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RESEARCH ARTICLE

Carbon to nitrogen stoichiometry of organic amendments influences the improvement of aggregate stability of a cropping vertisol

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Abstract

Soil aggregation is one of the key processes controlling air, water and gas transport in soil. Long-term cropping without returning organic matter in the soil can reduce the water stability of the aggregates. Microbial decomposition of organic matter plays a significant role in aggregate formation and hence can reverse the decline in the water stability of the aggregates. The inoculation of soil with beneficial microbes can improve the aggregate stability of cropping soil, potentially restoring its condition to healthy soil. However, the restoration of the aggregate stability may also be dependent on the C:N ratio of added organic matter. We hypothesize that a higher C:N ratio of added organic matter and microbial inoculation can trigger a more persistent improvement in aggregates. We treated pasture (aggregates were water-stable) and cropping (aggregates were unstable in water) vertisols with sugarcane (C:N = 104) and lucerne (C:N = 23) residues with and without microbial inoculant that had both bacteria and fungi. After 4 months of incubation, we found that the slaking index dropped by 46% in sugarcane-treated cropping soils, whereas the reduction was 27% in lucerne treatment. A similar reduction in the slaking index was also observed in the pasture soil but the magnitude of the reduction was lower than in the cropping soils. However, microbial inoculation did not show a statistically significant influence on reducing the slaking of cropping or pasture soils in this study. The reduction of slaking in both soils was supported by an increase in mean weight diameter (MWD), macro-aggregates and the aggregate-associated soil organic carbon. Our results demonstrated that organic carbon input with a high C:N ratio facilitates the restoration of water stability of the structurally unstable cropping soils.

KEYWORDS

aggregate formation, mean weight diameter, microbes, slaking index

1 | INTRODUCTION

Soil aggregates support many crucial functions, including water and nutrient cycling, storing carbon, and providing a habitat for plant roots and microbes (Creamer et al., 2022; Oades, 1984). Vertisols, soils with a high content of shrinking-swelling clays, cation exchange capacity and fertility, are one of the most productive soils when properly managed (Schweizer et al., 2022). Recent studies have proved that the type of clay minerals plays an essential role in the stabilization of the soil macroaggregates (Bravo-Garza et al., 2009). Bravo-Garza et al. (2010) reported that the wetting and drying cycle is a unique mechanism for the formation of aggregates, which promotes carbon storage and enhances the protection of organic residues in vertisols. However, the extensive cropping on these soils has caused the degradation of the soil aggregate structure, and the declining structural stability of vertisols has become a serious issue in Australia (Chan & Hulugalle, 1999). Generally, aggregate stability can be improved through agricultural practices, which allow the carbon input and limit the physical disturbance of soil, such as the minimum or zero tillage, crop residue retention, and cover crops (Abiven et al., 2009). Significantly, soil aggregation improvement and restoration have the potential to increase the soil's ability to store more carbon and water, and protect soil biodiversity (Minasny et al., 2017).

The quality and quantity of organic amendments influence soil aggregate formation, though the specific relationship between organic matter types and soil aggregation remains unclear (Sarker et al., 2022). The C:N ratio of the organic matter can be considered as an indicator of its quality (Sarker et al., 2022). It has been demonstrated that the C:N ratio influences the soil aggregation process, with residues of higher C:N ratios leading to more persistent improvements in aggregation (Hagedorn et al., 2003). The aggregation process is also regulated by microbial activities, microbial depolymerization of organic matter and production of extracellular polysaccharides. While fungal mycelium can physically enmesh and cluster soil particles to stabilize the aggregates, especially macroaggregates (Six et al., 2004), bacteria can influence micro-aggregates and organo-mineral formation through rapid decomposition of organic matter (Totsche et al., 2018). A laboratory study using pure clays demonstrated that microbial processes were the key to forming aggregates within just 6 weeks (Rabbi et al., 2020).

However, very few studies empirically translate this fundamental work on restoring structurally degraded soil produced by long-term cropping. Previous research either focuses on the agricultural management practices associated with organic matter or the mechanisms that allow microbes to form aggregates in soils. Inoculation of soil with agriculturally beneficial microbes may have a positive influence on the aggregation process in soil. Yet, from a single inoculation, bacteria and fungi exhibit relatively low activity because of the competitive pressures of survival, they struggle to thrive in nutrient-depleted soils (Singh, 2015). Nonetheless, the concurrent application of microbial inoculants and organic matter may represent a feasible approach for rehabilitating degraded soils (Song et al., 2015), since the addition of organic matter provides both carbon and nitrogen for bacterial and fungal growth and activity in soil (Rashid et al., 2016). We, therefore, hypothesized that C:N ratio of added organic matter and inoculation of soil with beneficial microbes can improve aggregate stability of cropping soil in vertisols.

