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Contrasting effects of maize straw and its biochar on aggregation and soil organic matter stabilization

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Abstract

Background and aims Crop straw and it derived biochar both increase soil fertility and stabilize soil structure. The contribution of these amendments to microbial biomass and aggregate formation, as well as carbon (C) and nitrogen (N) stabilization within aggregates is lacking.

Methods Maize straw that was labelled with ^{13}C and ^{15}N or its biochar (produced at 400 °C) were added to soil to quantify the incorporation of C and N into dissolved organic matter, microbial biomass, and aggregates throughout lab incubation.

Results After 20 days of incubation, 48% of microbial biomass C (MBC) and 18% of microbial biomass N (MBN) was derived from straw. In contrast, biochar contributed 4% to the MBC pool, with only a trace contribution to MBN. These contributions were reflected in the dissolved organic matter C and N pools. Soil aggregation (mean weight diameter) was 14% greater amended with straw than with biochar within the first 100 days, but without differences thereafter. Because the straw-derived binding agents for aggregation are short-lived, their effects on aggregate formation disappeared after 100 days resulting in a similar impact as that found in soil with biochar. Larger amounts of straw-derived C and N were occluded within aggregate compared to biochar.

Conclusion The amendments of soil with straw and biochar affected microbial processing, reflected in the uptake of C and N into microbial biomass, dissolved organic matter and aggregates. We conclude that straw has a short-term effect on aggregation while biochar has limited impact on aggregation.

Keywords: Maize straw return, Laboratory incubation, Aggregate formation and

stability, ^{13}C and ^{15}N methodology, Microbial biomass

Abbreviations: DOC: Dissolved organic carbon; TDN: Total dissolved nitrogen; MBC: Microbial biomass carbon; MBN: Microbial biomass nitrogen; C: Carbon; N: Nitrogen; SOM: Soil organic matter; MWD: Mean weight diameter; WHC: Water holding capacity.

1 Introduction

Plants and soil are interdependent, especially in agricultural ecosystems with relatively low biodiversity. Annual global plant straw production from cropping systems has been estimated at 3.12 billion ton (Li et al., 2018a). Crop plant straw contains considerable carbon (C), as well as nutrients such as nitrogen (N), phosphorus (P) and potassium (K) (Ji et al., 2022). Returning straw to soil can lift soil organic matter and nutrient content, improve soil structure, stimulate (micro)organisms, and thereby helps to sustain soil health (Fang et al., 2020). In the past several decades, opportunities to convert crop plant straw into biochar prior to application to soil have been proposed (Sohi et al., 2010; Zong et al., 2021). Biochar is well recognized for its role in C sequestration and climate change mitigation, and the remediation of soil pollution (Ding et al., 2018b; Shaaban et al., 2018; Lu et al., 2019; Lehmann et al., 2021; Zhang et al., 2023). After crop straw is transformed into biochar, the physicochemical properties are modified, i.e., straw has a high molar H/C ratio, while the C in biochar is condensed with a lower molar H/C ratio (Yakout, 2017). Therefore, it is expected that crop plant straw, and its biochar would have contrasting stability in soil, influencing microbial C and N assimilation and occlusion within aggregates.

Crop plant straw is mainly composed of cellulose, hemicellulose, lignin, and small amounts of other organic and inorganic compounds (Ji et al., 2022). The straw-derived biochar is composed of aromatic compounds, which increases the stability of the C (Keiluweit et al., 2010; Kuzyakov et al., 2014; Li et al., 2018b). As such, it is expected that larger amounts of C in plant straw can be utilized by soil microorganisms as compared to biochar. Microbial proliferation following straw amendment would promote the production of microbial products such as carbohydrates and glucoproteins that can act as binding agents for soil aggregates (Oades, 1988; Poirier et al., 2014). Thus, it is expected that crop straw would increase soil aggregation to a greater extent than its biochar. The extent of C and N occlusion within aggregates is also likely to be greater for crop straw than biochar. However, little information exists on the microbial assimilation and stabilization of C and N within aggregates when comparing the amendment to soil of straw with its biochar.

To address this paucity in knowledge, we conducted a 600-day incubation of soils amended with ^{13}C and ^{15}N labelled maize straw or its biochar and assessed microbial biomass assimilation and aggregate occlusion for the supplied C and N. We hypothesized that: 1) larger amounts of C in straw would be assimilated by soil microorganisms as compared to biochar 2) straw addition would increase soil aggregation to a greater extent compared to its biochar, because straw facilitates microbial proliferation and produce greater amounts of microbial binding agents; and

3) straw-derived C and N would be occluded within soil aggregates to a greater extent than biochar-derived C and N.

2 Materials and methods

2.1 Soils, ^{13}C and ^{15}N dual-labeled straw and biochar

In 2017, soils with two levels of organic matter (SOM) content were collected from the 0–20 cm depth in a long-term fertilization experiment (initiated in 1987) at Shenyang Agricultural University, Shenyang, Liaoning, China (41°49′N, 123°34′E). Low SOM soil was collected from the field plot with long-term nil fertilization, and high SOM soil was collected from the field plot with long-term manure application (27 t ha^{-1} yr^{-1}). The soils were characterized as brown earth according to Chinese Soil Taxonomy and Typic Hapludalf according to US Soil Taxonomy. The low and high SOM soils had organic C content of 10.1 g kg^{-1} and 17.8 g kg^{-1}, and total N content of 1.16 g kg^{-1} and 2.16 g kg^{-1}, respectively (Table 1). The roots and the other organic debris were manually removed from soil.

