Journal of Integrative Agriculture 2026, 25(3): 1194–1208

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Granulated organic amendment enhances recalcitrant carbon accumulation through soil aggregation in a barren paddy field

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Highlights

- The physical protection of MAOC from aggregates reduces recalcitrant carbon loss.
- Granulated organic amendment enhances the formation of intra-microaggregates within macroaggregates.
- Preservation of recalcitrant carbon occurs primarily within microaggregates.

Abstract

Barren paddy fields characterized by poor soil structure, shallow tillage layers and low organic carbon content are a common limitation to rice production in subtropical China. As a novel approach to soil improvement, granulated organic amendments offer significant potential. Previous studies have shown that granulated straw can improve soil physicochemical properties and rapidly increase the soil organic carbon (SOC) content. However, their effects on barren paddies remain underexplored. This study evaluated four soil amendment strategies: no organic amendments (CK), 10 t ha^{-1} of composted manure (M10), 20 t ha^{-1} of granulated organic amendment (G20), and 40 t ha^{-1} of granulated organic amendment (G40). The objective was to assess the effects of these amendments on soil structure, the contents of aggregate-associated carbon (AAC), particulate organic carbon (POC) and mineral-associated organic carbon (MAOC), and the chemical stability of MAOC among various size aggregates in both topsoil (0–20 cm) and subsoil (20–40 cm). The results demonstrated that organic amendment inputs significantly increased the macroaggregate (>250 μm) proportion and improved soil structural stability. These amendments also elevated the carbon concentration within aggregates of various sizes and facilitated the redistribution of organic carbon from microaggregates (53–250 μm) and silt+clay fractions (<53 μm) to macroaggregates. The proportion of POC to AAC declined with decreasing aggregate size, whereas the proportion of MAOC increased. In the topsoil, macroaggregate formation enhanced the protection of POC, supported the accumulation of non-hydrolyzable carbon within MAOC, and accelerated the formation of intra-microaggregates. In the subsoil, mineral-bound organic carbon remained the dominant form of carbon sequestration. In conclusion, the application of 40 t ha^{-1} of granulated organic amendment proved to be a successful tactic for enhancing soil physicochemical structure, increasing SOC content, and improving carbon stability. This approach offers a promising and innovative solution for the sustainable management and restoration of barren paddy fields.

Keywords: granulated organic amendment, soil aggregates, aggregate-associated carbon fraction, recalcitrant carbon

1. Introduction

Barren paddies are a typical example of medium- and low-yield fields, characterized by poor soil structure, shallow tillage layers, low organic carbon content, and nutrient deficiencies (Zhang *et al.* 2011; Zeng *et al.* 2014; Zhang and Li 2023). Therefore, upgrading and improving barren paddies is essential for promoting sustainable agricultural development and ensuring food security (Shahane and

Received 7 January 2025; Received in revised form 12 March 2025; Accepted 14 April 2025; Available online 14 May 2025
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doi: 10.1016/j.jia.2025.05.004

Shivay 2021; Rehman *et al.* 2022). Although extensive research has focused on soil improvement in the uplands and paddy fields, barren paddies have received relatively limited attention.

Organic amendments are a primary source of carbon input in arable soils (Marriott and Wander 2006; Diacono and Montemurro 2011). The addition of organic materials can directly supply a significant amount of carbon or release substantial nutrients during decomposition, thereby promoting plant growth (Di *et al.* 2018; Jia *et al.* 2024; Yang *et al.* 2024). This, in turn, increases crop biomass, enhances root exudates and crop residues entering the soil, and indirectly boosts soil carbon input (Chen *et al.* 2017; Zhao *et al.* 2021; Wu *et al.* 2024). In addition, the applied organic materials can improve soil structure, promote the formation of macroaggregates, and enhance the physical protection of soil organic carbon (SOC) (Bucka *et al.* 2019; Bai *et al.* 2023; Even and Cotrufo 2024). However, a major factor influencing SOC sequestration capacity is the chemical properties of the external organic amendment (Cotrufo *et al.* 2013; Yan *et al.* 2013; Kallenbach *et al.* 2016; Guan *et al.* 2019; Zhao *et al.* 2021). Studies have shown that the proportions of labile carbon pools in straw and manure are 76 and 43%, respectively, while the recalcitrant carbon pools account for 17 and 53%, respectively, indicating that straw decomposes faster than manure (Ma *et al.* 2019). Furthermore, increasing the degree of organic material fragmentation enhances the contact surface area between straw and soil, thereby accelerating decomposition and promoting SOC accumulation (Cong *et al.* 2019). A novel approach for farmland improvement and organic matter supplementation involves crushing crop residues, manure, and other farm wastes, followed by compression into 3–5 cm granules for field application (Wang X *et al.* 2023). Compared to traditional straw return incorporation, this technique accelerates microbial decomposition, minimizes negative impacts on soil structure, avoids hindering crop root development, and reduces the risk of pest and disease outbreaks (Wang *et al.* 2017; Cong *et al.* 2020). The granular amendment increases the surface area and alters the structural organization of the straw during the crushing and compression preparation processes, accelerating its decomposition and the rapid release of nutrients after soil application (Wang X *et al.* 2023; Duan *et al.* 2025). The decomposition rate and carbon release rate were reportedly 8.7 and 11% higher, respectively, compared to those of crushed straw (Cong *et al.* 2019; Fan and Wu 2020). In addition, under equal-weight field application conditions, the volume of granulated straw was only one-fifth that of conventional crushed straw, minimizing its negative impact on soil structure (Zhang *et al.* 2019). The deep application of granular amendments not only mitigates the gradual shallowing and compaction of the tillage layer caused by agricultural mechanization (Burger *et al.* 2023; Wang Q *et al.* 2023) but also replenishes the potential organic carbon (OC) loss in deeper soil (Li *et al.* 2021). The results of Wang X *et al.* (2023) demonstrated that high rates of granulated maize straw application (75 Mg ha^{-1} yr^{-1}) increased total soil carbon by 17 and 51% in the 0–20 and 20–40 cm soil layers, respectively, while reducing the surface soil bulk density by 8–14%. Furthermore, applying granulated straw at 6 Mg ha^{-1} increased wheat and maize yields by 10.3 and 16.9%, respectively, compared to the control (Zhang *et al.* 2018). Although granulated straw application has been implemented in Northeast China, field data on its use in paddy soils remain scarce. Its potential impacts on soil structure and SOC enhancement in barren paddies have yet to be fully explored. Consequently, investigating the large-scale application of granulated organic amendments in paddy soils is crucial for assessing their effects on soil fertility, grain yield, and economic viability, which can provide valuable insights for optimizing sustainable agricultural practices in these systems.

Soil aggregates are fundamental units of soil physical structure which play a cardinal role in the development of soil architecture as well as regulating interactions between minerals and organic substances (Tisdall and Oades 1982; Kemper and Rosenau 1986; Totsche *et al.* 2018). Soil aggregates are typically classified into macroaggregates and microaggregates based on a threshold of 250 μm (Six *et al.* 2004). Macroaggregates (>250 μm) are formed by the weak binding of microaggregates *via* gluing agents and exhibit greater sensitivity to soil management practices and faster turnover rates (Amézketa 1999; Elmholt *et al.* 2008). The formation of soil aggregates is primarily driven by organic matter from plant detritus and microbial by-products acting as binding agents (Totsche *et al.* 2018). These binding agents can be categorized into temporary, transient, and persistent types, based on their nature and size (Tisdall and Oades 1982; Amézketa 1999; Tang *et al.* 2011; Cai *et al.* 2024). These properties vary among the different agents and trigger aggregation processes at different aggregate sizes. Soil particles assemble into complex aggregates that drive SOC accumulation and protection, as the composition and properties of soil aggregates vary by size (Six *et al.* 2004; Six and Paustian 2014). Therefore, soil aggregation is the most pivotal of soil carbon sequestration mechanisms (Liu *et al.* 2019). Understanding the interactions between soil aggregates and SOC is crucial for sustaining soil fertility and boosting the carbon sink potential in agroecosystems.

