

Article

Combined Effects of Biochar and Milk Vetch with Reduced Nitrogen Application on Rice Yield, Soil Aggregation, and Carbon and Nitrogen Distribution in Reddish Paddy Soil

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Abstract

Although rice straw biochar (RSB) and milk vetch (MV) facilitate rice production and soil carbon and nitrogen (N) storage, their combined effects under N-reduction remain unclear, particularly at the aggregate-scale in reddish paddy soils. A two-year field experiment with four treatments: 100% N (N₁₀₀), 20% N-reduction + RSB (N_{80B}), 20% N-reduction + MV (N_{80M}), and 20% N-reduction + RSB + MV (N_{80BM}), was conducted in reddish paddy soil. Rice yield, soil organic carbon (SOC) and total N (TN) stocks, water-stable aggregate distribution and stability, and aggregate-associated C and N allocation were measured. Over the two-year study, N_{80BM} significantly increased grain yield by 11.2%, SOC_s by 5.42%, and TN_s by 6.92% compared with N₁₀₀ ($p < 0.05$). RSB and MV combined with N-reduction increased macroaggregate proportion by 7.92–11.8% and aggregate stability by 7.14–58.1%, while decreasing silt–clay fraction by 39.6–62.9%. Soil C and N enrichment occurred predominantly in macroaggregates. Pearson correlation and random forest analyses jointly identified aggregate distribution and stability as primary predictors of rice yield, SOC_s, and TN_s. Therefore, RSB + MV combined with 20% N-reduction sustained rice yield while enhancing soil aggregation and C, N sequestration, providing a rational strategy for N management in sustainable rice production.

Keywords: biochar; milk vetch; N-reduction; rice yield; soil aggregation; carbon and nitrogen stocks

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1. Introduction

Carbon (C) and nitrogen (N) are key elements in ecosystem biogeochemical cycles [1]. Paddy fields are important reservoirs of C and N [2]. Globally, C sequestration in these systems largely depends on the accumulation of recalcitrant organic C, and Chinese paddy soils contain over 20% greater C stocks per unit area than other croplands [3]. Originating mainly from Quaternary red clay, reddish paddy soils are an important anthropogenic wetland soil type in southern China. In these systems, C and N cycling strongly influence soil fertility and crop productivity [4]. Prolonged anaerobic conditions during rice cultivation slow organic matter decomposition [5]. Meanwhile, abundant Fe/Al oxides bind organic C and, through alternate wetting–drying cycles, promote macroaggre-

gate formation that physically encapsulates organic matter, providing both chemical and physical protection [6]. These interacting processes underpin the high C stocks in reddish paddy soils [7]. However, acidic soils are highly susceptible to further acidification and structural degradation under intensive N fertilization [8,9]. Excessive N inputs accelerate base cation depletion, weaken aggregate cementation, and intensify C and N mineralization. Consequently, C and N losses threaten long-term soil health and agroecosystem productivity [10]. Long-term intensive cultivation with excessive N fertilization further depletes soil organic matter, reduces aggregate stability, lowers C and N retention, and increases greenhouse gas (GHG) emissions [11]. Therefore, integrated C and N management has become a critical strategy for ensuring food security and achieving China's 2060 carbon neutrality target [10].

It is well established that sustainable rice production relies on a balanced supply of nutrients (e.g., nitrogen, phosphorus, and potassium) and beneficial elements such as silicon, which strengthens stem rigidity and reduces lodging risk [12]. While phosphorus and potassium are essential for root development and stress tolerance, their recommended application rates are generally sufficient to maintain soil fertility in reddish paddy soils [13]. In contrast, N remains the primary yield-limiting nutrient and poses the greatest environmental challenge because of its high mobility and susceptibility to gaseous and leaching losses [14]. Over the past decades, excessive N fertilization has become widespread in rice production systems in southern China, driven by the pursuit of maximum yields. However, this practice not only reduces N use efficiency but also accelerates soil acidification, organic matter depletion, and aggregate disintegration while increasing N₂O emissions and reactive N losses to water bodies [15,16]. Therefore, reducing N inputs without compromising productivity is a critical goal for sustainable rice production in this region.