2 | MATERIALS AND METHODS

2.1 | Soil samples

To test our hypothesis, we initiated a laboratory incubation study using vertisols (IUSS Working Group WRB, 2022). The soil was taken from the University of Sydney's farm in Narrabri, NSW, Australia (149°50′16″ E, 30°16′40″ S) with a clay content of 47% dominated by smectite. We collected top soil samples (0–10 cm) from a cultivated field, where wheat and sorghum were grown for over two decades. For comparison, we also collected samples of the same soil type from an adjacent pasture. The soil samples were air-dried, ground into fine homogeneous particles, and stored in sealed containers for subsequent experiments.

2.2 | Experimental design

The experiment was laid out following a 2 × 3 × 3 factorial design to investigate the dynamics of aggregate formation in soils. The experimental setup included 30 g of soils (pasture or cropping), 0.3 g of organic matter (sugarcane (*Saccharum officinarum L.*) mulch or lucerne (*Medicago sativa L.*) leaves or no addition), and 0.2 g inoculation resource (biofertilizer or sterilized biofertilizer or no addition).

We used the Retsch ZM1 Centrifugal Mill Grinder to mill the organic matter into powder to maximize its surface area. We used MycoGold® (BioStim Pty Ltd., QLD, Australia) as biofertilizer, which had mycorrhizal fungi, Trichoderma, Bacillus and humic substances (see Appendix S1: 1.1). The sterilized biofertilizer group was added to observe the effect of humic substance that was part of the inoculant. For the sterilization, the inoculant was autoclaved at 121°C for at least 15 min.

The soils (30 g) were uniformly mixed with each type of organic matter and the soil water content was adjusted to 80% of field capacity (i.e., water content at field capacity was 30% w/w). The containers were covered with Parafilm® to reduce the water evaporation. The samples were incubated for 4 months, and each treatment was replicated three times. Note that we did not add additional water during the incubation period. After 14, 28, 42, 56 and 112 days, a small amount of each sample was collected and oven-dried at 40°C for the wet-sieving and slaking tests.

2.3 | Physical and chemical properties

The concentration of soil organic carbon (SOC) in aggregates was determined by dry combustion using a LECO CN analyser (LECO Corporation, USA). The lucerne leaves and sugarcane mulch had a carbon content of 415 and 428 g C kg^{-1}, respectively, with C:N ratios of 22.59 and 103.74.

Soil respiration was measured using a non-dispersive infrared (NDIR) K30 CO_2 sensor, following the manufacturer's instruction (Joshi Gyawali et al., 2019). Soil and pH and electrical conductivity (EC) were determined by 1:5 soil: deionized water suspension. Soil particles were measured by the hydrometer methods (Gee & Or, 2002). Other properties such as exchangeable sodium, calcium, potassium and magnesium, were measured using Mid-infrared spectroscopy (MIR) using FTIR TENSOR 37 (Bruker Optics, Ettlingen, Germany) (Ng et al., 2022). The exchangeable sodium percentage (ESP) was calculated as the ratio of exchangeable sodium to the total exchangeable cations, expressed as a percentage. The electrochemical stability index (ESI) was calculated as the $EC_{1:5}$/ESP ratio. The results are presented in Table 1.

2.4 | Wet sieving

We performed wet sieving as described by Six et al. (2002) to assess the change in aggregate size distribution after 4 months of incubation. We measured two aggregate sizes – macroaggregates (250–2000 μm) and microaggregates (53–250 μm). Five grams of dried soil was used for the tests. The soil was taken on a 250 μm sieve and submerged in water in a 2 L beaker so that the soil remained 10 mm under water. The sieve was moved manually up and down in the water 50 times over 2 min. The macroaggregates were collected from the sieve after the sieving and this procedure was repeated for the microaggregates (aggregates <250 μm that passed through the sieve) with a 53 μm sieve. The collected aggregates were weighed after 24 h of oven-drying at 40°C.

TABLE 1 Soil physical and chemical properties.