Due to the high heterogeneity of soil organic material compositions, separating SOC through physical or chemical methods is essential (Hartemink *et al.* 2020). Physical separation methods isolate SOC into particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) based on their sizes. This widely adopted approach offers valuable insights into soil organci matter (SOM) distribution (Jagadamma and Lal 2010; Zhang *et al.* 2022; Peng *et al.* 2023). POC and MAOC are derived from distinct sources and exhibit varying degrees of physicochemical protection, leading to diversity in their chemical stability and turnover rates (Lavallee *et al.* 2020). MAOC constitutes the majority of SOC and is regarded as a stable carbon fraction due to its strong physicochemical interactions with mineral surfaces, which form organo-mineral complexes (Angst *et al.* 2021). These complexes are key structural components of soil aggregates and often exist as nanoparticulate fractions within microaggregates (Totsche *et al.* 2018; Lehndorff *et al.*

2021). Driven by SOM, soil aggregation preserves these complexes over long periods by physically isolating them within aggregates (Tisdall and Oades 1982). However, MAOC comes from a variety of sources, ranging from easily decomposable organic detritus to more recalcitrant organic matter tightly bound to clay particles (Golchin *et al.* 1996; Jastrow 1996; Chenu and Stotzky 2002). As a result, both labile and recalcitrant carbon fractions coexist within the MAOC fraction (Craig *et al.* 2018; Angst *et al.* 2021; Chen *et al.* 2023). Although MAOC is generally stable, it can contribute to SOC decomposition in the absence of POC or when MAOC lacks aggregate-based protection (Torn *et al.* 2013; Sokol *et al.* 2019; Li *et al.* 2024). Under such conditions, the chemical recalcitrance of MAOC becomes critical, as it contains molecules that are more resistant to natural degradation when physical protection is diminished (Márquez *et al.* 2017; Even and Cotrufo 2024; Jakab *et al.* 2024). However, our understanding of how the chemically recalcitrant carbon pool within organo-mineral complexes varies across different aggregate sizes is limited, particularly in response to shifts in aggregate distribution. When hydrolyzed by 6 mol L^{-1} HCl, MAOC can be separated into hydrolyzable carbon (HYC) and non-hydrolyzable carbon (NHC), representing the decomposable and recalcitrant fractions, respectively (Paul *et al.* 1997). Thus, a combination of physical and chemical methods to characterize organic carbon pools in organo-mineral complexes offers valuable insights into the mechanisms underlying carbon stabilization in soils (Nisar and Benbi 2024).

Although numerous research efforts have explored the effects of organic material additions on soil structure and SOC content, studies on improving the aggregate structure and enhancing the aggregate-associated carbon (AAC) content of barren paddy soils through the addition of organic amendments remain scarce. Therefore, the main objectives of this study were: 1) to investigate the effects of organic amendments on soil aggregate distribution and soil structural stability; 2) to assess their contributions to aggregate-associated organic carbon content and its various fractions; and 3) to evaluate the chemical stability of MAOC fractions in topsoil (0–20 cm) and subsoil (20–40 cm) of barren paddy soils.

2. Materials and methods

2.1. Study site description

This study was conducted in Kaihui Town, Hunan Province, China (113°32′E, 28°54′N), at an altitude of 80 m. This region is subject to a subtropical monsoon climate, with an average annual temperature of 17.0°C and average annual precipitation of 1,390 mm, predominately concentrated from March to August. The experimental field is representative of a typical double-cropping rice field, with granite weathering as the parent material. According to the USDA soil taxonomy (FAO 2015), the soil is classified as a clay loam Ultisol, with a particle size distribution of 21.3% sand, 38.9% silt, and 36.4% clay. The background physical and chemical properties of the soil are as follows: pH, 5.06; organic carbon (C), 18.7 g kg^{-1}; total nitrogen (N), 1.89 g kg^{-1}; total phosphorus (P), 0.50 g kg^{-1}; and total potassium (K), 37.8 g kg^{-1}.

2.2. Field experiment establishment

The field trial was established in 2022 and arranged in a randomized block with four treatments: 1) no organic amendments (CK); 2) 10 t ha^{-1} composted cattle manure (M10); 3) 20 t ha^{-1} granulated organic amendment (G20) and 4) 40 t ha^{-1} granulated organic amendment (G40). Two types of organic materials, composted cattle manure and granulated organic amendment, were applied in this experiment, and their basic properties are detailed in Appendix A. The cropping system comprised double-cropping rice with Chinese milk vetch as green manure during the winter season in all treatments. Early-rice seedlings were usually transplanted in April and harvested in July, followed by late-rice transplantation in August and harvesting in November. Following the rice harvest, Chinese milk vetch was planted as a cover crop and subsequently plowed back into the soil as green manure prior to the early rice planting in the following year. Prior to planting both early- and late-rice crops, compound fertilizer ($N\text{-}P_2O_5\text{-}K_2O$=22:12:16) was applied as a basal fertilizer at the rate of 75 kg N ha^{-1} per plot for each treatment. At the tillering and tasseling stages, urea was used as topdressing fertilizer at rates of 45 and 30 kg N ha^{-1} per plot, respectively. Composted cattle manure was applied annually before early rice planting, whereas the granulated organic amendment was applied once at the start of the experiment. All amendments were applied using a deep application method.

2.3. Soil sampling and analysis

Soil samples were collected from the 0–20 and 20–40 cm layers after the late rice harvest. In each plot, five undisturbed soil samples were collected from the two soil layers using a spade. The samples from the same layer in each plot were combined to form composite samples. After transporting wet field samples to the laboratory and air-drying them in the shade for 2 days, the composite samples were gently broken apart and passed through an 8 mm nylon sieve that was used for the subsequent wet sieving of soil aggregates after air-drying. An additional portion of each soil sample was used for subsequent analyses after the removal of small stones. The soil carbon fractions were separated using a combined fractionation approach, and the procedure is briefly detailed in Fig. 1.

Water-stable aggregate fraction separation The soil samples were fractionated into four water-stable aggregate classes: large macroaggregates (>2,000 μm), small macroaggregates (2,000–250 μm), microaggregates (250–53 μm), and silt+clay (<53 μm), according to Six *et al.* (1998). Briefly, a 60 g subsample (sieved to <8 mm) was placed on the top of a series of sieves and gradually moistened with water for at least 30 min. The sieves were then manually moved vertically by 3 cm at a frequency of 25 times per min. The particles retained on each sieve were carefully collected, while particles smaller than 53 μm were collected and centrifuged at 2,600×g for 12 min to isolate the silt+clay fraction. All separated aggregate fractions of different sizes were oven-

