as follows:

$$R_{0.25} = 100 \frac{\sum_{i=3}^n w_i}{w} \quad (4)$$

$$\text{GMD} = \exp \left(\frac{\log d_i \sum_{i=1}^n \frac{w_i}{w}}{\sum_{i=1}^n \frac{w_i}{w}} \right) \quad (5)$$

$$\text{MWD} = \frac{\sum_{i=1}^n d_i w_i}{W} \quad (6)$$

where: d_i (mm) – average diameter of the *i*th size fraction of the aggregate; w_i (g) – weight of *i*th size fraction of the aggregate; w (g) – total weight of all size fractions of the aggregate; *i* = 1, 2, ..., 4 – aggregate size > 2, 2~0.25, 0.25~0.053, and < 0.053 mm, respectively.

Experimental data was processed and presented as graphs using Origin (Northampton, USA). SPSS 26.0 software (SPSS Inc., Chicago, USA) was used for statistical analyses. Significance analysis was performed using the least significant difference (LSD) method, and differences with *P* < 0.05 were considered significant. AMOS 24.0 software (version 26.0; IBM, Chicago, USA) was used to perform structural equation modelling (SEM) for identifying the causal connections between SOC, aggregate stability, and the organic and humus carbon fractions in soil and aggregates under the five treatments. Probability (*P*), root mean square error of approximation (RMSEA), chi-square test (χ²/df), and goodness-of-fit index (GFI) were utilised to evaluate the fitness of the final model.

RESULTS

Maize yield. The variations in corn yield after different treatments between the years are shown in Table 2. In the first year (2015), no significant (*P* < 0.05) variations in yield were observed among

Table 2. Effect of different treatments on grain yield (t/ha) in Hala Hai City, Jilin Province (2015–2023)

Treatment	CK	NPK	NPKS	NPKC	NPKB
2015	7.4 ± 0.72 ^a	9.52 ± 2.38 ^a	9.32 ± 1.26 ^a	9.48 ± 0.96 ^a	8.60 ± 1.26 ^a
2016	4.89 ± 0.78 ^c	10.06 ± 1.97 ^b	10.77 ± 0.91 ^{ab}	12.8 ± 0.25 ^a	12.00 ± 1.87 ^{ab}
2017	5.01 ± 0.72 ^b	9.82 ± 0.32 ^a	10.25 ± 0.66 ^a	10.60 ± 0.54 ^a	10.24 ± 0.56 ^a
2018	4.87 ± 0.29 ^b	7.25 ± 2.16 ^{ab}	9.55 ± 0.91 ^a	9.24 ± 0.70 ^a	7.17 ± 2.94 ^{ab}
2019	3.98 ± 0.23 ^c	9.66 ± 1.04 ^b	9.82 ± 0.72 ^{ab}	11.34 ± 0.56 ^a	10.13 ± 1.16 ^{ab}
2020	4.09 ± 0.88 ^b	9.76 ± 2.74 ^a	11.33 ± 0.92 ^a	11.53 ± 0.76 ^a	10.56 ± 1.28 ^a
2021	5.26 ± 0.16 ^b	13.37 ± 0.09 ^a	13.48 ± 1.57 ^a	14.09 ± 0.18 ^a	13.10 ± 0.62 ^a
2022	5.38 ± 0.17 ^c	10.07 ± 0.26 ^{bc}	11.15 ± 1.19 ^{ab}	11.78 ± 0.09 ^a	9.66 ± 1.16 ^{bc}
2023	4.67 ± 0.17 ^b	9.66 ± 1.60 ^a	10.65 ± 1.10 ^a	10.40 ± 1.17 ^a	9.81 ± 1.05 ^a
Average grain yield	5.06 ± 0.34 ^b	9.91 ± 0.55 ^a	10.70 ± 0.80 ^a	11.25 ± 0.44 ^a	10.14 ± 1.11 ^a

All values are expressed as mean ± standard deviation ($n = 3$). Different letters mean significant differences between treatments in each soil depth ($P < 0.05$; least significant difference (*LSD*) test). CK – no fertiliser; NPK – mineral fertiliser; NPKS – mineral fertiliser + maize straw; NPKC – mineral fertiliser + compost; NPKB – mineral fertiliser + biochar

the different treatments. Over nine years, the average annual yields for the different treatments were ranked as NPKC > NPKS > NPKB > NPK > CK. Different treatments all significantly ($P < 0.05$) increased grain yield. The yearly average grain yield after treatment with NPK, NPKS, NPKC, and NPKB increased by 95.8, 111.5, 122.3, and 100.4%, respectively, compared to the control treatment.