Properties	Pasture soil	Cropping soil
pH (1:5)	7.75	7.35
$EC_{1:5}$ (dS m^{-1})	0.41	0.16
Total C %	1.25	0.75
Total N %	0.09	0.05
$C:N_{molar}$	15.93	18.49
Sand (2–0.05 mm) (%)	32.99	35.75
Silt (0.05–0.002 mm) (%)	13.05	12.33
Clay (<0.002 mm) (%)	53.96	51.92
Exchangeable Na ($cmol_c$ kg^{-1})	1.18	0.84
Exchangeable Ca ($cmol_c$ kg^{-1})	14.93	9.57
Exchangeable Mg ($cmol_c$ kg^{-1})	8.60	4.82
Exchangeable K ($cmol_c$ kg^{-1})	1.06	2.40
ESP%	4.59	4.78
ESI	0.03	0.08

The mean weight diameter (MWD) of the aggregates was calculated as follows:

$$\mathrm{MWD} = \sum_{i=1}^{n} \bar{x}_i w_i \quad (1)$$

where x_i represents the mean diameter size of the fraction and W_i represents the mass fraction of each aggregate.

2.5 | Slaking test

The slaking test was conducted using the Slakes App developed by the University of Sydney for use with smartphones. The test uses image recognition technology to record soil aggregation before and after being immersed in water (Fajardo et al., 2016). Three samples from each treatment were oven-dried at 40°C. The size of the aggregates ranged from 2 to 15 mm. The soil aggregates were placed in an empty petri dish where the first image was captured. We prepared another petri dish filled with deionized water, placing three soil aggregates from the same treatment into it in a similar orientation to the original image. Once the aggregates were submersed, we pushed the 'start' button on the SLAKES application to launch the imaging analysis procedure, which lasted for 10 mins. Once completed, the slaking index (SI) was calculated as the area after 10 min over the initial area. An SI of 0 indicates no change in the aggregates' area after immersion, and a value of 1 indicates the area was increased by 100%. The slake index value ranges from 0 to 14, with higher indices indicating less stability.

2.6 | Statistical analysis

The data were analysed using a mixed-effect model in the '*nlme*' package (Pinheiro et al., 2016) in R (R Core Team, 2022). In the mixed-effect model slaking, MWD, macro- and micro-aggregates were fixed-effect and days of incubation were considered as random-effect. We tested the interaction between soil × carbon × inoculation using the '*emmeans*' package in R (Lenth, 2022). Soil organic carbon data were treated statistically by the two-way analysis of variance with organic matter and microbial inoculation as factors in R (R Core Team, 2022). The models were tested for normality, homogeneity of means and heteroscedasticity in the random effects and the models satisfied the statistical assumptions.

3 | RESULTS AND DISCUSSION

Compared with the control, the slaking index was significantly reduced after 14 days and persisted over the 112 days of incubation (27% to 59%) following sugarcane and lucerne application in both soil types (Figure 1). The slaking reduction was larger in pasture than the cropping soils in all measurement periods with the greatest reduction in sugarcane (high C:N ratio) treated soils compared with control as indicated by the significant soil × organic matter interaction ($p < .001$).

Overall, the application of microbial inoculant (either sterile or non-sterile) also produced a significant reduction in slaking compared with no-inoculation control in both soil types ($p < .001$). However, no significant difference between non-sterile and sterile microbial inoculation was observed, suggesting that the allochthonous microbes had little effect on the reduction of slaking. The effect was mainly through additional organic matter (from the inoculant). It is not rare that the introduced microbes do not survive in soil as a result of competitive interactions with native species and the influence of physico-chemical properties of soil (Romdhane et al., 2022).

While inoculated microbes did not have a significant influence on the aggregation, the native soil microbes decomposed the added organic matter as indicated by higher CO_2 production in organic matter-treated soils compared with the control (see Supplementary Materials S1:1.2). The role of microbes in soil aggregation is widely acknowledged with a specific emphasis on fungi (Miller & Jastrow, 2000; Rillig & Mummey, 2006). Daynes et al. (2013) showed that organic matter, living plant roots and fungi are essential for stable soil structure formation. However, our study did not demonstrate a significant influence of inoculated microbes on aggregation. The processing of added organic matter by native soil microbes might be responsible for the reduction in slaking of the soils, since the microbial decomposition of the newly added organic carbon resource accelerates the growth of fungi and bacteria (Rabbi et al., 2020). Both bacteria and fungi can cement soil particles into aggregates by producing an array of extracellular organic macromolecules (Chenu & Cosentino, 2011). The reduction of slaking also indicated an increase in cohesion between soil particles through cementation by microbially derived or processed organic matter (Goebel et al., 2005; Oades, 1984).