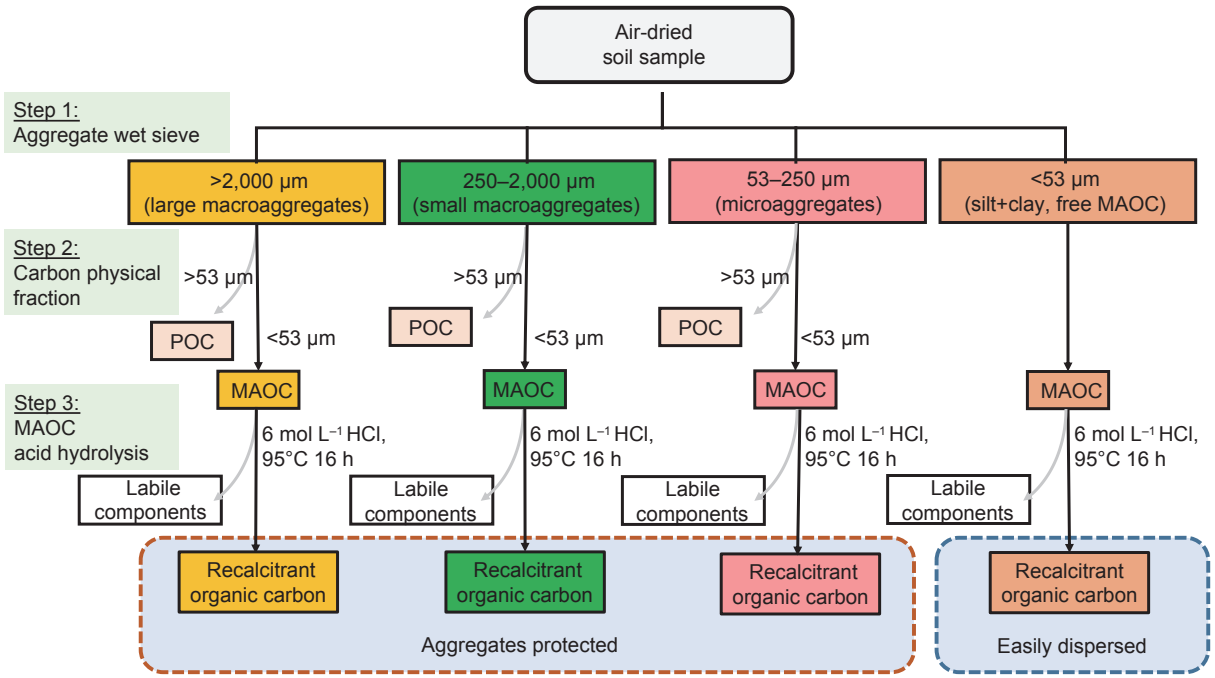


Fig. 1 Methodological framework for soil aggregate sieving, aggregate-associated carbon fractionation, and mineral-associated organic carbon (MAOC) chemical stability analysis. POC, particulate organic carbon.

dried at 55°C and weighed to calculate their mass proportions. For sand correction, a 0.5% sodium hexametaphosphate solution was added to subsamples of the large macroaggregate, small macroaggregate, and microaggregate fractions (approximately 5 g) and shaken at 180 r min^{-1} for 18 h. The calculations were performed as follows:

$$m_{corr-agg}=m'_{agg}\times(1-m_{sand}/m_0) \quad (1)$$

where m'_{agg} and $m_{corr-agg}$ represent the weight of each aggregate class before and after sand correction, respectively; and m_{sand} and m_0 denote the weight of sand and the weight used for sand correction, respectively.

Sand-corrected aggregates of various sizes were then passed through a 0.15 mm nylon sieve to measure the aggregate-associated C concentration. The C contents of different aggregates were calculated as:

$$Ci_{cont}=Ci_{conc}\times W_i \quad (2)$$

where Ci_{cont} denotes the carbon content (g kg^{-1} soil) of the *i*-sized aggregate in the bulk soil, Ci_{conc} indicates the carbon concentration (g kg^{-1} aggregate) of the *i*-sized aggregate, and *Wi* represents the proportion (w/w) of the *i*-sized aggregate.

The enrichment factor (*Ef*) was used to evaluate the carbon contents of different aggregate sizes as percentages of the total SOC content. The percentage of aggregate-associated carbon content of a given particle size to total SOC was calculated as Christensen (1992):

$$Ef_{agg}=(Ci_{cont}/C_{bs})\times 100\% \quad (3)$$

where Ci_{cont} and C_{bs} denote the C contents of *i*-sized aggregates and bulk soil, respectively.

Mean weight diameter (MWD) and geometric mean diameter (GMD) were used to assess aggregate stability and size distribution after aggregate isolation (Kemper and Rosenau 1986). Specifically, MWD and GMD were calculated as:

$$MWD=\sum_{i=1}^{n}(Xi\times Wi) \quad (4)$$

$$GMD=\exp[\sum_{i=1}^{n}(Wi\times \ln Xi)/\sum_{i=1}^{n}Wi] \quad (5)$$

where *Xi* and *Wi* represent the mean diameter (mm) and mass proportion (%) of the *i*-sized aggregate, respectively, and *n* denotes the number of sieved aggregate size classes.

Physical fractionation of aggregate-associated carbon The isolation of aggregate-associated carbon fractions followed the method described by Marriott and Wander (2006). Briefly, 10 g of aggregate samples were combined with 45 mL of 0.5% sodium hexametaphosphate and shaken at 180 r min^{-1} for 18 h with 12 glass beads (2 mm) to facilitate dispersion, ensuring that the organic carbon occluded within the aggregates was completely released. The fully dispersed suspensions were then separated using a 53 μm sieve and rinsed with deionized water three to four times until the underflow liquid was clear. Ultimately, what remains on the sieve is POC (which may include some sand) (>53 μm), and what passes through the screen is MAOC (<53 μm). All subsamples were oven-dried at 55°C, weighed, and passed through a 0.15 mm sieve for the determination of carbon concentration.

The calculations for aggregate-associated carbon fraction POC and MAOC contents (g kg^{-1} aggregate) were as follows:

$$POC_{cont}\ (g\ kg^{-1}\ aggregate)=C_{POC}\times f_i \quad (6)$$

$$MAOC_{cont}\ (g\ kg^{-1}\ aggregate)=C_{MAOC}\times(1-f_i) \quad (7)$$

where C_{POC} and C_{MAOC} denote the C concentrations of POC and MAOC fractions in different aggregates, respectively, and f_i refers to the weight fraction of POC in the *i*-sized aggregates.

Non-hydrolyzable C in the MAOC fraction Non-hydrolyzable carbon (NHC) in the MAOC fraction of different aggregate sizes was isolated using a modified acid hydrolysis method (Paul *et al.* 1997). Specifically, a 0.6 g soil sample

was added to 25 mL of 6 mol L^{-1} HCl at 95°C for 16 h. The solution was stirred at 1 h intervals to ensure an adequate reaction. After the reaction, the suspension was centrifuged at 4,000×g for 15 min and the supernatant was removed, while the precipitate was rinsed three times with deionized water to remove the residual Cl^-. After these steps, all samples were oven-dried at 55°C, weighed and ground for the determination of carbon concentration. The HYC was calculated as the difference between the carbon content of the MAOC fraction and NHC. The concentrations of NHC and HYC in the MAOC fractions of different aggregate samples were calculated as follows:

$$C_{NHC}=(C_{after}\times m_{after})/m_{before} \quad (8)$$

$$C_{HYC}=C_{MAOC}-C_{NHC} \quad (9)$$

where C_{after} is the C concentration (g kg^{-1}) after acid hydrolysis, and m_{before} and m_{after} refer to the masses of the soil samples before and after acid hydrolysis, respectively.

The difference in recalcitrant carbon concentration between the different aggregates and the bulk soil was expressed by ΔRC and calculated as:

$$\Delta RC=Ci_{conc-agg}-C_{conc-bs} \quad (10)$$

where $Ci_{conc-agg}$ and $C_{conc-bs}$ refer to the NHC concentration of the *i*-sized aggregates and bulk soil, respectively.

The percentage of recalcitrant C content in various aggregates relative to total organic carbon was calculated as:

$$Ef_{RC}=(C_{rec}\times Wi_{agg}/C_{bs})\times 100\% \quad (11)$$

where C_{rec} is the C concentration of MAOC in the different aggregate sizes, Wi_{agg} refers to the mass proportion of the *i*-sized aggregates, and C_{bs} is the carbon content of the bulk soil.

2.4. Statistical analysis

A one-way analysis of variance (ANOVA) was conducted to evaluate differences in measurement results among the treatments at a significance level of α=0.05. The statistical software SPSS 25.0 was employed for all analyses. Linear regression analyses were employed to explore the relationships between aggregate-related organic carbon concentrations, organic carbon fractions and non-hydrolyzable carbon and total organic carbon contents. All plots and graphical outputs were generated using SigmaPlot 15.0 (Systat Software Inc., Chicago, IL, USA).