SOC sequestration. The annual average carbon input from corn straw organic materials returned to the field between 2015 and 2023 is shown in Table 3. The sources of the external carbon input primarily included root systems, rhizosphere deposition, and input from organic fertiliser carbon. Compared with the CK treatment, the carbon input increased significantly after NPK treatment ($P < 0.05$). The addition of organic materials led to marked increases in the total carbon input. Compared with the NPK treatment, the carbon input in the NPKS, NPKC, and

NPKB treatments increased 2.08-, 2.24-, and 2.09-fold, respectively, after the addition of organic materials.

The results revealed the significant impact of treatments on SOC content (Table 4). In both soil layers, SOC content across the treatment groups was in the following order: NPKB > NPKC > NPKS > NPK > CK. At 0–20 cm soil depth, SOC contents were significantly ($P < 0.05$) higher in NPKS, NPKC, and NPKB groups than in CK and NPK. Furthermore, SOC content in the NPKB group was substantially ($P < 0.05$) higher than that in NPKS.

The SOC stock in the topsoil (0–20 cm) across all treatments ranged from 37.01 to 52.58 t/ha, ranked in the order of NPK + BR > NPK + CP > NPK + ST > NPK > CK (Table 4). Compared to the topsoil SOC stock in 2015, continuous application of NPK had no significant ($P < 0.05$) effect on the SOC stock, while continuous application of NPKS, NPKC, and NPKB significantly ($P < 0.05$) enhanced the SOC stock in the

Table 3. Annual average carbon input (t/ha, 2015–2023) under different treatments in Hala Hai, Jilin Province

Treatment	Straw biomass	Root		Rhizodeposition carbon	Total carbon input
		biomass	carbon input		
CK	6.61 ± 1.36 ^b	1.52 ± 0.31 ^b	0.61 ± 0.13 ^b	0.61 ± 0.13 ^b	3.86 ± 0.80 ^c
NPK	8.54 ± 0.27 ^a	1.96 ± 0.06 ^a	0.79 ± 0.02 ^a	0.79 ± 0.02 ^a	4.99 ± 0.16 ^b
NPKS	8.29 ± 0.48 ^a	1.91 ± 0.11 ^a	0.76 ± 0.04 ^a	0.76 ± 0.04 ^a	8.04 ± 0.28 ^a
NPKC	9.35 ± 0.17 ^a	2.15 ± 0.04 ^a	0.86 ± 0.02 ^a	0.86 ± 0.02 ^a	8.66 ± 0.10 ^a
NPKB	8.35 ± 0.42 ^a	1.92 ± 0.10 ^a	0.77 ± 0.04 ^a	0.77 ± 0.04 ^a	8.08 ± 0.25 ^a

All values are expressed as mean ± standard deviation ($n = 3$). Different letters mean significant differences between treatments in each soil depth ($P < 0.05$; least significant difference (*LSD*) test). CK – no fertiliser; NPK – mineral fertiliser; NPKS – mineral fertiliser + maize straw; NPKC – mineral fertiliser + compost; NPKB – mineral fertiliser + biochar

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Table 4. Effects of applying mineral nitrogen, phosphorus, and potassium fertiliser (NPK); NPK + straw (NPKS); NPK + compost (NPKC); NPK + biochar (NPKB), and no fertiliser (CK) for 9 consecutive years (2015–2023) on the soil organic carbon (SOC) content, SOC_{stock}, and carbon sequestration rates in the topsoil (0–20 cm) in Hala Hai City, Jilin Province, China

Item	Soil (depth/cm)	Initial soil	CK	NPK	NPKS	NPKC	NPKB
SOC content (g/kg)	0–10	12.98 ± 0.70	13.01 ± 1.28 ^c	14.29 ± 0.38 ^c	17.68 ± 0.40 ^b	18.89 ± 1.76 ^{ab}	20.07 ± 0.13 ^a
	10–20		12.54 ± 1.02 ^c	12.95 ± 0.45 ^c	16.69 ± 0.13 ^b	18.06 ± 1.78 ^{ab}	19.81 ± 0.66 ^a
Bulk density (g/cm ³)	0–10	1.41 ± 0.03	1.43 ± 0.03 ^a	1.43 ± 0.02 ^a	1.33 ± 0.03 ^b	1.35 ± 0.02 ^b	1.34 ± 0.02 ^b
	10–20		1.47 ± 0.03 ^a	1.44 ± 0.03 ^a	1.32 ± 0.02 ^b	1.29 ± 0.03 ^b	1.30 ± 0.02 ^b
SOC _{stock} (t/ha)	0–10	36.52 ± 2.20	18.55 ± 1.63 ^c	20.39 ± 0.51 ^c	23.46 ± 0.75 ^b	25.47 ± 2.69 ^{ab}	26.90 ± 0.25 ^a
	10–20		18.46 ± 1.43 ^c	18.61 ± 0.90 ^c	22.03 ± 0.34 ^b	22.31 ± 2.59 ^{ab}	25.68 ± 0.60 ^a
Sequestered carbon (t/ha)	0–20		0.49 ± 3.06 ^c	2.47 ± 1.38 ^c	8.97 ± 1.06 ^b	12.26 ± 5.28 ^{ab}	16.06 ± 0.35 ^a
Carbon sequestration rate (t/ha)	0–20		0.05 ± 0.34 ^c	0.27 ± 0.15 ^c	1.00 ± 0.12 ^b	1.36 ± 0.59 ^{ab}	1.78 ± 0.04 ^a