Soil aggregates, the basic structural units of soil, are key determinants of C and N stabilization through physical protection and chemical binding, thereby regulating C and N cycling and crop productivity [17]. Stable aggregates account for more than 60% of soil organic C (SOC) retention by reducing mineralization losses through physical encapsulation and chemical adsorption [18]. However, long-term excessive and unbalanced N fertilization causes soil acidification, base cation leaching, microbial community shifts, and altered microbial metabolism. These changes weaken aggregate cementation, promote fragmentation, and accelerate C and N mineralization losses [19,20]. Conversely, reducing N inputs and integrating organic amendments can decrease nitrate leaching, ammonia volatilization [12,21], and N₂O emissions [22], while sustaining productivity and improving environmental quality [23].

Biochar and green manure (GM) are effective organic amendments that improve soil health, crop productivity, and stabilize C and N pools in paddy soils [18]. Biochar, a porous, carbon-rich material, enhances soil organic carbon, total N, and cation exchange capacity, while its recalcitrant fraction promotes C sequestration and aggregate stability [24]. Owing to its high specific surface area and aromatic structure, biochar directly increases soil C and N storage [25,26]. Its abundant oxygen-containing functional groups (e.g., carboxyl and phenolic hydroxyl groups) enhance organo-mineral interactions, while its fine particles promote macroaggregate formation and structural stability, thereby improving SOC distribution among aggregate fractions [27,28]. Moreover, biochar application significantly increases total and recalcitrant organic C in aggregates, enhancing aggregate stability and SOC persistence [29]. It also improves N retention, increases N use efficiency and crop productivity, and enriches bacterial diversity, particularly N-cycling functional microbes [30].

Milk vetch (MV), a widely used leguminous green manure (GM), improves soil aggregation and soil C and N status by utilizing agricultural resources (e.g., heat, light, and

water) during winter fallow periods, thereby enhancing soil C and N stocks and rice productivity [31]. As a legume, MV enriches soil N through symbiotic biological N fixation by *Rhizobium*, thereby reducing reliance on synthetic N fertilizers [32,33]. Previous studies have shown that MV increased soil NH_4^+ and total N through enhanced biological N fixation and autotrophic nitrification [34,35]. Meanwhile, MV residues can partially substitute for chemical N fertilizers and improve rice yield and SOC storage [31,36]. However, approximately 60% of GM-derived C and N is mineralized during the growing season [37]. This process increases soluble and oxidizable C and N pools, potentially triggering a priming effect that destabilizes SOC, reduces SOC stabilization, and increases C and N loss risk [38].

Although the individual effects of biochar and MV on soil C and N dynamics are well documented [22,31], their combined impact under reduced N application for rice productivity and aggregate-associated C and N distribution in reddish paddy soils remains poorly understood. Therefore, we hypothesized that: (i) the combined application of RSB and MV under 20% reduced N input would enhance rice yield compared with traditional N fertilization, (ii) RSB and MV would promote macroaggregate formation and stability, thereby increasing C and N distribution in >0.25 mm aggregates; and (iii) aggregate distribution and stability would be the primary predictors of SOC and TN stocks and rice yield. This study, therefore, aimed to provide a practical N management strategy for sustainable rice production while demonstrating the value of recycled organic resources for improving soil structure, enhancing rice productivity, and promoting long-term C sequestration.

2. Materials and Methods

2.1. Experimental Site, Soil and Climate

The two-year field experiment was conducted from 2021 to 2022 at the Jiangxi Institute of Red Soil and Germplasm Resources, Jinxian County, Jiangxi Province, China ($116^{\circ}16'$ E, $28^{\circ}20'$ N; 137 m above sea level). The region has a subtropical monsoon climate. Meteorological data for the rice growing seasons in 2021 and 2022, provided by the Jiangxi Meteorological Bureau, are presented in Table S1.

The experimental soil was classified as a Hydragric Anthrosol, derived from Quaternary reddish clay with a clay loam texture, according to the World Reference Base for Soil Resources (WRB, 2022) classification [39]. Before the experiment began, initial topsoil (0–20 cm) samples were collected using the five-point sampling method, and their physicochemical properties are presented in Table S2.

2.2. Milk Vetch, Rice Straw Biochar Preparation and Characterization

The commonly cultivated local variety of milk vetch (*Astragalus sinicus* L., var. Ganzi No. 1; MV) was used in this study. The MV seeds were obtained from Guilong Rice Family Farm, Dengyuan, Yujiang District, Yingtan City, Jiangxi province, China. Seeds were broadcast uniformly across the field without row spacing at a rate of 30 kg ha^{-1} in the N_{80}M and N_{80}BM plots during mid-to-late October of each year. Because seeds were broadcast, no fixed plant spacing was used. MV was grown throughout the winter fallow period and incorporated into the 0–20 cm soil layer at the full-bloom stage in mid-April, 10–15 days before rice transplanting. No fertilizers were applied during the winter fallow period, regardless of whether the plots were left fallow or planted with MV.