The ^{13}C and ^{15}N dual-labeled maize straw was obtained using a pulse labeling method as described in An et al. (2015) and Zheng et al. (2018). Briefly, maize plants at seedling stage placed in a sealed transparent chamber enriched with $^{13}CO_2$, and the soils dosed with $(^{15}NH_4)_2SO_4$ solution. The ^{13}C-enriched CO_2 was generated through a reaction between 30 mL of 2 mol L^{-1} HCl and 1.89 g $Na_2{}^{13}CO_3$ (99 atom% ^{13}C, Sigma–Aldrich) to obtain a $^{13}CO_2$ concentration of approximately 400 μL L^{-1}. The labeling process started at 8:00 am on a sunny day and lasted for 6 hours, the process was repeated on three consecutive days during the maize seedling stage. The labeled maize straw was harvested in autumn, then oven-dried to constant weight. A portion of the harvested maize straw was cut into 3~5 mm-length pieces, and pyrolyzed with a temperature ramp of 5 °C min^{-1} to the maximum temperature at 400 °C and kept at this temperature for 2 hours in a muffle furnace (3XL-1008, China) to produce biochar (Ge et al., 2018). This pyrolysis temperature and time were chosen because it had the highest biochar yield (32% of the original biomass) (Ge et al., 2018). Both straw and biochar were crushed to pass a 1 mm sieve for the incubation. A small portion of straw and its biochar were pulverized using a ball mill (GT200, Beijing Grinder Instrument Co., Ltd, China) to measure C and N, and $\delta^{13}C$, and $\delta^{15}N$ through the dry combustion method. The properties of straw and biochar are shown in Table 1.

Table 1 Properties of maize straw, its derived biochar, and the two soils

Materials	Total C (g kg^{-1})	Total N (g kg^{-1})	C/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$pH_{(H2O)}$
Maize straw	422	11.5	36.7	209	4544	–
Biochar	766	24.7	31.0	236	3369	7.68
Low SOM soil	10.1	1.16	8.68	-17.8	9.35	6.42
High SOM soil	17.8	2.16	8.25	-19.6	5.08	6.37

2.2 Experimental layout and incubation conditions

Fresh soil samples (kept at 4 °C) equivalent to 100 g oven-dried weight were placed into 500-mL glass flasks (five treatments with three replicates for six destructive sampling times) both for low and high SOM soils. These soils in flasks were preincubated at 40% water holding capacity (WHC) and 20 °C in the dark for 7 days for the stabilization of soil microbial activity. 40% WHC condition was chosen at pre-incubation stage because this moisture is not too wet and thereby easy to evenly mix crop residues with soils at next step. After the pre-incubation, the soils were amended with 0.626 g (1 fold) or 1.248 g (2 fold) straw, or 0.343 g (1 fold) or 0.686 g (2 fold) biochar, and mixed evenly. The 1-fold (i.e., 0.626% relative to soil weight) dose of straw addition was equivalent to all the straw returned to the field in our region (i.e., 22.5 t straw ha^{-1}), assuming incorporation into the top 30 cm of soil at a bulk density of 1.2 g cm^{-3}. The doses of 1 fold and 2 fold of biochar were calculated based on equal C input with straw addition. There were 5 treatments for each of the SOM level: nil amendment, and 2 rates of straw and 2 rates of biochar. Each treatment had 3 replicates for each of the destructive sampling events. The soil samples were brought to 60% WHC and incubated at 20 °C in a dark environment. WHC was measured based on only soil, and it was the same for the incubated soil water content for biochar and straw amendment. Soil moisture was adjusted to 60% WHC by weight test every week.

2.3 Aggregate fractionation and measurements

Three replicates for each treatment were randomly selected and destructive sampled on days 20, 60, 100, 200, 400 and 600. On days 20 and 200, 20 g soil was sampled for the assessment of MBC and MBN using the chloroform fumigation-extraction (K_2SO_4) method with extracts analyzed with a Multi N/C 3100-TOC/TN Analyzer (Analytik Jena, Jena, Germany). The K_2SO_4-extractable organic C and N from the non-fumigated soil samples were reported as dissolved organic carbon (DOC) and total dissolved nitrogen (TDN). The K_2SO_4 extracts from the fumigated and non-fumigated soil samples were both freeze-dried to analyze their ^{13}C and ^{15}N values.

Soil aggregate fractionation was undertaken using the wet-sieving method (Elliott, 1986). Briefly, a 50 g soil sample was divided into macroaggregates (2000–250 μm), microaggregates (250–53 μm), and silt and clay sized aggregates (< 53 μm) using two sieves (250 μm and 53 μm). Floating organic material was removed and discarded during the sieving process to avoid free straw or biochar being fractionated into soil aggregate fractions. The isolated aggregate fractions were transferred to an aluminum tray, and oven-dried at 40 °C until the weight did not change. Sand was manually removed in macroaggregates using tweezers. The percentage of aggregates in the total soil mass was calculated based on weight. Soil aggregate fractions were finely ground and a 10 mg sample of each fraction weighted into a tin cap before being assayed for C, N, $\delta^{13}C$ and $\delta^{15}N$ contents. Soil aggregate fractionation was conducted for the soils at each sampling point, but isotope measurements were only conducted for the soil samples on days 20, 60 and 100. All isotope measurements were conducted using an elemental analyzer-isotope ratio mass spectrometer (Elementar vario PYRO cube and Isoprime 100, Germany).

2.4 Calculations and statistical analyses

Mean weight diameter (*MWD*), the index of soil aggregate stability, was calculated via Eq. (1):

$$MWD = \sum_{i=1}^{3} X_i \times W_i \quad (1)$$

where i represents the three aggregate fractions (2000–250 μm, 250–53 μm, and < 53 μm); X_i is the percentage of a given aggregate fraction in total soil mass (%); W_i is mean diameter of corresponding aggregate fraction (μm).