All values are expressed as mean ± standard deviation ($n = 3$). Different letters mean significant difference between treatments in each soil depth ($P < 0.05$; least significant difference (*LSD*) test)

soil (0–20 cm). Compared with CK and NPK, NPKS, NPKC, and NPKB all markedly increased soil SOC stock, carbon sequestration, and the sequestration rate. Notably, after treatment with NPKB, the soil SOC stock, carbon sequestration, and sequestration rate were significantly ($P < 0.05$) higher than those following the NPKS treatment.

At 0–20 cm soil depth, humic acid carbon (HAC) and humin carbon (HUC) contents in the groups

were in the following order: NPKB > NPKC > NPKS > NPK > CK (Table 5), with HAC and HUC contents in NPKS, NPKC, and NPKB groups being significantly ($P < 0.05$) higher than those in CK and NPK. HAC and HUC contents in the NPKB group were remarkably ($P < 0.05$) higher than those in NPKS.

Aggregate stability and distribution of particle sizes.

At 0–10 cm depth, microaggregates (0.25–0.053 mm) were found to be dominant in the CK and NPK groups,

Table 5. Effects of mineral nitrogen, phosphorus, and potassium fertiliser (NPK); NPK + straw (NPKS); NPK + compost (NPKC); NPK + biochar (NPKB) and no fertiliser (CK) applications on humic contents in different soil layers over a 9-year period (2015–2023) in Hala Hai, Jilin Province, China

Soil depth (cm)	Treatment	HAC	FAC	HUC
		(g/kg)		
0–10	CK	2.08 ± 0.16 ^c	1.30 ± 0.08 ^a	9.28 ± 1.02 ^c
	NPK	2.10 ± 0.09 ^c	1.30 ± 0.10 ^a	10.50 ± 0.30 ^c
	NPKS	2.63 ± 0.10 ^b	1.14 ± 0.11 ^a	13.36 ± 0.43 ^b
	NPKC	2.84 ± 0.21 ^{ab}	1.15 ± 0.10 ^a	14.29 ± 1.48 ^{ab}
	NPKB	3.07 ± 0.06 ^a	1.19 ± 0.11 ^a	15.37 ± 0.16 ^a
10–20	CK	1.96 ± 0.08 ^c	1.33 ± 0.07 ^a	8.93 ± 1.17 ^c
	NPK	1.97 ± 0.02 ^c	1.35 ± 0.12 ^a	9.33 ± 0.56 ^c
	NPKS	2.54 ± 0.09 ^b	1.18 ± 0.13 ^a	12.46 ± 0.05 ^b
	NPKC	2.71 ± 0.23 ^{ab}	1.20 ± 0.05 ^a	13.57 ± 1.51 ^{ab}
	NPKB	2.91 ± 0.11 ^a	1.28 ± 0.08 ^a	15.24 ± 0.61 ^a

All values are expressed as mean ± standard deviation ($n = 3$). Different letters mean significant difference between treatments in each soil depth ($P < 0.05$; least significant difference (*LSD*) test); HAC – humic acid carbon; FAC – fulvic acid carbon; HUC – humin carbon