Rice straw used for biochar production was collected from the same experimental plots under identical management practices (including cultivation conditions, fertilization history, and agronomic management), to ensure feedstock consistency. Air-dried rice straw (3–5 cm pieces) was pyrolyzed in an electric muffle furnace (SX2-12-10N, Shanghai

Yiheng Technology Instrument Co., Ltd., Shanghai, China) under oxygen-limited conditions at a heating rate of about 20 °C min⁻¹ to 450–500 °C, maintained for 2 h, and then allowed to cool naturally to room temperature, resulting in a biochar yield of approximately 35% (*w/w*). The resulting RSB was passed through a 5 mm sieve before field application. RSB was prepared annually following rice harvest.

The total C and N contents of MV and RSB were determined using an elemental Analyzer (Vario Max, Elementar, Langenselbold, Germany) following Zhou et al. (2026) [40,41]. The pH of RSB was tested at a biochar-to-water ratio of 1:2.5 (*w/v*) [42]. Electrical conductivity (EC) was measured using a conductivity meter (Mettler 175 Toledo, FE30, Columbus, OH, USA) at a biochar-to-water ratio of 1:10 (*w/v*), and ash content (AC) was determined according to the method described by Morais et al. (2024) [43]. Volatile matter (VM) content was determined by thermogravimetric analysis following the method described by Babinszki et al. (2024) [44]. The specific surface area of RSB was determined by the Brunauer–Emmett–Teller (BET) method based on N adsorption at 77 K with a surface area analyzer (TriStar II 3020, Micromeritics, Norcross, GA, USA) [41]. The physicochemical properties of MV and RSB are presented in Table S3.

2.3. Experimental Design and Field Management

A two-year field experiment (2021–2022) was conducted using a randomized complete block design with four treatments and three replications. The treatments were: (1) 100% recommended N fertilizer (N₁₀₀), (2) 20% N-reduction + RSB (N₈₀B), (3) 20% N-reduction + MV (N₈₀M), and (4) 20% N-reduction + RSB + MV (N₈₀BM). The N₁₀₀ treatment received the recommended chemical N fertilizer rate (150 kg N ha⁻¹), whereas the remaining treatments received 20% less N (120 kg N ha⁻¹). The 20% N reduction was selected based on our previous studies indicating that this reduction can be compensated by organic amendments without compromising grain yield and is consistent with China's national policy to reduce fertilizer use while maintaining food security [38]. Each plot measured 20 m² and was separated by plastic-film-covered ridges with 1 m buffer zones.

The 25-day-old rice seedlings (*Oryza sativa* L., var. Xiangzaoxian 45) were transplanted at a spacing of 15 cm × 30 cm on 25 April 2021 and 26 April 2022, and harvested on 20 July 2021 and 22 July 2022, respectively. The field was managed under an alternating flooding-drainage regime during the first two growth stages, followed by intermittent irrigation from grain filling until physiological maturity.

The recommended fertilizer rates were 150 kg N ha⁻¹ (urea, 46.4% N; Sinopec, Beijing, China), 75 kg P₂O₅ ha⁻¹ (superphosphate, 12.0% P₂O₅), and 120 kg K₂O ha⁻¹ (potassium chloride, 60.0% K₂O). Chemical fertilizers were applied only to rice crops, whereas P and K were applied at the same recommended rates across all treatments (Table S4). MV was mechanically in situ incorporated into the 0–20 cm soil layer at a rate of 22.5 t ha⁻¹ at the full-bloom stage, 5 days before rice transplanting, after which the field was flooded to a depth of approximately 5 cm. RSB was simultaneously applied at 4 t ha⁻¹, and the same fertilizer management practices were followed in the second year (Table S4).

For urea application, 50%, 20%, and 30% of the total N were applied as basal, tillering, and panicle initiation fertilizers, respectively. Basal chemical P and K fertilizers were applied uniformly across all treatments 1 day before transplanting (Table S4).