The proportion (F) of straw- or biochar-derived C (or N) to C (or N) of a given soil aggregate fraction was calculated via Eq. (2):

$$F = \frac{\delta^{13}\mathrm{C}\ (^{15}\mathrm{N})_{\mathrm{aggregate-treatment}} - \delta^{13}\mathrm{C}\ (^{15}\mathrm{N})_{\mathrm{aggregate-control}}}{\delta^{13}\mathrm{C}\ (^{15}\mathrm{N})_{\mathrm{residue}} - \delta^{13}\mathrm{C}\ (^{15}\mathrm{N})_{\mathrm{aggregate-control}}} \quad (2)$$

where δ^{13}C (^{15}N)$_{\mathrm{aggregate\text{-}treatment}}$ is the abundance of ^{13}C (or ^{15}N) of a given soil aggregate fraction with amendment (‰); δ^{13}C (^{15}N)$_{\mathrm{aggregate\text{-}control}}$ is the abundance of ^{13}C (or ^{15}N) of this soil aggregate fraction in the corresponding control soil (‰); δ^{13}C (^{15}N)$_{\mathrm{residue}}$ is the abundance of ^{13}C (or ^{15}N) of the straw or biochar (‰).

The amount of straw- or biochar-derived C (or N) occluded in soil aggregate was calculated via Eq. (3):

$$\mathrm{C\ (N)_{residue}} = \mathrm{M_{aggregate}} \times \mathrm{C\ (N)_{aggregate}} \times \mathrm{F}/1000 \quad (3)$$

Where C (N)$_{\mathrm{residue}}$ is the amount (g) of straw- or biochar-derived C (or N) in a given soil aggregate fraction (g); M$_{\mathrm{aggregate}}$ and C (N)$_{\mathrm{aggregate}}$ are the mass (g) and C content (g kg^{-1}) of this soil aggregate fraction.

The calculations for the proportion of straw- or biochar-derived C (or N) entering dissolved organic matter (DOM) and microbial biomass were similar to those entering soil aggregate fractions as Eq. (3). The δ^{13}C or δ^{15}N of microbial biomass were calculated following the method in An et al. (2015):

$$\delta^{13}\mathrm{C_{MBC}} = \left(\delta^{13}\mathrm{C_{fum}} \times \mathrm{C_{fum}} - \delta^{13}\mathrm{C_{nfum}} \times \mathrm{C_{nfum}}\right)/(\mathrm{C_F} - \mathrm{C_{nfum}}) \quad (4)$$

$$\delta^{15}N_{MBN} = (\delta^{15}N_{fum} \times N_{fum} - \delta^{15}N_{nfum} \times N_{nfum})/(N_F - N_{nfum}) \quad (5)$$

where C_{fum}, C_{nfum}, N_{fum} and N_{nfum} refer to the amount of dissolved organic C (mg kg^{-1} soil), total N (mg kg^{-1} soil) from the fumigated and the non-fumigated K_2SO_4 extracts, respectively. $\delta^{13}C_{fum}$, $\delta^{13}C_{nfum}$, $\delta^{15}N_{fum}$ and $\delta^{15}N_{nfum}$ refer to the $\delta^{13}C$, $\delta^{15}N$ values (‰) of the fumigated and the non-fumigated K_2SO_4 extracts, respectively.

Comparisons among treatments were performed using one-way analysis of variance (ANOVA) on high and low SOM soils for soil aggregate dynamics, straw- or biochar-derived C and N in aggregates, DOM, and microbial biomass at a given sampling time. Where significant differences ($p < 0.05$) were observed, treatment means were compared using Duncan tests at 5% probability. The effects of all three factors (SOM level, type and dose of added material) and their interactive effects were tested using a three-way ANOVA. Normality of residuals and homogeneity of the variances of the residuals across groups were checked for each ANOVA. All the analyses were conducted by SPSS 20.0 (SPSS, Chicago, IL).

3 Results

3.1 Straw- or biochar-derived C and N in DOM and microbial biomass

Amendment of soil with straw or biochar had varying influences on DOC, TDN, MBC, and MBN contents (Table 2). DOC and MBC were not affected by treatment ($p > 0.05$), except for high SOM soil at day 200. Biochar amendments generally had an average 79% higher TDN than straw amendment or the nil amendment at day 20 ($p < 0.05$), but tended to have similar or lower TDN at day 200 (Table 2). High SOM soil had larger DOC, TDN, MBC, and MBN contents than low SOM soil ($p < 0.05$, Table S1).

The amendment type altered the contributions of exogenous C to the DOC and MBC pools and exogenous N to the TDN and MBN pools ($p < 0.01$, Table S1), with straw-derived C and N having a greater contribution than biochar (Fig. 1). Specifically, straw-derived C had a 2.5% and 2.4% contribution to DOC at day 20 and 200, respectively; while biochar-derived C had a negligible contribution (Fig. 1). Straw-derived N provided 3.0% and 10% contribution to TDN at day 20 and 200, respectively; while biochar-derived N contributed 0.1% and 0.3%, respectively. Straw-derived C had a 48% and 59% contribution to MBC at day 20 and 200, respectively; while biochar-derived C contributed 4.2% and 14% to the MBC. Straw-derived N had a 18% contribution to MBN at day 20, increasing to 20% at day 200. Biochar-derived N provided a negligible contribution to MBN. Soil organic matter level did not alter the content of exogenous C and N in these pools ($p > 0.05$, Table S1), expect for TDN on day 20 ($p < 0.01$, Table S1). Generally, the higher dose of straw gave a larger contribution to these pools (Fig. 1).