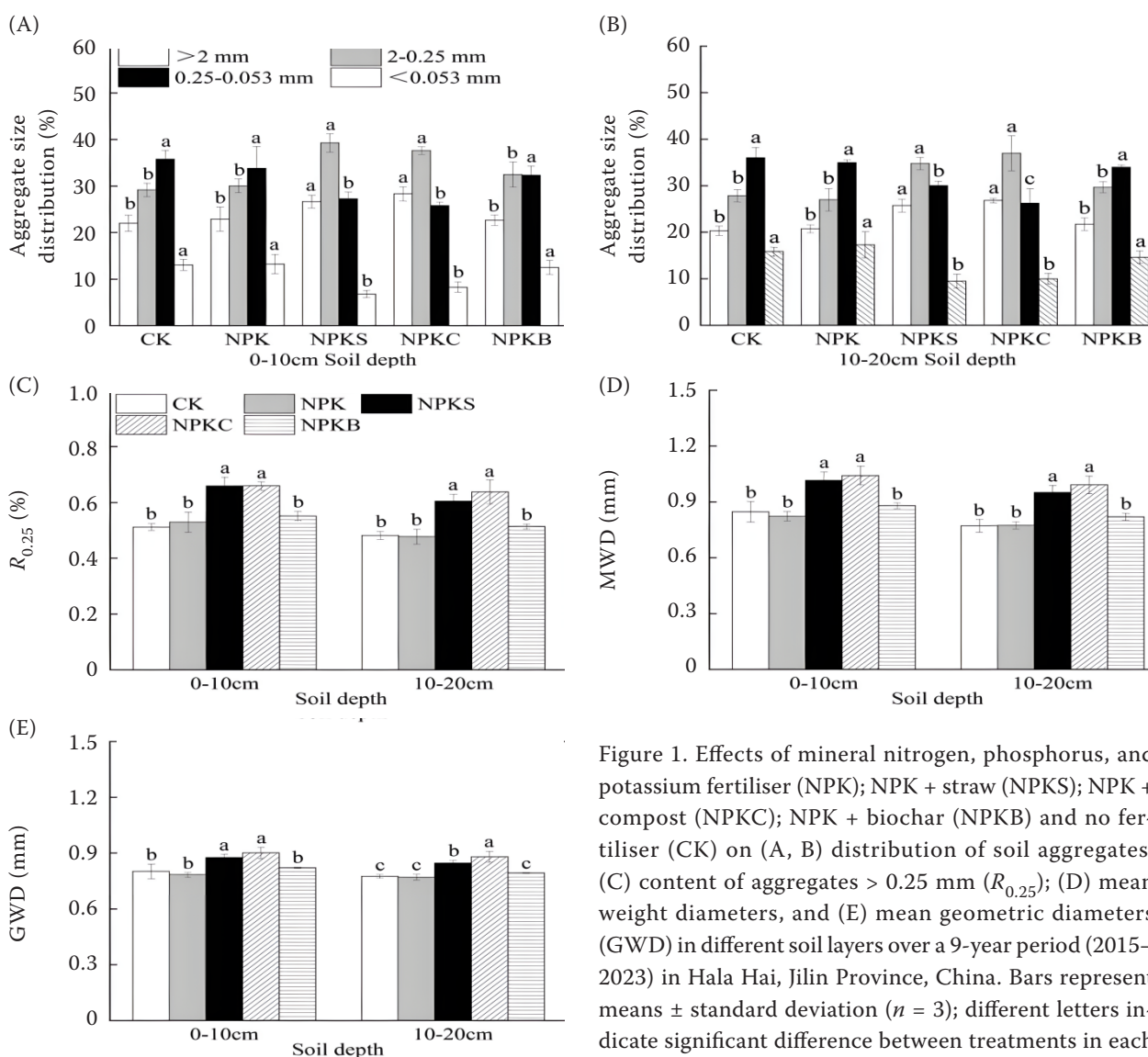


Figure 1. Effects of mineral nitrogen, phosphorus, and potassium fertiliser (NPK); NPK + straw (NPKS); NPK + compost (NPKC); NPK + biochar (NPKB) and no fertiliser (CK) on (A, B) distribution of soil aggregates; (C) content of aggregates > 0.25 mm ($R_{0.25}$); (D) mean weight diameters, and (E) mean geometric diameters (GWD) in different soil layers over a 9-year period (2015–2023) in Hala Hai, Jilin Province, China. Bars represent means $\pm$ standard deviation ($n = 3$); different letters indicate significant difference between treatments in each soil depth ($P < 0.05$; least significant difference (LSD) test)

with a 34~36% proportion (Figure 1A). Results revealed the impact of fertilisation on the size distribution of soil aggregates. Under NPKS, NPKC, and NPKB treatments, small macroaggregates were dominant, showing the highest proportion of 34~36% among all aggregates. At 10~20 cm depth, microaggregates were observed to be dominant in CK, NPK, and NPKB, accounting for 34~36% of aggregates. NPKS and NPKC treatments resulted in the highest percentage (35~37%) of small macroaggregates among all aggregates (Figure 1B).

NPK and NPKB treatments did not cause any significant variation in the proportions of soil aggregates of each size in both soil layers (Figure 1A, B). NPKS and NPKC treatments resulted in a noticeable rise ($P < 0.05$) in the proportions of small and large macroaggregates compared with CK, NPK, and NPKB. In

contrast, silt + clay and microaggregates proportions decreased significantly ($P < 0.05$). These changes in the proportions of soil water-stable aggregates of different sizes after NPKS and NPKC treatments further caused a substantial surge ($P < 0.05$) in the values of $R_{0.25}$ (Figure 1C), MWD (Figure 1D), and GMD of these aggregates at both soil depth (Figure 1E).