2.4. Soil Sampling and Analysis

To preserve the natural soil structure, soil samples were collected and handled carefully to minimize aggregate disturbance. Three undisturbed soil samples (0–20 cm) were collected from each plot immediately after rice harvest in early-mid July 2022. The samples were gently broken along natural fracture planes into approximately 1 cm soil clods, cleared of debris (e.g., stones and plant roots), air-dried, passed through an 8 mm sieve,

and stored for aggregate analysis. In addition, bulk soil samples (0–20 cm) were collected using the five-point sampling method with a soil auger to determine soil pH, SOC, and TN contents according to the methods described by Huang et al. (2025) [45]. The C:N ratio was calculated simultaneously. Soil bulk density (SBD) was measured by the 100 cm³ core method after drying the samples at 105 °C to constant weight [32]. Aggregate samples were collected adjacent to the bulk-soil sampling locations.

Water-stable aggregates were separated using the wet sieving method [46]. Briefly, 100 g of air-dried soil was placed in an aggregate analyzer (DM200-II, manufactured by Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China) fitted with a nest of 2, 0.25, and 0.053 mm sieves. After soaking for 20 min with the water level maintained above the upper sieve, the samples were oscillated with a 3 cm amplitude at 30 cycles min^{−1} for 15 min. Four aggregate fractions were obtained; mega macroaggregates (>2 mm), macroaggregates (0.25–2 mm), microaggregates (0.053–0.25) and the silt + clay fraction (<0.053 mm). Each fraction was oven-dried at 105 °C for 12 h, and its dry weight was recorded.

The sand fraction (>0.053 mm) in each aggregate fraction was corrected using the pipette method described by Elliott (1986) [46]. The proportions of four aggregate fractions were expressed as D₁ (>2 mm), D₂ (0.25–2 mm), D₃ (0.053–0.25 mm), and D₄ (<0.053 mm). SOC and TN contents of each aggregate fraction were determined according to Huang et al. (2025) [45] and expressed as SOC₁–SOC₄ and TN₁–TN₄, respectively. The corresponding C:N ratios (R₁–R₄) were calculated simultaneously.

Rice grain was separated from straw using a plot thresher at physiological maturity. Grain yield was determined for the entire plot in 2021 and 2022 and standardized to 14% grain moisture content. Straw biomass was measured from the entire plot. A 500 g sub-sample was oven-dried at 65 °C to constant weight to determine moisture content (ϕ_i), and total straw dry matter was calculated accordingly. Total aboveground biomass was calculated as the sum of grain yield and straw biomass.

2.5. Data Processing

2.5.1. Parameters Related to Soil Water-Stable Aggregates Stability

The mean weight diameter (MWD, mm) and geometric mean diameter (GMD, mm) were calculated by Formulas (1) and (2), respectively [47]:

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

$$GMD = \exp \left[\frac{(\sum_{i=1}^n w_i \ln \bar{d}_i)}{(\sum_{i=1}^n w_i)} \right] \quad (2)$$

where $\bar{d}_i$ is the average diameter of water-stable aggregates of each particle size (mm). w_i is the weight percentage of water-stable aggregates of each particle size (%).

The content of water-stable aggregates with a particle size larger than 0.25 mm ($WR_{>0.25}$, %) was calculated by Formula (3) [47]:

$$WR_{>0.25} = \frac{M_{w>0.25}}{M_t} \times 100\% \quad (3)$$

where $M_{w>0.25}$ is the weight of water-stable aggregates with particle size > 0.25 mm (g), M_t is the total weight of water-stable aggregates (g).

2.5.2. Soil Organic C Sequestrations and Total N Stocks

The soil organic C sequestration (SOC_s , Mg C ha^{−1}) and the total N stocks (TN_s , Mg N ha^{−1}) were calculated by Formulas (4) and (5) respectively [48]:

$$SOC_s = SOC \times BD \times h \times 10 \quad (4)$$

$$TN_s = TN \times BD \times h \times 10 \quad (5)$$

where SOC or TN are the contents of organic C or total N (g kg^{-1}) of bulk soil, respectively (0–20 cm), BD is the soil bulk density (g cm^{-3}), h is the soil layer depth (0.20 m), and 10 is the conversion factor.