The organic matter added soils also showed a greater increase in MWD in pasture than cropping soils compared

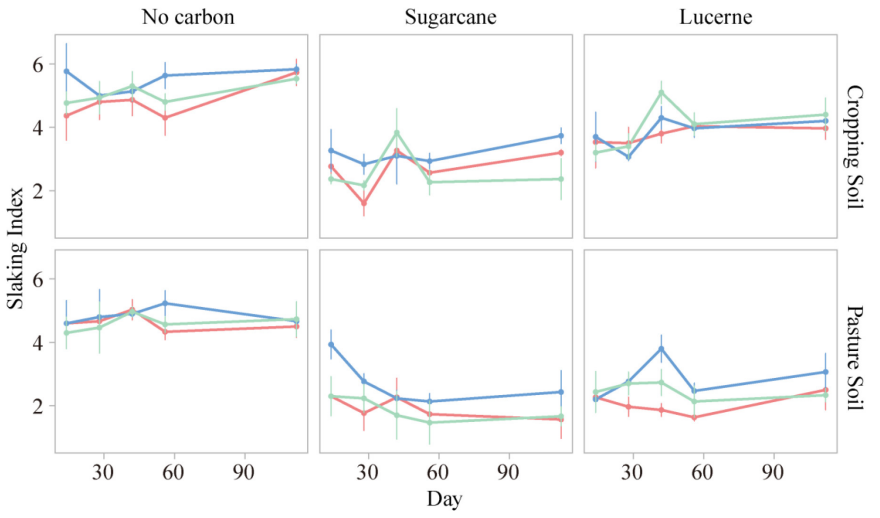

FIGURE 1 The slaking index of soils with organic matter amendment and microbial inoculation. Blue is control; red is inoculated treatment; green is inoculated sterile. The vertical bars denote mean ± standard deviations ($n = 3$).

with the no carbon control with non-significant differences between sugarcane and lucerne (low C:N ratio) treatments in each soil type (i.e., soil × organic matter interaction $p < .001$) (Figure 2). No significant increase in MWD was observed between non-sterile and sterile microbial inoculations in both soils. It should be noted that at 112 days of incubation, the sugarcane mulch had a significant increase in MWD compared with lucerne. This apparent discrepancy was also observed in the fluctuation of MWD over time under lucerne treatment compared with sugarcane mulch, which might be as a result of transient or weak binding of soil particles by microbial depolymerization products (Thuriès et al., 2001).

In addition to the change in MWD, the newly added organic matter significantly increased the proportion of macro-aggregates in both soils compared with the no-addition control ($p < .001$) (Figure 3). Particularly, the effect of sugarcane and lucerne on macro-aggregate formation was statistically significant after 112 days of incubation ($p < .05$). The proportion of macro-aggregates was significantly higher in pasture than cropping soil ($p < .001$), whereas a significantly greater proportion of macro-aggregates was observed in non-sterile and sterile inoculation compared with the no-inoculation control ($p < .001$). Conversely, the proportion of microaggregates, particularly under sugarcane mulch, showed an opposing trend and declined throughout incubation, which might indicate that aggregates formation might be a hierarchical process, where microaggregates form first that are then further aggregated into macroaggregates (Totsche et al., 2018).

Organic amendment plays a pivotal role in aggregate stability, with the impact hinging on both the quantity and quality of the matter added. A low C:N ratio will usually form macro-aggregates quickly since this indicates a highly decomposable product, while high C:N ratios are beneficial for forming aggregates over longer timeframes (Abiven et al., 2007; Le Guillou et al., 2011). Slaking indices of the current work support this conclusion. We observed lucerne, with its low C:N ratio, rapidly formed aggregates, but they were not particularly stable, which is consistent with previous research (Degens, 1997; Zhu et al., 2017), while the sugarcane, with a high C:N ratio, formed aggregates more slowly but much more stable (Zhu et al., 2023).