3. Results

3.1. Aggregate size distribution and structural stability

The type and quantity of organic amendments significantly influenced the soil aggregate size fractions, with varying degrees of impact among the treatments (Appendix B). Compared to the control (no organic amendments), manure (M10) and granulated organic amendments (G20 and G40) increased the proportions of large macroaggregates (LMA, >2,000 μm) and small macroaggregates (SMA, 2,000–250 μm), while reducing the proportions of microaggregates (MIA, 53–250 μm) and silt+clay fractions (S+C, <53 μm) in both the 0–20 and 20–40 cm soil layers. Among the treatments, the G40 treatment had the most pronounced impact on soil aggregate structure, increasing the proportion of macroaggregates from 25.9% (CK) to 45.1% in the topsoil and from 16.7% (CK) to 36.1% in the subsoil. The MWD and GMD also varied significantly among the treatments in both soil layers (Appendix B). Granulated organic amendments resulted in substantial increases in MWD and GMD compared to the control, with the highest values observed under the G40 treatment.

3.2. Aggregate-associated carbon concentration and content

The aggregate-associated carbon (AAC) concentration increased with aggregate size classes (Fig. 2-A and C). The granulated organic amendments increased the AAC concentrations in LMA and SMA more significantly than in the MIA and S+C fractions (Appendix C-a). Furthermore, granulated organic amendments significantly influenced the carbon content across the aggregate size fractions (Fig. 2-B and D), increasing the carbon content in LMA and SMA while reducing it in the MIA and S+C fractions of the topsoil (Appendix C-b). Among the treatments, the G40 treatment had the most pronounced effect, resulting in the greatest increases in carbon concentrations across all size aggregate fractions and the carbon content of macroaggregates (Fig. 2). Furthermore, the addition of granulated organic amendments increased the enrichment factor in macroaggregates while reducing it in microaggregates and silt+clay particles (Appendix D).

3.3. Analysis of aggregate-associated POC and MAOC

The carbon contents of the aggregate-associated POC and MAOC fractions increased with aggregate size (Fig. 3). The granulated organic amendments increased the POC and MAOC contents more significantly in LMA and SMA compared to the MIA and S+C fractions. Most carbon was stored in the form of organo-mineral complexes, accounting for over 80 and 90% of AAC content in the topsoil and subsoil, respectively. The G40 treatment exhibited the most significant enhancements in the POC and MAOC contents across different aggregate sizes, with the magnitude of increase diminishing as aggregate size decreased (Fig. 3-A). In addition, the MAOC fraction in a free state (unprotected) had a significantly lower carbon content than that protected within aggregates (Fig. 3-B and D). Linear regression analyses revealed strong positive correlations between AAC content and both the POC and MAOC contents (Appendix E). The regression slope for the MAOC fraction was steeper than that for the POC fraction. Furthermore, as aggregate size decreased, the regression slope for the POC fraction declined, while it increased for the MAOC fraction (Appendix E).

3.4. Recalcitrant carbon accumulation and aggregate protection

The NHC content in the MAOC fraction within aggregates increased with decreasing aggregate size and was the highest in microaggregates (Fig. 4-A and B). Within LMA, SMA, and MIA, the NHC concentrations of MAOC were higher than in bulk soil (ΔRC>0), whereas it was lower in the silt+clay particles than in bulk soil (ΔRC<0). The granulated organic amendments increased the NHC content and enrichment factor in LMA and

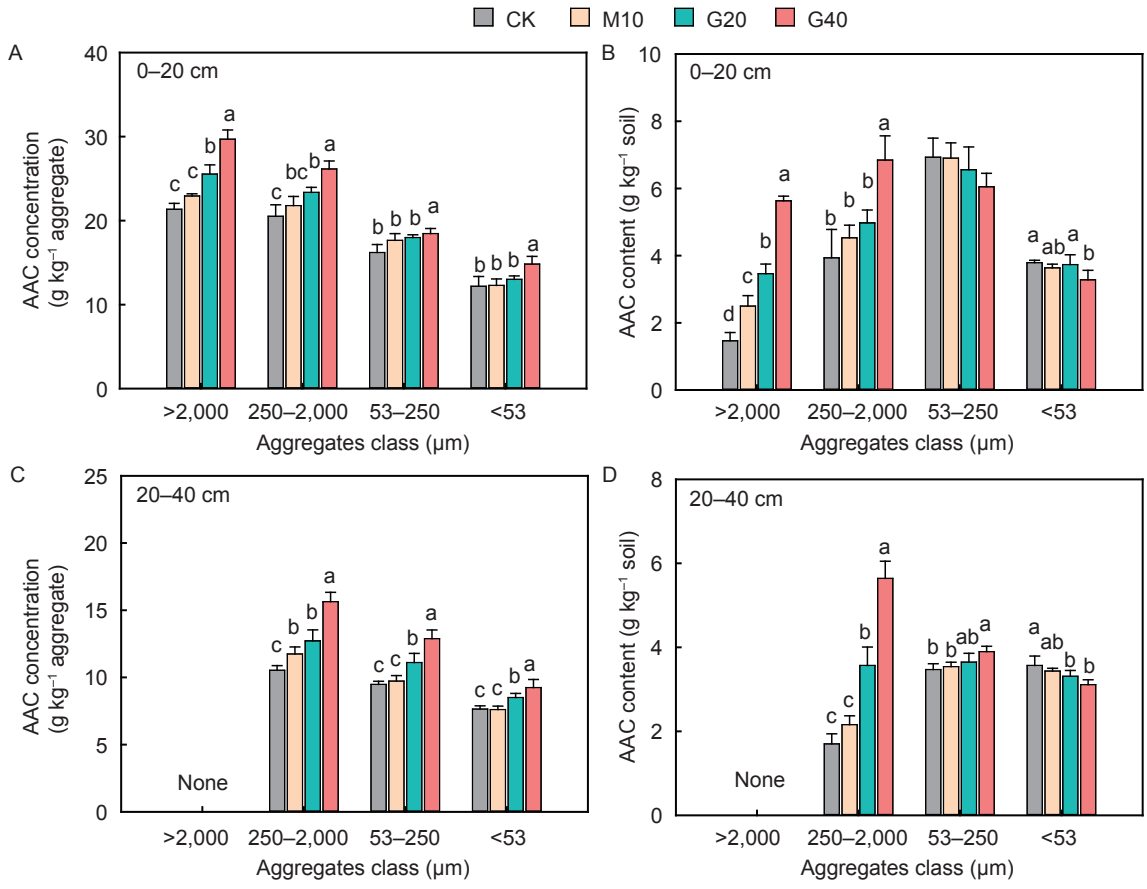


Fig. 2 Aggregate-associated carbon (AAC) concentrations (A and C) and contents (B and D) across different aggregate size classes under different organic amendments treatments in the topsoil (0–20 cm) and subsoil (20–40 cm). CK, no organic amendments; M10, 10 t ha^{-1} composted cattle manure; G20, 20 t ha^{-1} granulated organic amendment; G40, 40 t ha^{-1} granulated organic amendment. Bars with different letters denote statistically significant differences (P<0.05). Error bars represent the standard deviation of the means (n=3).

SMA, while reducing the NHC content in microaggregates and silt+clay particles, with the most significant changes observed under the G40 treatment (Fig. 4-D). Furthermore, the regression gradients for the G40 treatment were significantly higher than for the other treatments. Granulated organic amendments also increased the proportion of intra-microaggregates within macroaggregates from 39.0% (CK) to 62.7% (G40). In contrast to the topsoil, where NHC was primarily associated with macroaggregates, the NHC content in the subsoil was predominantly distributed in the microaggregate and silt+clay fractions (Fig. 5).

Both NHC and HYC showed strong positive linear correlations with MAOC content, and the gradient for HYC was steeper than that for NHC (Fig. 6-A and B). With increasing MAOC in the topsoil (0–20 cm), the NHC and HYC contents protected by aggregates increased exponentially and logarithmically, respectively (Fig. 6-C and D). In the subsoil (20–40 cm), both the NHC and HYC contents increased linearly, and HYC had a steeper gradient than NHC (Fig. 6-C–F).