2.5.3. Soil C and N Stock Rates

The sequestration rate of C (CSR , $\text{Mg C ha}^{-1} \text{ yr}^{-1}$) and N (NSR , $\text{Mg N ha}^{-1} \text{ yr}^{-1}$) were calculated by the Formulas (6), (7), (8), and (9), respectively [48]:

$$CSR = \frac{\Delta SOC_s}{t} \quad (6)$$

$$\Delta SOC_s = SOC_{ss} - SOC_{is} \quad (7)$$

$$NSR = \frac{\Delta N_s}{t} \quad (8)$$

$$\Delta N_s = TN_{ss} - TN_{is} \quad (9)$$

where ΔSOC_s and ΔN_s are changes in $SOCs$ (Mg C ha^{-1}) and TNs (Mg N ha^{-1}), respectively. SOC_{ss} and TN_{ss} represent the $SOCs$ and TNs of the sampling year in 2022, while the SOC_{is} and TN_{is} represent the $SOCs$ and TNs of the initial year of the field trial in 2021, respectively.

2.5.4. Contribution Rates of Aggregate-Associated Organic C and Total N to That of Bulk Soil

The contribution rates of aggregate-associated organic C or total N in particle sizes at >2 mm (CRC_1 or CRN_1), 0.25–2 mm (CRC_2 or CRN_2), 0.053–0.25 mm (CRC_3 or CRN_3) and <0.053 mm (CRC_4 or CRN_4) to that of bulk soil organic C or TN, respectively, were calculated by Formula (10) [48]:

$$CR = \frac{C_i \times W_i}{C_t} \quad (10)$$

where CR was the contribution rate (%), c_i was the contents of SOC or TN in soil aggregates of a certain particle size (g kg^{-1}), w_i was the weight percentage of each particle size of soil water-stable aggregate (%), c_t was the contents of SOC or TN (g kg^{-1}) of topsoil.

The relative accumulation of SOC and TN in soil aggregates with different particle sizes at >2 mm (EFC_1 or EFN_1), 0.25–2 mm (EFC_2 or EFN_2), 0.053–0.25 mm (EFC_3 or EFN_3) and <0.053 mm (EFC_4 or EFN_4) was calculated using the enrichment factors of SOC (EFC) and total N (EFN), and were calculated by the Formulas (11) and (12), respectively [49]:

$$EFC = \frac{SOC_{\text{certain aggregate}} (\text{g} \cdot \text{kg}^{-1})}{SOC_{\text{bulk soil}} (\text{g} \cdot \text{kg}^{-1})} \quad (11)$$

$$EFN = \frac{TN_{\text{certain aggregate}} (\text{g} \cdot \text{kg}^{-1})}{TN_{\text{bulk soil}} (\text{g} \cdot \text{kg}^{-1})} \quad (12)$$

where $SOC_{\text{certain aggregate}}$ and $TN_{\text{certain aggregate}}$ were the SOC and TN contents (g kg^{-1}) of a certain particle size of water-stable aggregates, $SOC_{\text{bulk soil}}$ and $TN_{\text{bulk soil}}$ were the SOC and TN contents (g kg^{-1}) of bulk soil, respectively.

2.6. Statistical Analysis

All data were analyzed using the SAS package (SAS Institute, v.9.1, Cary, NC, USA). Two-way analysis of variance (ANOVA) was used to evaluate the effects of “Treatment, Year” and their interaction on grain yield, straw biomass, and shoot biomass. Because soil samples were collected only in 2022, one-way ANOVA was used to assess the effects of

four treatments on soil properties, including soil pH, SOC content, TN content, soil bulk density, SOC_s, TN_s, the N storage rates (NSR) and C storage rates (CSR), aggregate size distribution (four fractions), aggregate stability indices (MWD, GMD, $WR_{>0.25}$), aggregate-associated SOC and TN contents, C:N ratios of aggregates, contribution rates (CRC and CRN), and enrichment factors (EF_{SOC} and EF_{TN}). Normality was confirmed by the Shapiro–Wilk test, and Levene’s test indicated homogeneity of variances ($p > 0.05$). Tukey’s HSD multiple range test was performed for post hoc pairwise comparisons ($p < 0.05$). Pearson correlation analysis was conducted to estimate the relationships among SOC_s, TN_s, grain yields, and soil aggregate stability indices.