Changes in carbon fractions of water-stable agglomerates. In all treatments, SOC was mainly distributed in the aggregates with a grain size > 0.25 mm (Figure 2A, B). NPKS, NPKC, and NPKB treatments resulted in a significant rise ($P < 0.05$) in the SOC content of large and small macroaggregates, as well as microaggregates at 0~20 cm depth, compared to NPK and CK; moreover, SOC contents in large and small macroaggregates under NPKB treatment were significantly ($P < 0.05$) higher than

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those under NPKS. In addition, NPKB treatment caused a significant surge in the SOC content of silt and clay at 0–10 cm depth.

In all treatment groups, HAC, FAC, and HUC fractions were largely disseminated in aggregates with a grain size > 0.25 mm at 0–20 cm soil depth (Figure 2).

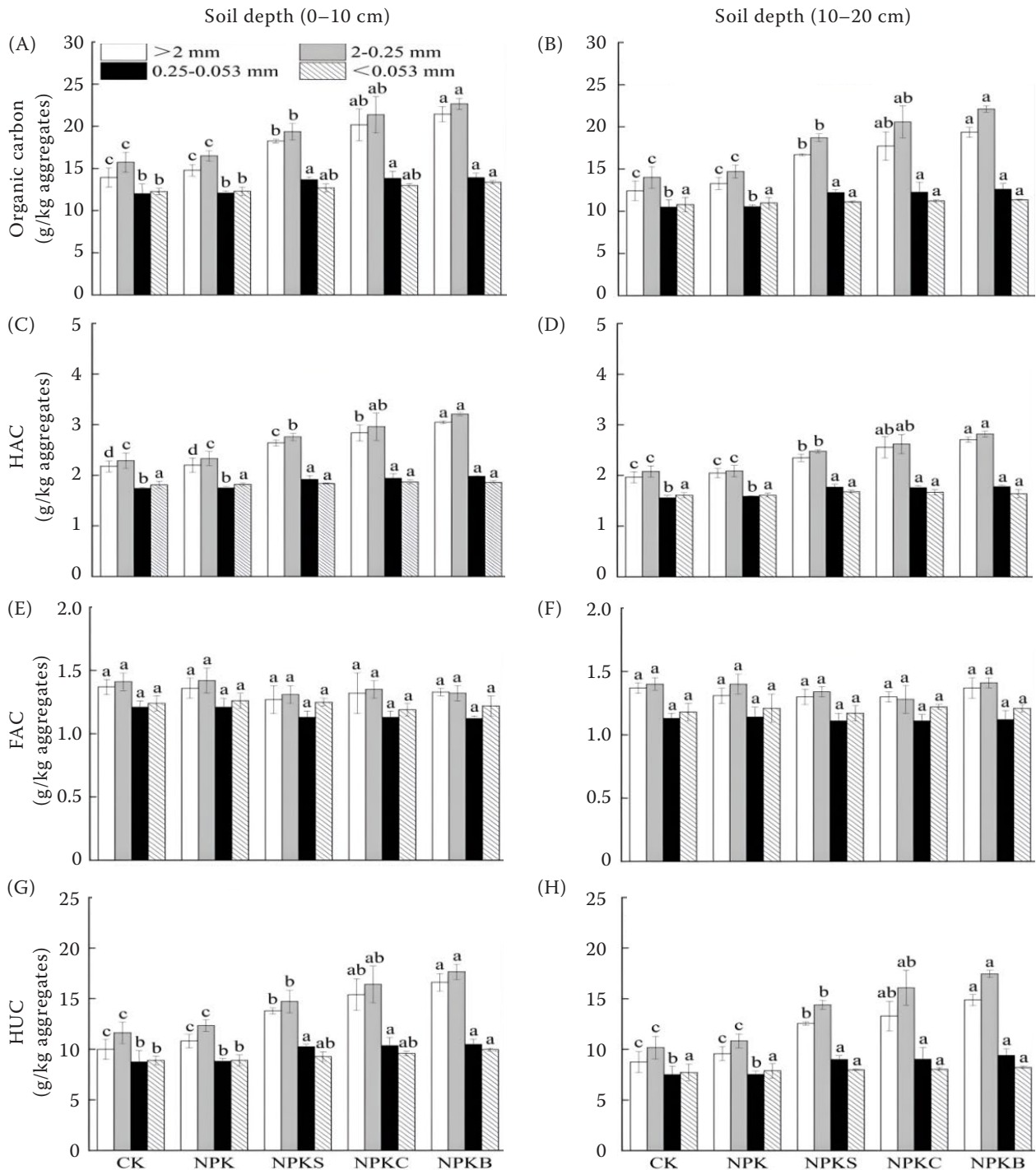


Figure 2. Effects of the application of mineral nitrogen, phosphorus, and potassium fertiliser (NPK); NPK + straw (NPKS); NPK + compost (NPKC); NPK + biochar (NPKB) and no fertiliser (CK) on (A, B) contents of organic carbon; (C, D) humic acid carbon (HAC); (E, F) fulvic acid carbon (FAC) and (G, H) humin carbon (HUC) in soil water-stable aggregates in different soil layers over a 9-year period (2015–2023) in Hala Hai, Jilin, China. Bars represent means $\pm$ standard deviation ($n = 3$); different letters indicate significant difference between treatments in each soil depth ($P < 0.05$; least significant difference (LSD) test)

NPKS, NPKC, and NPKB treatments resulted in a significant ($P < 0.05$) rise in the contents of HAC and HUC in large and small macroaggregates, as well as in microaggregates, compared to CK and NPK. HAC and HUC contents in large and small macroaggregates and microaggregates of the NPKB group were significantly ($P < 0.05$) higher than those in NPKS.

Correlations between SOC, humus concentrations, and stability of soil agglomerates. SEM

analysis (Figure 3) results showed that with NPKS treatment, the SOC content was most influenced by the soil humic content (0~10 cm, $\lambda = 0.910$; 10~20 cm, $\lambda = 0.786$), followed by aggregate stability (0~10 cm, $\lambda = 0.574$; 10~20 cm, $\lambda = 0.604$) and the SOC content in aggregates (0~10 cm, $\lambda = 0.546$; 10~20 cm, $\lambda = 0.477$). With the NPKC treatment, the SOC content was most strongly influenced by the soil humic content (0~10 cm, $\lambda = 0.955$; 10~20 cm, $\lambda = 0.955$),