4. Discussion

4.1. Granulated amendment promotes soil aggregation and stability

The formation and stabilization of soil aggregates are essential for physically protecting organic matter from rapid decomposition and enhancing soil stability (Six *et al.* 2002; Verchot *et al.* 2011). Granulated organic amendment significantly elevated the proportion of macroaggregates and improved soil structural stability in both topsoil and subsoil compared to the control, indicating that granulated organic amendment is a valid strategy for enhancing soil aggregate structure and soil stability. Granulated organic amendments provide a rapid nutrient supply while exerting a lesser impact on the soil structure (Zhang *et al.* 2019; Fan and Wu 2020; Wang *et al.* 2021; Xue *et al.* 2022). Based on the aggregate hierarchy model, macroaggregates form through the binding of mineral particles and microaggregates *via* multi-stage agents, such as polysaccharides and microbial hyphae (Oades 1984; Six *et al.* 2004; Tamura *et al.* 2017). The POC derived from granulated organic amendments can either serve as a direct nucleus for aggregate formation or undergo microbial decomposition and transformation, creating numerous adsorption sites for minerals (Liang *et al.* 2019; Witzgall *et al.* 2021; Situ *et al.* 2022). Subsoils typically experience slower aggregation due to their diminished organic matter inputs, reduced microbial metabolism, and a lack of gluing agents (Totsche *et al.* 2018; Button *et al.* 2022). However, the deep application of granulated organic amendment can enhance organic matter inputs into the

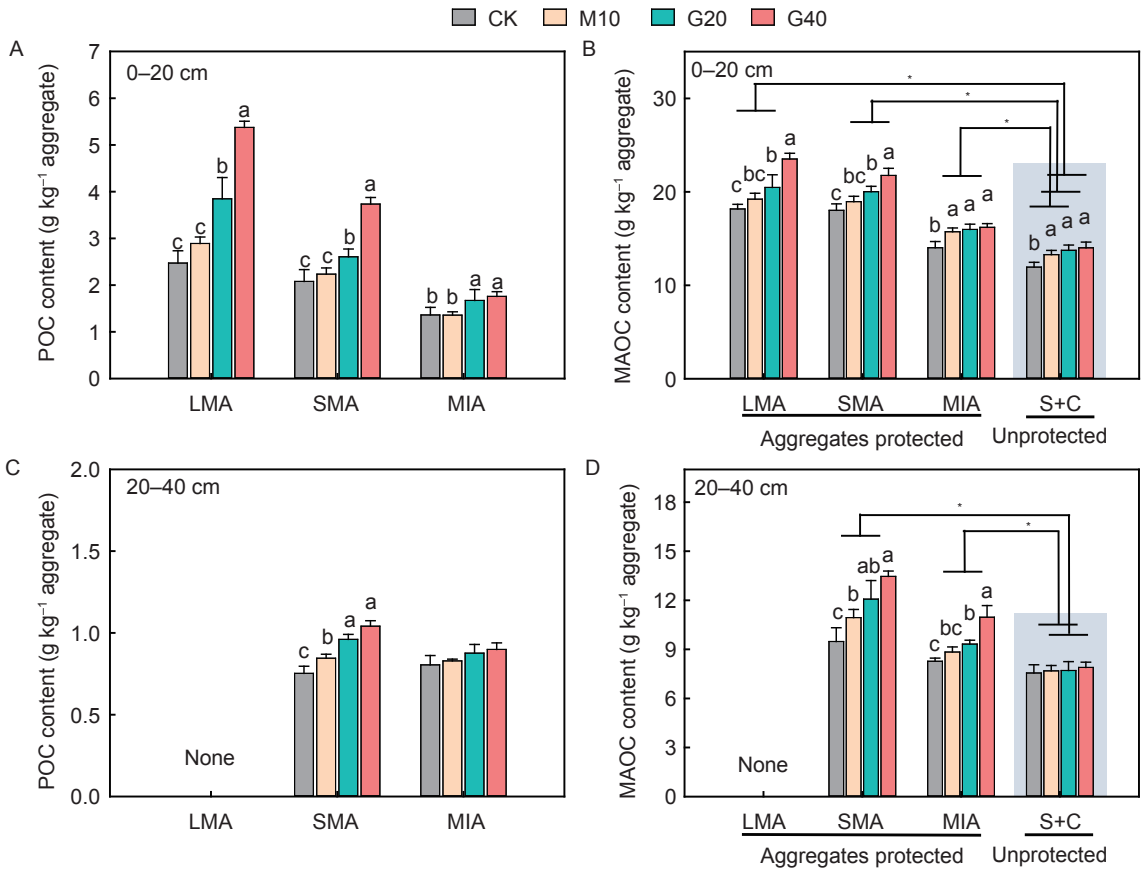


Fig. 3 Carbon contents of the particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) fractions across different aggregate size classes under different organic amendment treatments in the topsoil (0–20 cm) and subsoil (20–40 cm). CK, no organic amendments; M10, 10 t ha^{-1} composted cattle manure; G20, 20 t ha^{-1} granulated organic amendment; G40, 40 t ha^{-1} granulated organic amendment. LMA, large macroaggregates (>2,000 μm); SMA, small macroaggregates (250–2,000 μm); MIA, microaggregates (53–250 μm); S+C, silt and clay (<53 μm). Bars with different letters indicate significant differences (P<0.05). Asterisks (*) denote significant differences between aggregate-protected MAOC and unprotected MAOC. Error bars represent the standard deviation of the means (n=3).

deeper soil layers, and improve the soil environment, thereby promoting the formation of aggregates (Cong *et al.* 2020; Weidhuner *et al.* 2021).

4.2. Granulated amendment facilitates POC and MAOC accrual within various aggregate sizes

Soil aggregates play a key role in regulating carbon accrual and microbially mediated SOC decomposition. Organic matter encapsulated within aggregates of different sizes exhibits varying degrees of decomposition and stability (Balabane and Plante 2004; Bronick and Lal 2005; Qiu *et al.* 2023). Organic material inputs, especially plant residues, significantly increase the AAC concentrations across various particle sizes. Plant residues serve as temporary binding agents, promoting macroaggregate formation (Bucka *et al.* 2019; Cooper *et al.* 2020). Organic carbon encapsulated within large macroaggregates is typically fresher and less stable, leading to rapid carbon cycling and microbial transformations, either through *ex vivo* modification or *in vivo* turnover (Six *et al.* 2000b; Liang *et al.* 2017). As a result, macroaggregates exhibit a higher rate of organic carbon accrual, resulting in distinct carbon content and enrichment factors across aggregate sizes (Yu *et al.* 2015). In this study, organic amendments increased the carbon contents associated with LMA and SMA while reducing them in the MIA and S+C fractions. The organic amendments also facilitated macroaggregate formation by promoting the aggregation of microaggregates and silt+clay particles, which serve as the fundamental building units, thereby redistributing the proportions of different aggregate sizes (Six *et al.* 2000b; Totsche *et al.* 2018). This redistribution of soil aggregate structure ultimately led to greater organic carbon in macroaggregates and corresponding declines in microaggregates and silt+clay particles.