Table 2 Dissolved organic carbon (DOC), total dissolved nitrogen (TDN), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) after straw or biochar amendments.

Treatments	Low SOM soil				High SOM soil			
	DOC	TDN	MBC	MBN	DOC	TDN	MBC	MBN
	(mg kg^{-1})				(mg kg^{-1})			
				Day 20				
Soil	235±46	18±5^{ab}	242±148	24±6	305±76	50±21^{b}	257±127	99±8^{a}
Soil+1S	245±48	11±1^{b}	218±75	12±5	331±58	30±4^{b}	440±123	109±21^{a}

Soil+2S	307±60	18±2^{ab}	175±74	14±4	393±37	21±6^{b}	413±71	94±7^{a}
Soil+1B	317±2	30±2^{a}	77±2	7±1	363±9	92±8^{a}	157±44	43±14^{b}
Soil+2B	146±1	26±3^{a}	168±8	9±1	291±47	90±9^{a}	347±59	113±12^{a}
Day 200								
Soil	401±9	86±5	101±25	15±13	429±28^{bc}	220±7^{b}	132±56^{b}	103±3^{b}
Soil+1S	418±56	91±7	97±12	32±12	414±33^{c}	225±18^{b}	396±66^{a}	91±11^{b}
Soil+2S	413±34	106±9	138±31	31±16	507±32^{ab}	273±14^{a}	162±36^{b}	61±39^{b}
Soil+1B	400±42	91±1	141±67	28±14	507±16^{ab}	211±2^{b}	243±47^{ab}	175±11^{a}
Soil+2B	456±58	84±4	84±24	56±6	520±15^{a}	213±3^{b}	178±60^{b}	119±28^{b}

Soil+1S and Soil+2S: soils amended with one and two- fold of straw; Soil+1B and Soil+2B: soils amended with one and two- fold of biochar. Values represent mean ± standard deviation (n = 3). Different lowercase letters among treatments devoted significant different ($p < 0.05$). No marked letters meant no statistical difference ($p > 0.05$).