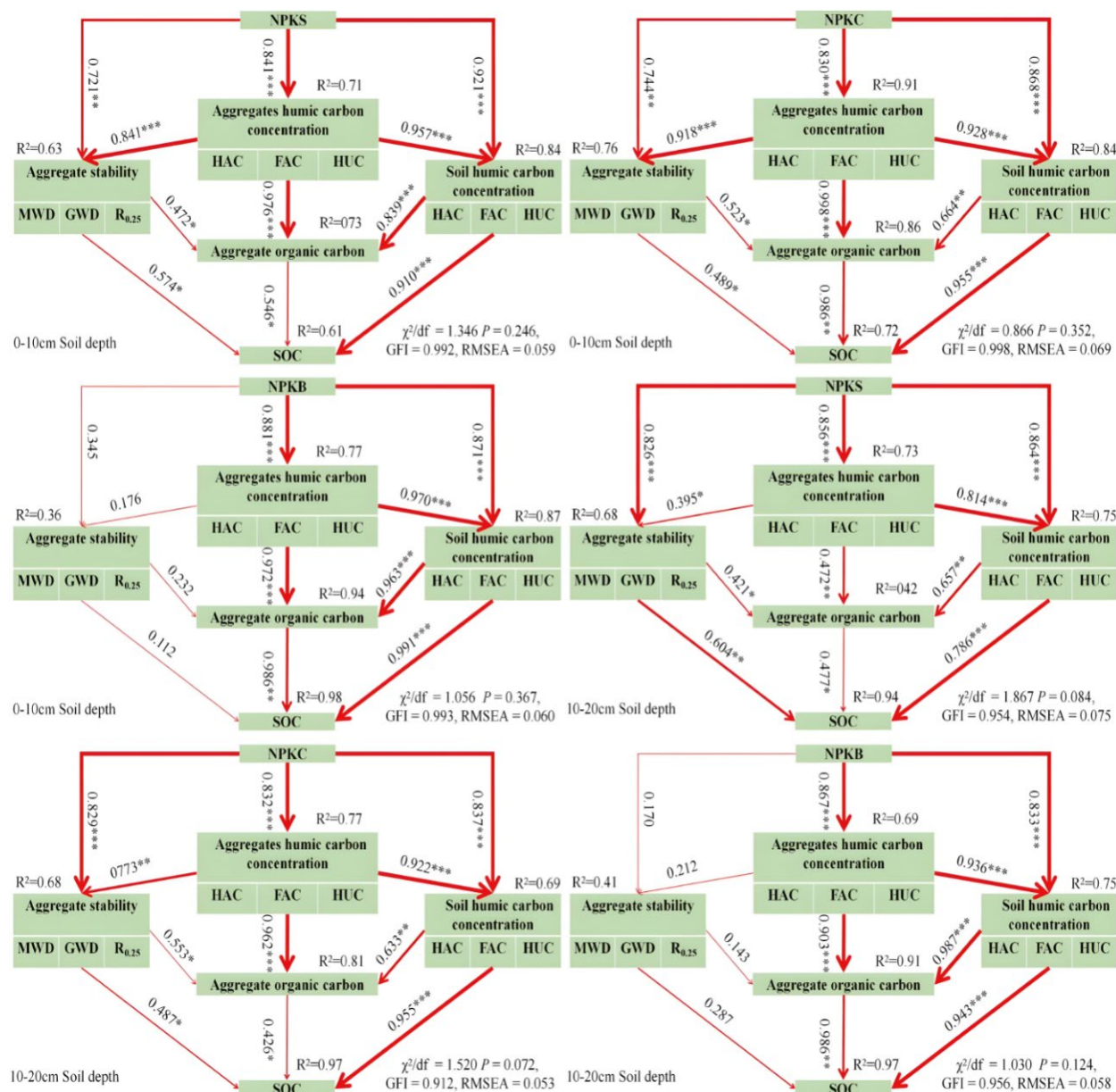


Figure 3. Structural equation model of causal relationships among aggregate humic carbon concentrations, aggregate stability, humic concentrations, humic concentrations and organic carbon concentrations, under maize straw (MS), and its biochar (MSB) amendment in each soil depths, over a 9-year period (2015–2023) in Hala Hai, Jilin Province, China. Numbers on arrows are standardised path coefficients. Arrow thickness represents the magnitude of standardised path coefficient. Significance levels are denoted with * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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followed by the SOC content in aggregates (0~10 cm, $\lambda = 0.986$; 10~20 cm, $\lambda = 0.426$) and aggregate stability (0~10 cm, $\lambda = 0.489$; 10~20 cm, $\lambda = 0.487$), while with the NPKB treatment, the SOC content was most influenced by soil humic content (0~10 cm, $\lambda = 0.991$; 10~20 cm, $\lambda = 0.943$), followed by the SOC content in the aggregates (0~10 cm, $\lambda = 0.986$; 10~20 cm, $\lambda = 0.986$). Furthermore, the humus content in aggregates also indirectly affected SOC content under the three treatments.

DISCUSSION

Effects of straw-derived carbon input on maize yield. In this study, it was found that the application of mineral fertilisers alone or in combination with organic materials significantly ($P < 0.05$) increased crop yields (Table 2). Bilgili et al. (2009) showed that long-term application of NPK increased crop yields by 2.3- to 4-fold compared to no fertilisation. Similarly, Zhang et al. (2019) found that combining mineral fertilisers with organic materials led to marked improvements in crop yields and biomass. Previous studies have indicated that applying mineral fertilisers together with organic fertilisers is more beneficial for increasing crop yields than applying NPK alone. However, the results of the present study indicated that compared to NPK, the combination of mineral and organic fertilisers only led to increased crop yields in some years. Crop yields are known to be strongly influenced by both natural and management factors, including climate, soil fertility, and management practices (Sun et al. 2016). This would explain the observation that corn yields under different organic treatments varied significantly across different years, and further research is needed to understand the long-term effects of these organic amendments and other factors and their interactions on corn yields.