SOC consists of carbon fractions with varying decomposition rates and turnover times (Leite *et al.* 2004; Poeplau *et al.* 2018). To investigate the stabilization process of organic carbon, the two fractions of POC and MAOC were isolated based on their stability (von Lützow *et al.* 2007). MAOC accounted for more than 84% of AAC in the topsoil and over 90% in the subsoil, highlighting its dominance in these soils and its critical role in carbon accrual in barren paddies (Briedis *et al.* 2012; Cotrufo *et al.* 2019). The granulated organic amendments linearly increased the MAOC content across aggregate sizes, suggesting that the soils were in a carbon-deficit state and had significant potential for further sequestration (Di *et al.* 2018). The MAOC content protected by aggregates was notably higher than that of

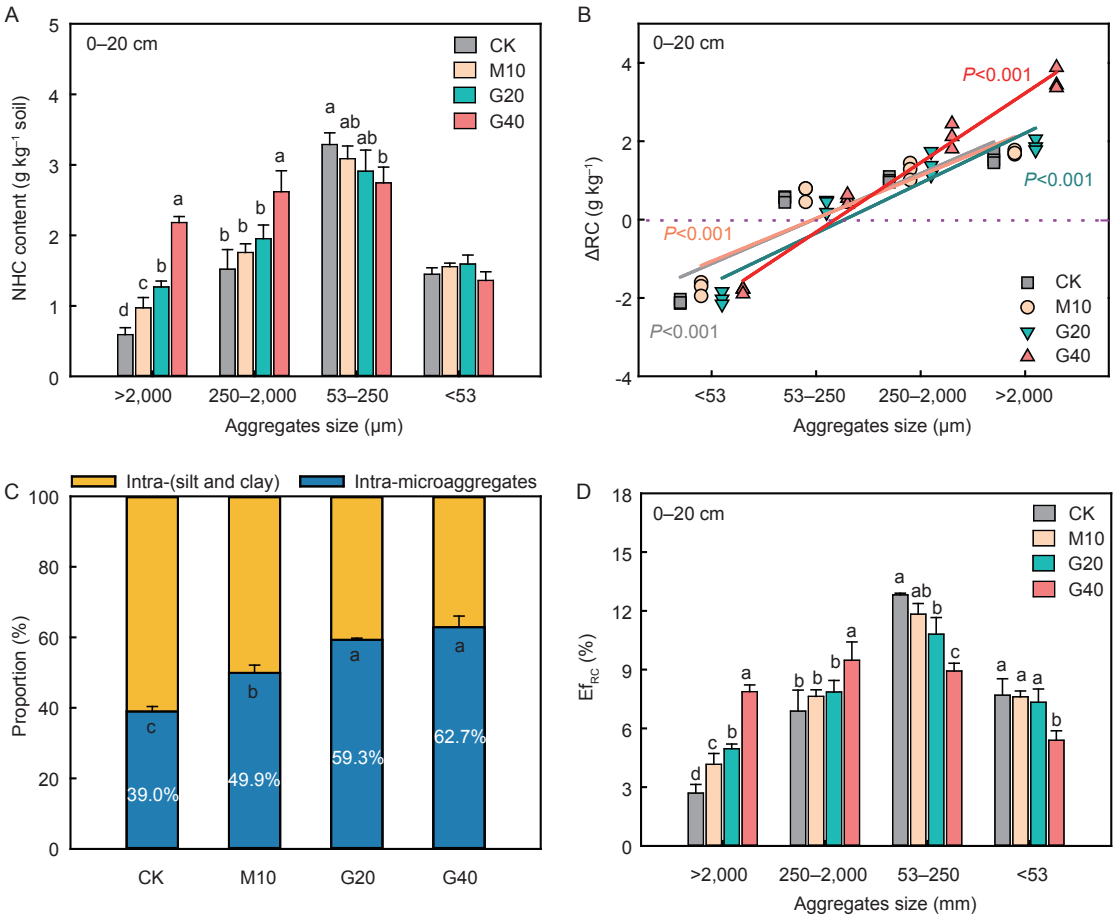


Fig. 4 Effects of different organic amendments on the non-hydrolyzable carbon (NHC) contents within the aggregate associated mineral-associated organic carbon (MAOC) fractions (A), differences in recalcitrant carbon concentrations between various aggregate sizes and bulk soil (B), percentages of microaggregates and silt and clay particles within macroaggregates (C) and the recalcitrant carbon enrichment factor (D) in topsoil (0–20 cm). CK, no organic amendments; M10, 10 t ha^{-1} composted cattle manure; G20, 20 t ha^{-1} granulated organic amendment; G40, 40 t ha^{-1} granulated organic amendment. ΔRC, difference in recalcitrant carbon between aggregates and bulk soil; Ef_{RC}, enrichment factor of recalcitrant carbon. Bars with different letters denote statistically significant differences (P<0.05). Error bars represent the standard deviation of the means (n=3).

easily dispersed MAOC, emphasizing the importance of aggregate protection. The increase in MAOC content with aggregate size (Fig. 3-B) can be attributed to two factors: 1) enhanced physical protection within microaggregates, which reduces carbon loss to microbial metabolism; and 2) the continuous decomposition and fragmentation of fresh organic materials within macroaggregates, which promotes the formation of intra-microaggregates and facilitates the carbon flow towards the silt+clay fraction (Briedis *et al.* 2012; Peng *et al.* 2023; Even and Cotrufo 2024; Fu *et al.* 2024; Ma *et al.* 2024). Without aggregate protection, easily dispersed MAOC components are susceptible to microbial decomposition, resulting in carbon loss (Six *et al.* 2000a; Wiesmeier *et al.* 2012; Keiluweit *et al.* 2015; Han *et al.* 2024). The granulated organic amendment significantly enhanced the contents of aggregate-associated POC and MAOC, consistent with previous findings (Lan *et al.* 2022; Zhang *et al.* 2022; Jia *et al.* 2024). This enhancement can be attributed to the high organic fragment content in granulated amendments, which decompose rapidly and possess a higher specific surface area compared to straw amendment (Wang *et al.* 2017; Cong *et al.* 2020; Wang X *et al.* 2023). Over time, microbial decomposition transforms these organic fragments into smaller, more stable particles, which are incorporated into the MAOC pool (Islam *et al.* 2022; Jia *et al.* 2024). Furthermore, greater organic material inputs stimulate microbial activity, accelerating organic carbon sequestration (Liang *et al.* 2017; Bhattacharyya *et al.* 2022; Tian *et al.* 2022).

In this study, the contribution of POC to AAC content gradually decreased with diminishing aggregate size, dropping from 35% in large macroaggregates to 15% in microaggregates, while MAOC increased from 50 to 85%. This shift reflects the decomposition of fresh residues and their stabilization as intra-aggregate POC (from coarse POC to intra-microaggregate POC), which eventually transitions into microaggregates (Kumar *et al.* 2013; Shen *et al.* 2021). This process involves multiple stages of decomposition and fragmentation of POC, which enhances its chemical stability but ultimately leads to a lower proportion of POC (Amézketa 1999; Violante *et al.* 2008). As a result, macroaggregate-associated carbon has a faster turnover and lower stability compared to microaggregate-associated carbon (An *et al.*