The increase in MWD and macro-aggregation was also supported by the increase in SOC concentration in sugarcane and lucerne treatments compared with control in both soils ($p < .05$) (Figure 4). While the SOC concentration in macro-aggregates in sugarcane treatment was significantly higher than the lucerne treatment in cropping soil ($p < .001$), non-sterile and sterile microbial inoculant did not produce any significant change in SOC concentration of the macro- and micro-aggregates. This aligns with a previous study that the efficiency of organic amendment in increasing SOC concentration varies with aggregate size, typically showing a stronger effect on the macroaggregates formation than on the microaggregates (Sarker et al., 2022). Organic amendments have been widely recognized as an effective way of increasing the organic carbon content of soil (Crystal-Ornelas et al., 2021). Our research demonstrated that organic amendments significantly increase the SOC in macro-aggregates that displayed a ca. 137% improvement for cropping soil and a 13% increase for pasture soil (Figure 4).

Previous research emphasized the importance of the fungi in aggregate formation in the vertisol (Bearden & Petersen, 2000; Rahman et al., 2017), but our 3-month

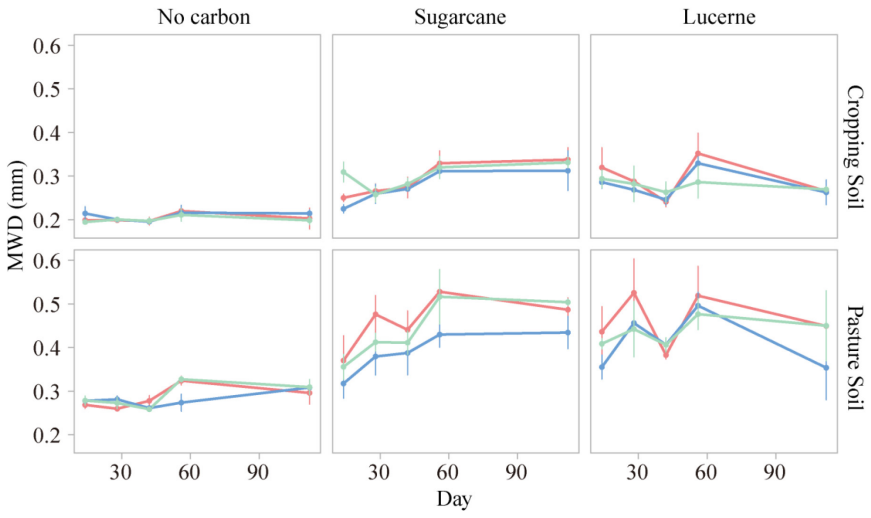

FIGURE 2 The MWD of the soils during the incubation period given organic matter amendments and microbial inoculation. Blue is control; red is inoculated treatment; green is inoculated sterile. The vertical bars denote mean ± standard deviations ($n = 3$).