Random forest modeling, implemented in R (version 4.3.3) using the “randomForest” and “caret” packages, was employed to evaluate the primary predictors of rice yield, SOC_s and TN_s. The dataset comprised 35 predictors (distributions of soil aggregates with particle size at >2 mm, 0.25–2 mm, 0.053–0.25 mm and <0.053 mm; aggregate stability; aggregate-associated SOC and TN contents, as well as their C:N ratios with particle size at >2 mm, 0.25–2 mm, 0.053–0.25 mm and <0.053 mm; contribution rates of aggregate-associated organic C and total N with particle size at >2 mm, 0.25–2 mm, 0.053–0.25 mm, and <0.053 mm to that of bulk soil; enrichment factor of aggregate-associated organic C and total N with particle size at >2 mm, 0.25–2 mm, 0.053–0.25 mm and <0.053 mm.) with 12 observations per rice season (four treatments $\times$ three replicates) per rice season. Model hyperparameters were optimized with mtry tested at 2, 3, and 4, while ntree was fixed at 500. Predictive stability was evaluated using leave-one-out cross-validation (LOOCV), repeated 10 times, with final R^2 and root mean square error (RMSE) averaged across runs. Variable importance was quantified by the mean percentage increase in mean squared error (%IncMSE) with 95% confidence intervals derived from 1000 bootstrap iterations. The final model, trained on all 12 observations, was used for interpretation. The figures and tables were prepared by using Sigmaplot 14.0 (Systat Software, San Jose, CA, USA) and MS Excel (Microsoft Corporation, Redmond, WA, USA), respectively.

3. Results

3.1. Straw Biomass, Grain Yields, and Shoot Biomass of Rice Plants

According to the two-way ANOVA analysis (Table 1), the effects of “Treatment” and “Year” on grain yield, straw, and shoot biomass were highly significant ($p < 0.01$ or $p < 0.001$). However, the interaction between the two factors was not significant for any of the three variables ($p > 0.05$).

The combination of RSB and MV with 20% N reduction effectively increased rice shoot biomass (Table 1). Relative to N₁₀₀, although the N_{80B} treatment showed no significant difference in grain yield or straw biomass ($p > 0.05$), the N_{80M} and N_{80BM} treatments significantly enhanced grain yield by 6.83% and 11.2%, and straw biomass by 5.67% and 10.0%, respectively. Consequently, shoot biomass in both treatments increased by 6.25% and 10.6%, respectively, compared with the N₁₀₀ treatment ($p < 0.05$).

In addition, interannual variation was pronounced, with the grain yield, straw biomass, and shoot biomass in 2021 being significantly higher than those in 2022, by 11.0%, 8.31%, and 9.68%, respectively.

Table 1. Two-way ANOVA and Tukey’s multiple comparison of the effects of RSB and MV applied with N reduction on rice grain yields, straw biomass, and shoot biomass.

Variables	Grain (kg ha ⁻¹)	Straw (kg ha ⁻¹)	Shoot (kg ha ⁻¹)
F-values			
Treatments	16.7 ***	7.09 **	18.2 ***
Years	113.4 ***	26.4 ***	95.3 ***

Treatments × Years	0.66 ^{ns}	0.41 ^{ns}	0.30 ^{ns}
Treatment marginal means			
N ₁₀₀	5972.1 ± 131.3 c	6035.0 ± 100.6 c	12,007.1 ± 52.9 c
N _{80B}	6197.0 ± 88.9 bc	6082.6 ± 119.3 bc	12,279.7 ± 201.1 bc
N _{80M}	6380.2 ± 192.6 ab	6377.3 ± 54.1 ab	12,757.5 ± 218.0 ab
N _{80BM}	6638.3 ± 92.6 a	6641.1 ± 292.5 a	13,279.4 ± 315.7 a
Year marginal means			
2021	6664.6 ± 327.9 a	6556.4 ± 382.7 a	13,221.0 ± 646.6 a
2022	5929.2 ± 263.9 b	6011.6 ± 298.9 b	11,940.8 ± 502.6 b

Values are means ± SD ($n = 3$). The means of the same indicator followed by different letters indicate significant differences (Tukey's HSD, $p < 0.05$). ** $p < 0.01$, *** $p < 0.001$, and ns $p > 0.05$.

3.2. Soil pH, Organic C and Total N Contents, and Bulk Density

Co-applying RSB and MV, along with 20% N reduction, significantly increased soil pH and the contents of soil organic C (SOC) and total N (TN) ($p < 0.05$), while having no significant effect on soil bulk density (SBD) or the C:N ratio ($p > 0.05$; Table 2). Compared with the N₁₀₀ treatment, the N_{80BM} treatment significantly increased soil pH, SOC, and TN by 8.16%, 6.86%, and 8.29%, respectively.

Table 2. Soil pH, organic C and total N contents, and bulk density under the application of RSB and MV with N reduction.