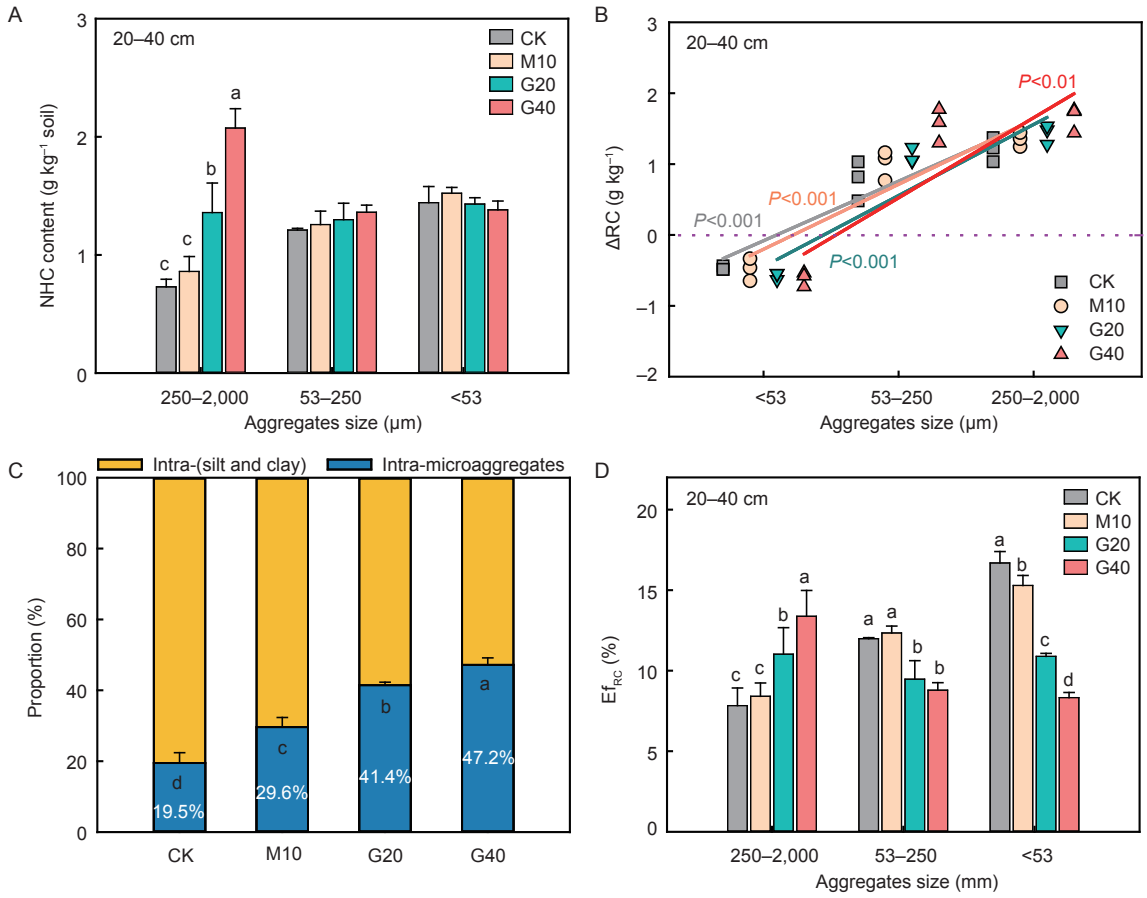


Fig. 5 Effects of different organic amendments on the non-hydrolyzable carbon (NHC) contents within aggregate associated mineral-associated organic carbon (MAOC) fractions (A), differences in recalcitrant carbon concentrations between various aggregate sizes and bulk soil (B), percentages of microaggregates and silt and clay particles within macroaggregates (C) and the recalcitrant carbon enrichment factor (D) in subsoil (20–40 cm). CK, no organic amendments; M10, 10 t ha^{-1} composted cattle manure; G20, 20 t ha^{-1} granulated organic amendment; G40, 40 t ha^{-1} granulated organic amendment. ΔRC, difference in recalcitrant carbon between aggregates and bulk soil; Ef_{RC}, enrichment factor of recalcitrant carbon. Bars with different letters denote statistically significant differences (P<0.05). Error bars represent the standard deviation of the means (n=3).

2010; Liu *et al.* 2023; Even and Cotrufo 2024). In the subsoil, POC contributes less than 5% to AAC, with MAOC being the primary form of SOC storage (Angst *et al.* 2021; Islam *et al.* 2022). This layer typically receives less organic matter input, consisting mainly of dissolved organic carbon (DOC) or small POC fragments transported from the surface through soil capillaries (Fig. 7). These small molecular organics are directly immobilized through mineral adsorption, which serves as the dominant mechanism of carbon accumulation in subsoils (Poirier *et al.* 2020; Kleber *et al.* 2021; Wang Q *et al.* 2023).

4.3. Physical protection of MAOC by microaggregates reduces recalcitrant carbon loss

Organo-mineral complexes formed through the adsorption of SOC onto mineral surfaces represent a crucial mechanism for soil carbon stabilization (Marschner *et al.* 2008; Rumpel *et al.* 2010; Tian *et al.* 2022). However, the composition of MAOC is highly variable, encompassing both easily decomposable organic detritus and recalcitrant organic matter with complex molecular structures (Jilling *et al.* 2021; Villarino *et al.* 2023). This variability implies that MAOC serves as a major substrate for microbial decomposition, particularly when POC is absent or not protected by aggregates (Hall *et al.* 2015; Keiluweit *et al.* 2015; Even and Cotrufo 2024; Zhou *et al.* 2024). MAOC can be classified into two categories: aggregate-protected occluded MAOC (oMAOC) and easily dispersed free MAOC (fMAOC) (Lavallee *et al.* 2020). In this study, the silt+clay fraction (<53 μm) obtained through wet sieving was considered as fMAOC and subjected to acid hydrolysis (6 mol L^{-1} HCl) along with oMAOC to investigate the effects of aggregate protection on the chemical stability of MAOC (Plante *et al.* 2006). Acid treatment separates carbon into two fractions based on chemical stability: NHC representing recalcitrant carbon, and HYC representing labile carbon (Paul *et al.* 1997). Our results indicated that microaggregates were the primary storage site for recalcitrant carbon and had the highest NHC concentrations (Fig. 4-A). Granulated organic amendments increased NHC concentrations across all aggregate sizes and, coupled with enhanced aggregation, promoted the physical protection of recalcitrant carbon (Abiven *et al.* 2011; Nisar and Benbi 2024). Compared with fMAOC, the NHC concentration was significantly higher in oMAOC, which might be ascribed to the rapid carbon turnover

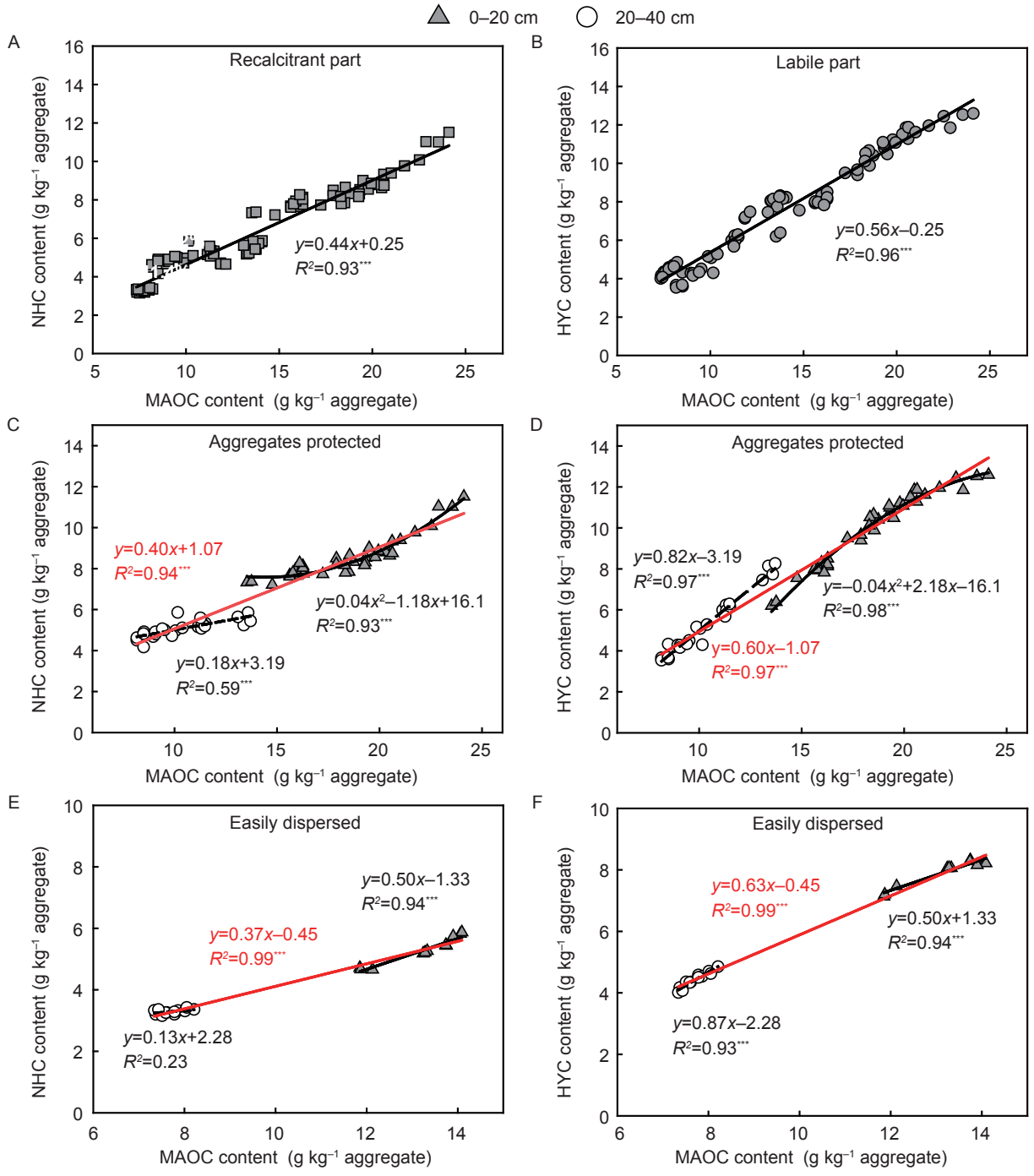


Fig. 6 Linear regression analysis of mineral-associated organic carbon (MAOC) content with non-hydrolyzable carbon (NHC) (A) and hydrolyzable carbon (HYC) (B) concentrations (with all treatments, depths and aggregates combined); and linear regression of MAOC content with the NHC (C) and HYC (D) concentrations for aggregate-protected fractions, as well as the NHC (E) and HYC (F) concentrations for readily dispersible fractions. ***, $P<0.001$.