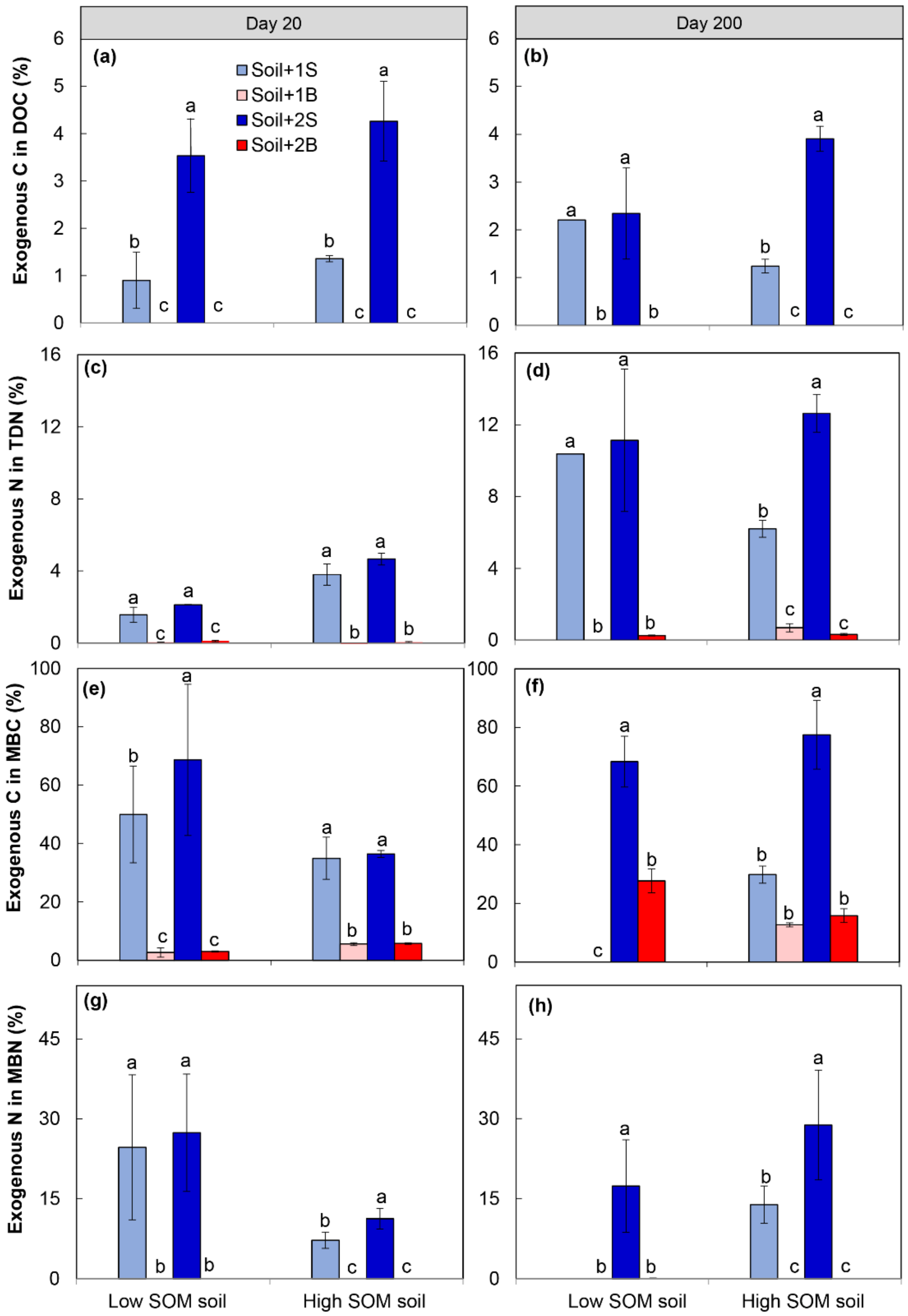
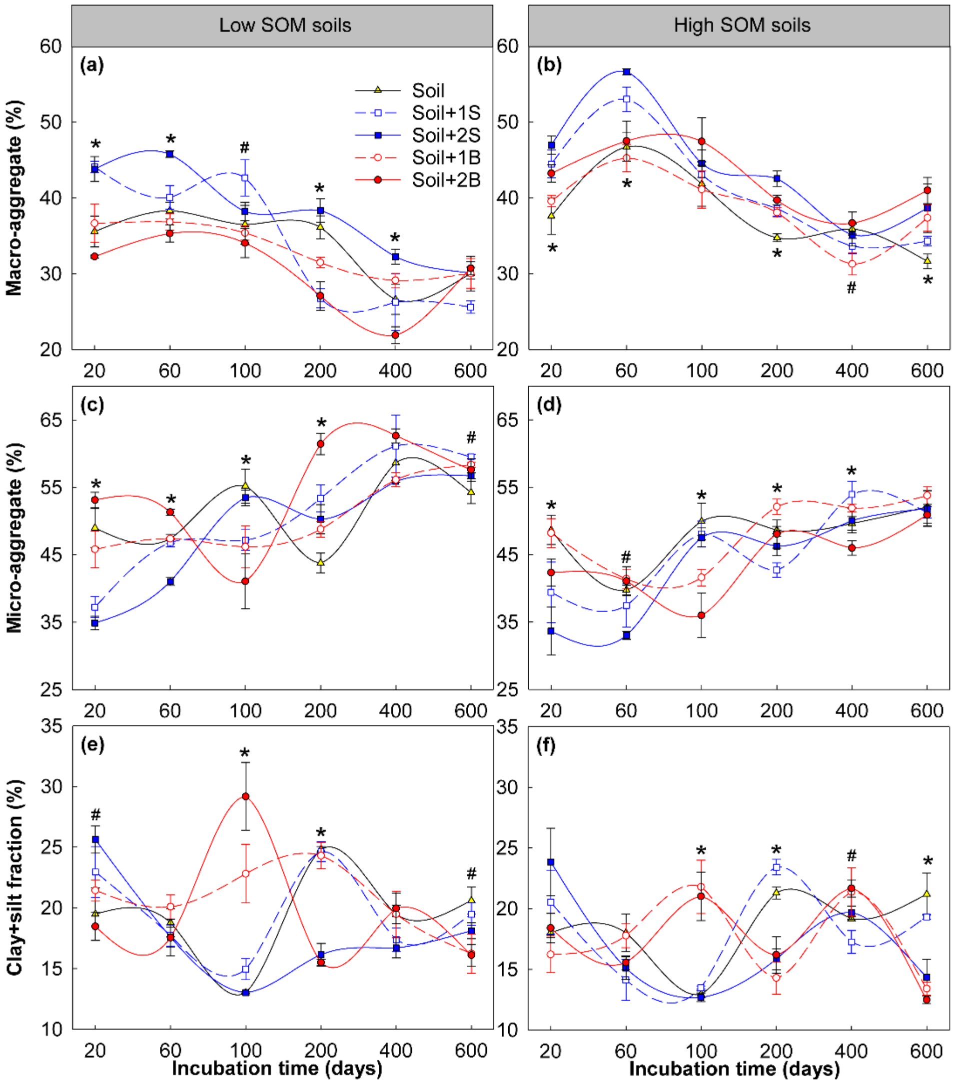


Fig. 1 Contributions of C or N from straw and biochar to the various pools at day 20 (a, c, e, g) and day 200 (b, d, f, h). DOC: dissolved organic carbon, TDN: total dissolved nitrogen, MBC: microbial biomass carbon and MBN: microbial biomass nitrogen. Soil+1S and Soil+2S: soils amended with one and two-fold of straw; Soil+1B and Soil+2B: soils amended with one and two- fold of biochar. MBC and MBN data in low SOM soils were not obtained.

3.2 Soil aggregate dynamics

Generally, across all the treatments, the proportion of macroaggregates increased from day 20 to 60, and then decreased to day 600 (Fig. 2). The proportions of microaggregates generally increased along with the incubation, and no change in silt and clay fractions were observed. The amendment type affected the proportion of soil aggregate fractions at most sampling times ($p < 0.05$, Table S2). The soils amended with maize straw had 15%~21% more macroaggregates than those amended with biochar ($p < 0.05$) within the first 100 days of the incubation in low SOM soils and before 60 days in high SOM soils (Fig. 2). Conversely, the amounts of microaggregates were smaller in soils amended with straw than with biochar (Fig. 2). The higher dose of either of the amendments resulted in larger proportions of macroaggregates, except for biochar addition in the low SOM soils. Amendment type affected MWD at most of incubation time ($p < 0.05$, Table S2). The MWD in the soils amended with straw was 14% larger than those amended with biochar during the early stages of the incubation ($p < 0.05$, Fig. 3). Notably, biochar amendment only increased MWD at 200 and 600 days in high SOM soil ($p < 0.05$, Fig. 3).