Effects of straw-derived carbon input on SOC sequestration. In our study, organic matter input in CK and NPK treatments was primarily derived from root biomass (Table 3). Although the application of mineral fertiliser resulted in better crop growth, it did not lead to a significant ($P < 0.05$) increase in SOC stocks in the top layer of soil compared to CK or the baseline (Table 4). This may be because mineral fertiliser application alone is not enough to uphold the carbon levels in soil (Chen et al. 2010, Yuan et al. 2025). The addition of straw, compost, and biochar into soil resulted in a significant ($P < 0.05$)

rise in SOC stocks, HAC, and HUC contents, as compared to mineral fertilisation alone or no fertilisation. Straw has a high organic carbon content, and its application to the field provides a high amount of exogenous organic carbon to soil (Hao et al. 2022), leading to higher SOC stocks in the soil. In addition, straw serves as a nutrient source for soil microorganisms (Wu et al. 2019). The addition of straw leads to higher microbial activity and accelerated decomposition of straw in the soil, which further improves the ability of soil to accumulate the organic carbon resulting from straw decomposition (Spaccini et al. 2000, Guan et al. 2020). In this study, SOC stocks, HAC, and HUC contents in the NKPC group were higher than in the NKPS group, indicating that compost strongly influenced soil carbon fractions more than the straw. Diacono and Montemurro (2011) demonstrated that the humification process of compost is higher, which may be the main reason for higher SOC accumulation in soil compared to straw fertiliser. Furthermore, SOC stocks, HAC, and HUC increased further in the NPKB group. This may be due to the large amount of carbon in biochar, which causes a direct increase in the SOC concentration. Simultaneously, after entering the soil, aliphatic carbon portions of biochar are converted into substances such as HA, resulting in a rise in the proportions of humus fractions in the soil (Cheng and Lehmann 2009). Moreover, biochar has a high proportion of aromatic structures similar to soil humus (Guo et al. 2021), which are difficult to mineralise and decompose. Therefore, the application of biochar is more favourable for soil carbon sequestration.