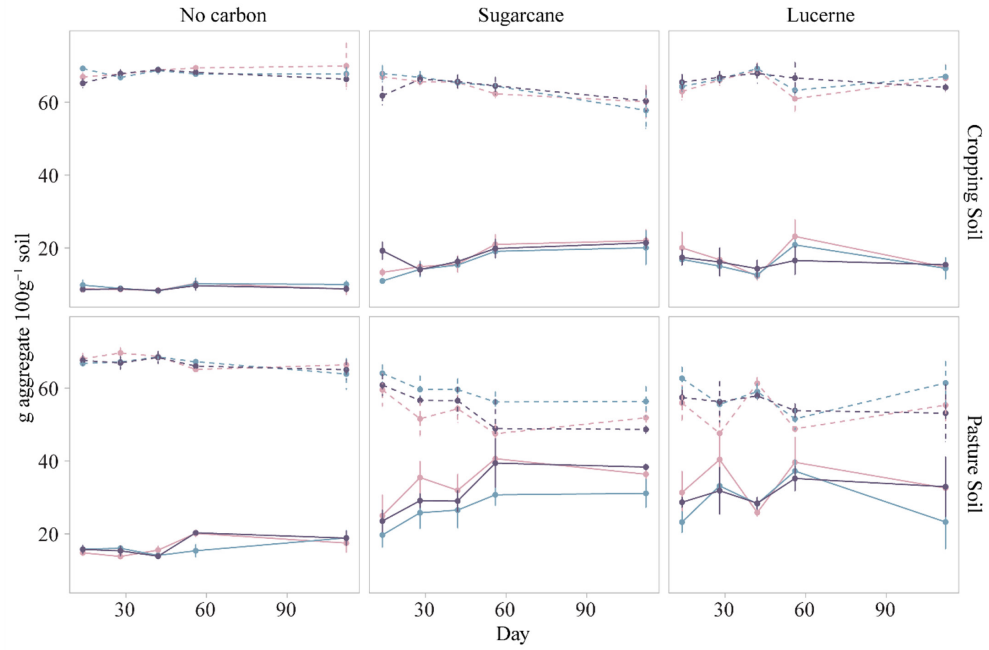

FIGURE 3 The macroaggregate (Solid line) and microaggregates (Dashed line) in the soils amended with organic matter and microbial inoculation during the incubation period. Red is inoculated; blue is control; Purple is inoculated sterile. The value shown means ± standard deviations ($n = 3$).

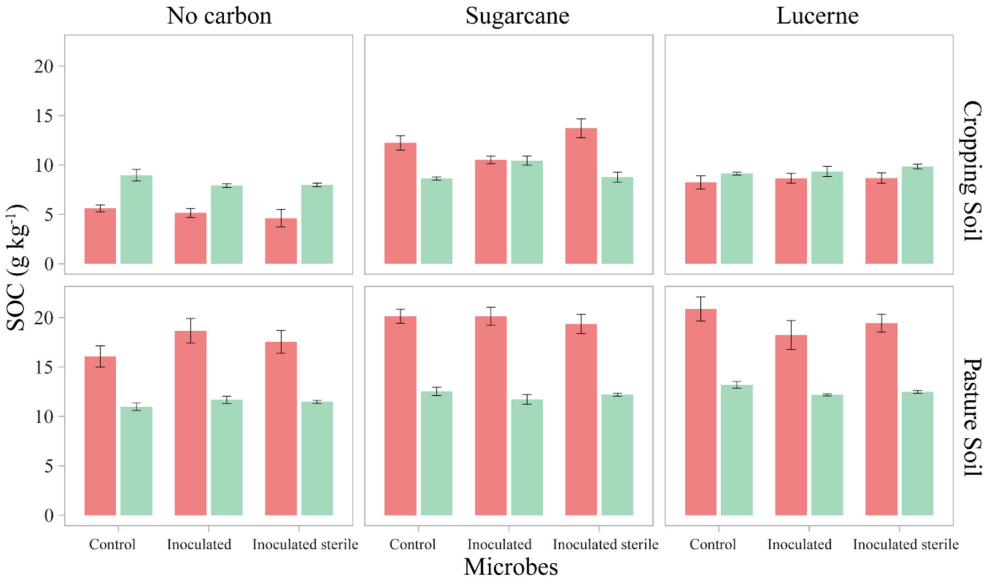

FIGURE 4 Mean (±SD) of soil organic carbon content after 112 days given added carbon and microbial inoculation. Red is macroaggregate; green is microaggregate. The value show means ± standard deviations ($n = 3$).

experiment did not yield a significant enhancement in microbial inoculation outcomes. This lack of observed improvement may be attributed to several factors. It is possible that the introduced microbial community could not withstand the competitive pressures from the native microorganisms. The incubation duration was insufficient for the microbial community to achieve stabilization and formation of aggregates. Additionally, our experimental conditions were carefully controlled within a laboratory setting, maintaining a constant room temperature of 20°C. We employed Parafilm® to seal the incubation tubes, ensuring an effective moisture barrier against evaporation and contamination, and chose not to introduce additional water throughout the incubation period. Consequently, the soil underwent minimal shrink-swell cycles because of the absence of wetting and drying events.

4 | CONCLUSIONS

Our research indicates that decomposing sugarcane (high C:N ratio) and lucerne (low C:N ratio) both positively influence aggregation in soil. However, sugarcane creates aggregates that are both more stable and persist. Introducing carbon into cropping soil at 0.75 g/100 g carbon (i.e., soil C plus added C) resulted in aggregate formation comparable to that in pasture soil with 1.25 g/100 g carbon. This demonstrates that degraded soil can be restored through organic amendment. Our research also concurrently confirms that the newly added organic matter contributes more to the formation of macroaggregates. The pathways of macroaggregate formation are controlled by the C:N ratio of the organic matter. In this study, we did not observe a significant improvement in the microbial inoculation on the soil aggregate. Nevertheless, further research is essential to elucidate the interaction between introduced and native microbes and changes in microbial community structure during soil aggregation.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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