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25 **Fig. 2** Dynamics of aggregate size classes (mass proportion) in soils with low (a, c, e) and high SOM (b,
26 d, f) amended with maize straw or biochar. Error bars represent standard errors (n = 3). Soil+1S and
27 Soil+2S: soil amended with one and two- fold of straw, respectively; Soil+1B and Soil+2B: The soils
28 amended with one and two- fold of biochar, respectively. "*" and "#" represent $p < 0.05$ and $p < 0.1$ for
29 the effect of amendment treatments in one-way ANOVA at a given sampling time, respectively.

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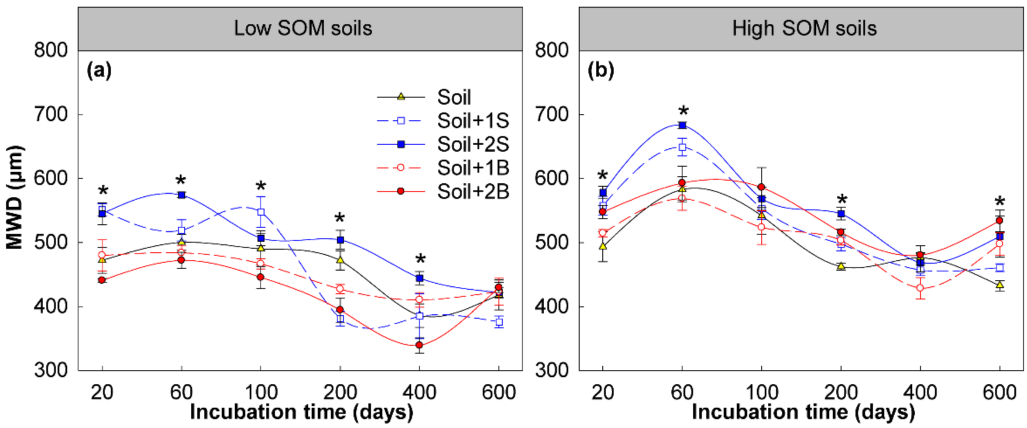

Fig. 3 Dynamics of mean weight diameter (MWD) of aggregates in soils with low (a) and high SOM (b) amended with maize straw or biochar. Error bars represent standard errors (n = 3). Soil+1S and Soil+2S: soil amended with one and two- fold of straw, respectively; Soil+1B and Soil+2B: The soils amended with one and two- fold of biochar, respectively. "*" represents $p < 0.05$ for the effect of amendment treatments in one-way ANOVA at a given sampling time.

3.3 Amendment-derived C and N in aggregates and their C/N ratios

Amendment type affected the content of exogenous C and N in the majority of aggregate size classes (most $p < 0.05$, Table S3). Specifically, larger amounts of straw-derived C and N was found in all aggregates compared to biochar derived C and N (Fig. 4). The higher amendment dose resulted in greater C and N entering into the aggregates (all $p < 0.05$, Table S3, Fig. 4). Soil organic matter level only affected the amount of exogenous C and N entering into soil aggregates at day 20 (most $p < 0.05$, Table S3).

The dynamics of exogenous C and N entering into soil aggregates generally showed similar trends (Fig. 4), suggesting that the stabilization of C and N elements were relatively synchronous. The C:N ratios of exogenous organic matter incorporated into soil aggregates were calculated based on the C and N amounts occluded in aggregates (Table 3). Exogenous C:N ratios in aggregates ranged from 5:1 to 20:1 across all treatments, and generally decreased with decreasing aggregate size (Table 3). Biochar amendment had larger exogenous C:N ratios in soil aggregates than straw (most $p < 0.01$ for effect of amendment type, Table S4). High SOM soils tended to have larger exogenous C:N ratios than low SOM soils for all the sized aggregates at early stage, i.e., at 20 and 60 days of incubation (most $p < 0.05$ or < 0.1 for effects of SOM level, Table S4), but this difference did not occur at late incubation stage, i.e., at 100 days of incubation (all $p > 0.1$, Table S4). In addition, amendment doses did not change exogenous C:N ratios (most $p > 0.05$, Table S4).