Effects of straw-derived carbon input on particle-size distribution and stability of soil aggregates. $R_{0.25}$, MWD, and GMD serve as crucial indicators to assess the aggregate stability in soil (Sun et al. 2021). Higher values of MWD and GMD indicate more stable soil aggregates, with a relatively high percentage of large macroaggregates (Sheng et al. 2023). After the application of mineral fertilisers, there was no noticeable change in the distribution of particle sizes of soil aggregates across different layers (Figure 1A, B). This observation was consistent with the findings reported by Liang et al. (2021), and compared to CK and NPK treatments, NPKS and NPKC treatments resulted in significant increases ($P < 0.05$) in the proportion of soil macroaggregates as well as the MWD and GMD values. According to some previous studies, returning waste straw to the field is an effective and direct approach to increasing SOC levels in the soil.

Degradation of straw by soil microorganisms contributes to the generation of a large quantity of reinforcing substances, such as sugars, organic acids, and amino acids. These substances further promote the formation of soil macroaggregates from soil sand, clay, and soil microaggregates through agglomeration, leading to a rise in the values of $R_{0.25}$, MWD, and GMD (Zhao et al. 2020). There was no noticeable difference in the stability of aggregates in the NPKB, NPK, and CK groups. This study's results agreed with the previous findings (Heikkinen et al. 2019, Zhou et al. 2019, Liang et al. 2021). This may be because biochar contains high proportions of esters and aromatic compounds, which are not easily degradable by microbes. Thus, biochar addition leads to relatively less production of cementing substances due to the slow decomposition of aromatic organic compounds (Kuz'yakov et al. 2014).

Effects of straw-derived input on humus fractions and agglomeration of soil. In this study, SOC and humus fractions were mainly distributed in the aggregates with > 0.25 mm size (Figure 2), which was consistent with the results observed by Huang et al. (2017). This may be because large aggregates generally contain more fungal mycelia than the other small-size aggregates. The decomposition of mycelia leads to a higher concentration of organic carbon in bigger aggregates (Huang et al. 2017). Furthermore, the highly porous structure of bigger aggregates is conducive to oxygen and water flow, leading to improved microbial activity and metabolism. These facts explain the higher concentrations of humus and organic carbon in the aggregates with > 0.25 mm size (Figure 2). Furthermore, fertilisation also led to a significant rise in the proportion of soil aggregates with a grain size > 0.25 mm. However, as the aggregate size decreased, differences between the soil SOC concentrations across different treatments also decreased. This may be due to the higher occurrence of organic materials in large aggregates and the lowest in microaggregates (Yin et al. 2018). Large soil aggregates display better soil carbon capacity (Jastrow 1996). Another reason may be that small aggregates can bind fewer organic carbon compounds. Under the long-term application of fertilisers, the carbon pool of small soil aggregates can quickly reach saturation level. Previous studies also reported the sequential saturation of carbon pools from small to larger aggregates (Kool et al. 2007, Gulde et al. 2008). Thus, the effect of different fertilisers on SOC decreases with the decrease in the particle sizes of soil aggregates.

Underlying mechanisms governing the effects of different straw-derived fertilisers on SOC. SEM analysis demonstrated that straw, compost, and biochar contribute to carbon sequestration mainly by altering the concentration of humus fractions in soil (Figure 3). Humus accounts for more than half of soil organic matter and is crucial for sequestration and fixation of SOC (Wei et al. 2022, Zhang et al. 2022). In contrast, straw and compost can improve the sequestration of soil carbon sequestration by improving soil stability. This may be because straw or compost addition into soil results in higher activity of soil microbes, which leads to the generation of cementing substances required for aggregate formation (Zhao et al. 2020). In this study, biochar did not improve soil stability, and therefore, biochar cannot achieve soil carbon sequestration through physical protection. We hypothesised that this could be due to the relatively lower efficiency of biochar to promote biological activity in soil. A recent report has suggested that biochar may inhibit the growth of microbes due to its biochemical recalcitrance and toxicity (Chen et al. 2024). Microorganisms significantly influence the stability of aggregates and can promote aggregate formation by releasing extracellular polymers (Lin et al. 2018).

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