within macroaggregates, and this fosters the formation and accumulation of recalcitrant carbon (Fig. 6-C). Conversely, fMAOC, which lacks aggregate protection, is more susceptible to microbial activity, leading to greater recalcitrant carbon loss (Keiluweit *et al.* 2015; Giannetta *et al.* 2019). These findings emphasize the importance of aggregate protection in recalcitrant soil carbon accrual (Adu and Oades 1978; Su *et al.* 2020). However, the fMAOC fraction in the subsoil had the highest recalcitrant carbon content and enrichment factor (Fig. 5-A and D), which can be primarily attributed to the subsoil's lower carbon saturation and higher bulk density inhibiting microbial activity and organic carbon decomposition (Pulido-Moncada *et al.* 2020; Button *et al.* 2022; Liebmann *et al.* 2022). Consequently, despite the absence of aggregate protection, organic carbon in the subsoil demonstrates high chemical stability, with mineral adsorption serving as the primary stabilization mechanism.

Overall, the proportion of recalcitrant carbon in MAOC

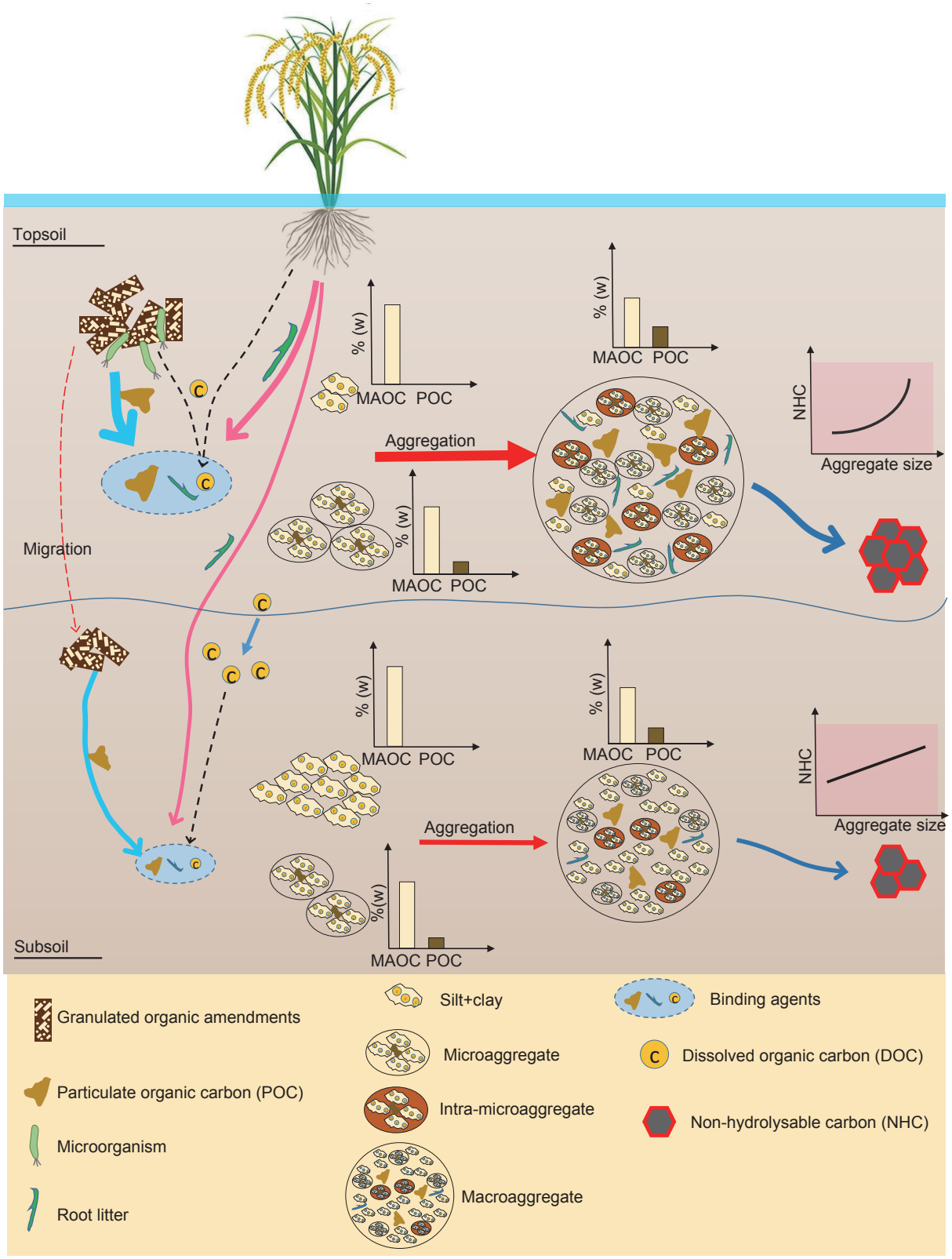


Fig. 7 Conceptual framework illustrating the coupling of organic amendments with aggregation processes and organic carbon accumulation in the topsoil (0–20 cm) and subsoil (20–40 cm). MAOC, mineral-associated organic carbon; % (w), mass percentage of MAOC or POC in aggregates. Differences in organic material inputs to the surface and sub-surface layers lead to differing intensities of soil aggregation processes and distinct mechanisms of organic carbon stabilization.

was slightly lower than that of labile carbon, suggesting that labile carbon contributes substantially to MAOC formation. Within aggregates, the accumulation rate of recalcitrant carbon increased with aggregate size (Fig. 6). Larger macroaggregates, enriched with POC, facilitate the carbon transformation and stabilization processes, which favors

the formation of recalcitrant carbon. Furthermore, these POCs contribute to the formation of microaggregates within macroaggregates (Fig. 7). Even when macroaggregates are disrupted or fragmented, stabilized carbon within the microaggregates is retained, which ensures long-term organic carbon conservation.

5. Conclusion

Granulated organic amendments significantly enhanced soil aggregation and structural stability, leading to elevated aggregate-associated carbon concentrations. The formation of macroaggregates improved the protection of POC and facilitated the development of intra-microaggregates. Recalcitrant carbon predominantly formed within macroaggregates and was subsequently preserved in microaggregates over the long term, accompanied by aggregate turnover processes. The aggregate protection not only accelerated the stabilization of organic carbon within macroaggregates but also prevented carbon loss through mineralization. In the subsoil, where organic matter inputs are limited and organic carbon saturation is low, mineral adsorption emerged as the primary mechanism for carbon stabilization. The addition of 40 t ha^{-1} of granulated organic amendment demonstrated the most pronounced effects of significantly enhancing macroaggregate formation, organic carbon sequestration, and carbon stability. This approach provides a reliable and effective strategy for improving soil quality and promoting carbon storage in barren paddy soils.

Acknowledgements

This work was financially supported by the National Key Research and Development Program of China (2024YFD1900104 and 2021YFD1901203), the National Natural Science Foundation of China (42177293, 42130716 and U23A2009), and the Chinese Academy of Sciences Talent Plan Program.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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

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

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.05.004

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