Treatments	pH	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	SBD (g cm ⁻³)	C:N Ratios
N ₁₀₀	4.90 ± 0.09 b	20.4 ± 0.15 b	1.93 ± 0.02 b	1.08 ± 0.01 a	10.6 ± 0.06 a
N _{80B}	5.31 ± 0.09 a	21.0 ± 0.25 ab	1.98 ± 0.01 b	1.07 ± 0.02 a	10.7 ± 0.08 a
N _{80M}	5.11 ± 0.26 ab	21.0 ± 0.12 ab	2.03 ± 0.02 ab	1.06 ± 0.01 a	10.3 ± 0.12 a
N _{80BM}	5.30 ± 0.13 a	21.8 ± 0.16 a	2.09 ± 0.03 a	1.06 ± 0.02 a	10.4 ± 0.05 a

SOC, soil organic C; TN, total N; SBD, soil bulk density. Soil data were obtained in July 2022 after rice harvest. Values are means ± SD ($n = 3$). The means followed by different letters within the same column indicate significant differences among treatments (Tukey's HSD, $p < 0.05$).

3.3. Soil Organic C Sequestrations and Total N Stocks in Reddish Paddy Field

SOCs and TNs in the plow layer (0–20 cm) under different N management practices are shown in Figure 1. Compared with the N₁₀₀ treatment, the N_{80B} and N_{80M} treatments showed no statistically significant differences in SOC ($p > 0.05$). In contrast, the N_{80BM} treatment significantly increased SOC and TNs by 5.42% and 6.92%, respectively, compared with N₁₀₀ (Figure 1, $p < 0.05$).

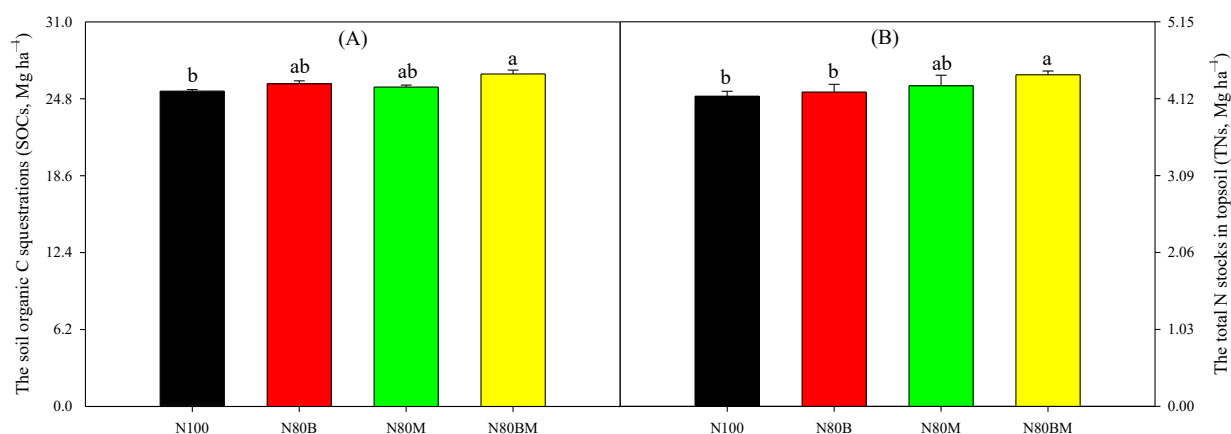


Figure 1. Effects of RSB and MV applied with N reduction on soil organic C sequestration (SOCs, (A)) and total N stocks (TNs, (B)) in the (0–20 cm) plow layer of reddish paddy soil. Error bars represent standard deviation.

represent the standard deviation of a measurement ($n = 3$). Different letters indicate significant differences among treatments (Tukey's HSD, $p < 0.05$).

The N storage rate (NSR) and C storage rate (CSR) in the rice–MV rotation system are shown in Figure 2. The highest NSR was observed in the N₈₀BM treatment (0.54 Mg N ha^{−1} yr^{−1}), followed by N₈₀M (0.47 Mg N ha^{−1} yr^{−1}) and N₈₀B (0.43 Mg N ha^{−1} yr^{−1}), whereas the lowest NSR was observed in N₁₀₀ (0.40 Mg N ha^{−1} yr^{−1}). A similar trend was observed for CSR. Compared with N₁₀₀ treatment, CSR increased by 1.06-, 0.58-, and 2.44-fold in N₈₀B, N₈₀M, and N₈₀BM treatments, respectively ($p < 0.05$).