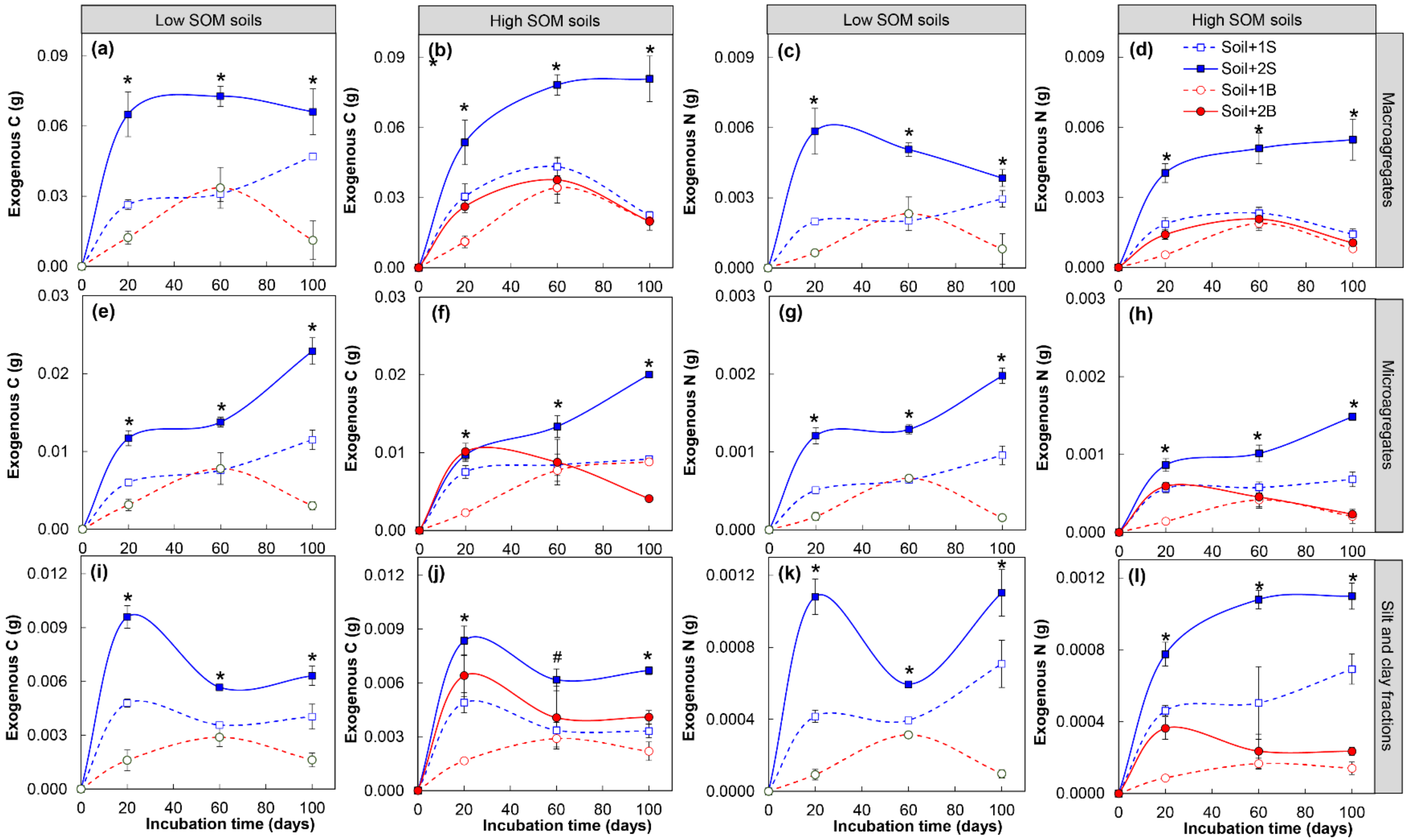

Fig. 4 Amount of C from straw or biochar-derived (a, b, e, f, i, j) and N (c, d, g, h, k, l) incorporated into soil aggregate size classes. Bars represent standard error (n = 3). Soil+1S and Soil+2S: soils amended with one and two- fold of straw, respectively; Soil+1B and Soil+2B: soils amended with one and two- fold of biochar, respectively. Data for the Soil+2S treatment in low SOM soils were not obtained. "*" and "#" represent $p < 0.05$ and $p < 0.1$ for the effect of amendment treatments in one-way ANOVA at a given sampling time, respectively.

Table 3 Mass C/N ratios of exogenous organic matter incorporated in soil aggregate size classes.

Treatments	20 d		60 d		100 d	
	Low SOM soils	High SOM soils	Low SOM soils	High SOM soils	Low SOM soils	High SOM soils
			Macroaggregates			
Soil+1S	13.2±0.1	16.5±0.8	15.3±0.8	18.8±0.5	27.5±4.7	15.8±0.1
Soil+2S	11.3±0.7	13.3±0.3	14.4±0.2	15.4±0.4	17.1±1.0	17.4±0.6
Soil+1B	18.7±1.0	20.2±1.7	15.6±2.5	18.4±0.6	16.0±1.3	24.7±2.8
Soil+2B	ND	19.0±1.0	ND	17.9±0.7	ND	19.2±1.0
			Microaggregates			
Soil+1S	11.8±0.2	13.4±0.6	11.8±0.2	14.8±0.7	12.1±0.2	13.4±0.4
Soil+2S	9.7±0.3	11.2±0.3	10.7±0.2	13.2±0.1	11.6±0.4	13.5±0.1
Soil+1B	19.2±2.0	16.1±1.0	14.0±1.4	18.8±0.7	19.8±1.3	17.0±2.7
Soil+2B	ND	17.0±0.5	ND	19.1±0.4	ND	17.5±0.4
			Clay and silt fractions			
Soil+1S	11.6±0.5	10.6±0.5	9.0±0.1	8.1±1.5	5.8±0.1	5.1±0.9
Soil+2S	8.9±0.3	10.8±0.2	9.5±0.2	5.7±0.3	5.9±0.8	6.1±0.2
Soil+1B	17.1±1.1	19.1±0.2	9.9±1.0	17.6±0.4	16.5±0.2	15.7±0.1
Soil+2B	ND	17.6±0.2	ND	14.1±2.3	ND	17.4±0.1

Values represent mean ± standard error (*n* = 3). Soil+1S and Soil+2S: soil amended with one and two-fold of straw, respectively; Soil+1B and Soil+2B: The soils amended with one and two- fold of biochar, respectively. ND: not determined.

4 Discussion

4.1 Microbial assimilation of straw- or biochar-derived C and N

Supporting our first hypothesis, straw-derived C and N had much greater contribution to microbial biomass than biochar (Fig. 1). The results are consistent with Singh et al. (2014) who reported that raw biomass was more easily assimilated by the microbial biomass compared to biochar derived from the same biomass. Straw contains larger amounts of labile organic compounds (Kuzyakov et al., 2009) that can be more easily assimilated by microorganisms compared to biochar (Singh et al., 2014; Yin et al., 2014; Xu et al., 2020). Similarly, plant straw resulted in more C in the DOM fraction and more N in the TDN fraction compared to biochar (Fig. 1). This is reflected by the ‘DOC-microbial pathway’, i.e., the transformation of litter to SOM occurs via the DOM formation, which is utilized through microbial processing into SOM (Cotrufo et al., 2015). The microbial mechanism was supported by the ratio of straw or biochar derived C:N of 5:1-20:1 within the aggregates, suggesting greater retention of exogenous N compared to C and this retention being microbial processed due to the similarity of this ratio with microbial needs (Weil and Brady 2017; Fahey et al., 2011). SOM levels did not affect C and N assimilation by microorganisms (Table S1), suggesting that soil fertility was not a key factor determining the incorporation of crop residues into microbial biomass.

4.2 Soil aggregation

Straw addition increased soil aggregation to a greater extent (i.e., larger mean weight diameter) compared to biochar within the first 100 days of the incubation (Fig. 3), supporting our second hypothesis. This is consistent with Rahman et al. (2018), who reported that straw increased soil aggregation but biochar did not within 56 days of incubation. Larger mean weight diameter of aggregate in soil amended with plant straw is derived from the development of macroaggregates (Fig. 2), but macroaggregates generally have a short lifetime as the binding agents are degraded with time (Tisdall and Oades, 1982; Ouyang et al., 2013; Yudina and Kuzyakov, 2023). Microorganisms utilizing straw release carbohydrates and glucoproteins, which are the two common binding agents for aggregate formation (Fang et al., 2021; Agnihotri et al., 2022). Other binding agents including microbial necromass and extracellular secretion or mycelial cementation which may have occurred after plant straw amendment (Jien and Wang, 2013; Leifheit et al., 2014; Agnihotri et al., 2022). Our data revealed that straw had substantial contribution to microbial biomass C and N, but not biochar (Fig. 1), suggesting microbial processing of the straw, along with the greater short term effect on aggregation, influence the production of binding agents in soil.

The dose of organic amendments is an important controlling factor on soil aggregation (Kallenbach and Grandy, 2011; Islam et al., 2021). The higher dose of amendments generally resulted in larger amounts of macroaggregate (Fig. 2), which was supported by previous research (Curaqueo et al., 2014; Zhang et al., 2017). High application dose of organic materials generally facilitate microbial activity and possibly produce larger amounts of binding material (e.g., mucilage and hyphae), thereby benefiting for the development of macroaggregates (Jien and Wang, 2013).

4.3 Occlusion of straw- or biochar-derived C and N within aggregates and their C/N ratios

Supporting our third hypothesis, straw-derived C and N are occluded within soil aggregates to a greater extent than with biochar amendment, despite similar amounts of exogenous C applied (Fig. 4). This is consistent with a mesocosm study in temperate forest, where wood C was distributed in aggregate occluded light fractions (33%) and mineral associated fractions (27%), while 20% and 6% of wood-derived biochar C were found in corresponding fractions (Singh et al., 2014). Using a dual ^{13}C and ^{15}N tracing technique, Weng et al. (2020) showed that straw amendment stimulated the priming of SOC with more N being made available for plants from the soil organic matter compared to biochar.

The higher dose of straw amendment had larger amounts of exogenous C and N detected in all aggregates (Fig. 4). Although the proportion of macroaggregates also had a similar dependence on the dose, the proportion of microaggregates and silt and clay factions did not (Fig. 2). The proportions of these two small aggregate fractions were even lower at the larger dose of amendment at certain sampling time (Fig. 2). This suggested that the larger amounts of microaggregates were aggregated to macroaggregates at larger dose of straw amendment (Yudina and Kuzyakov, 2023).

In our study, C:N ratios of exogenous organic materials incorporated into soil aggregates ranged from 5 to 20 and decreased with the size of aggregates (Table 3). This is comparable with Núñez et al. (2022) who also used plant residues amendment and found exogenous C/N ratios as 18.4, 15.9, and 13.0 in macroaggregates, microaggregates and mineral associated organic matter fractions, respectively. This agrees with the decrease of C/N ratios of SOM with decreasing aggregate size (Ding et al., 2014, 2018a), suggesting that SOM in smaller-sized aggregate is usually older and in a more decomposed state (von Lützow et al., 2007). In our study, biochar amendment had larger exogenous C:N ratios in soil aggregates than straw (Table 3), though biochar itself had a numerically lower C:N than straw (Table 1). This is possibly because aggregates formation after straw amendment is more related with microbe-derived cementing materials (Le Guillou et al., 2012), which generally have a strongly lower C/N ratio than amendments; whereas biochar-derived aggregates are mainly formed through the chemical adsorptive interactions between the surface of biochar itself and soil particles (Yoo et al., 2017; Weng et al., 2022).

5 Conclusions

Maize straw and its biochar (pyrolyzed at 400 °C) have contrasting effects on C and N uptake by microorganisms and aggregate formation in a Typic Hapludalf. Straw addition increased soil aggregation within the first 100 days, whereas straw effects disappeared thereafter. In contrast, biochar only had minor influence on aggregation within this timeframe, supporting the recalcitrant nature of biochar and its resistance to microbial transformations. The dynamics of C and N from straw and biochar entering into soil aggregates showed similar trends, indicating that the stabilization of C and N elements were synchronous. Results from this 600-day experiment confirmed our hypotheses that straw increases short-term aggregation via microbial processing of labile organic matter like DOM originated from straw and leading to the release of binding agents (e.g. glucoproteins), while biochar had limited impact on soil aggregation. Therefore, crop straw should be used to fast improve soil structure. The combined straw application with biochar may be a promising soil engineering technique, as straw can increase aggregation and biochar the soil carbon content.

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