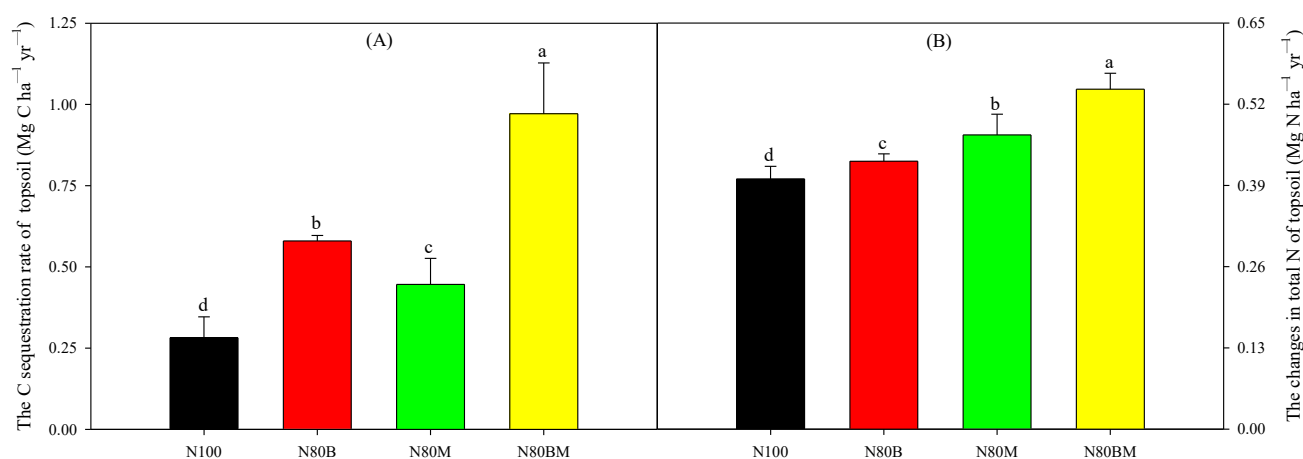


Figure 2. Effects of RSB and MV applied with N reduction on the C sequestration rate (CSR, (A)) and changes in total N stock (ΔN , (B)) in the topsoil (0–20 cm) from 2021 to 2022. Error bars represent the standard deviation of a measurement ($n = 3$). Different letters indicate significant differences among treatments (Tukey's HSD, $p < 0.05$).

3.4. Distribution and Stability of Water-Stable Aggregates of Reddish Paddy Soil

The distribution of water-stable aggregates in reddish paddy soil differed among N management strategies (Table 3). Overall, mega- and macroaggregates were dominant, accounting for more than 65% of the topsoil. Compared with N₁₀₀ treatment, the proportion of mega-aggregates (>2 mm) increased by 4.01% in N₈₀B ($p > 0.05$), 9.33% in N₈₀M treatment ($p < 0.05$), and 15.7% in N₈₀BM treatment ($p < 0.05$). The proportions of macroaggregates (0.25–2 mm) and microaggregates (0.053–0.25 mm) increased significantly in all three organic-amended treatments ($p < 0.05$), with the largest increases observed in the N₈₀BM treatment (10.6% and 20.2%, respectively). In contrast, N₈₀B, N₈₀M, and N₈₀BM treatments significantly reduced the silt–clay fraction by 39.6%, 47.8%, and 62.9%, respectively, relative to N₁₀₀ treatment ($p < 0.05$).

In addition, the combined application of RSB and/or MV, along with N reduction, significantly enhanced aggregate stability (Figure 3). Compared with N₁₀₀ treatment, the N₈₀B, N₈₀M and N₈₀BM treatments significantly increased MWD by 7.14%, 13.0% and 15.7%; GMD by 29.3%, 46.9% and 58.1%; and the $WR_{>0.25}$ by 7.92%, 9.42% and 11.8%, respectively ($p < 0.05$).

Table 3. Particle size distribution of soil aggregates under the application of RSB and MV with N reduction.

Treatments	>2 mm (%)	0.25~2 mm (%)	0.053~0.25 mm (%)	<0.053 mm (%)
N ₁₀₀	18.3 ± 0.57 c	47.5 ± 0.38 b	16.1 ± 0.20 c	17.5 ± 0.22 a
N ₈₀ B	19.0 ± 0.66 bc	52.0 ± 0.60 a	17.9 ± 0.31 b	10.6 ± 0.42 b
N ₈₀ M	20.0 ± 0.51 b	52.2 ± 0.42 a	18.4 ± 0.34 b	9.14 ± 0.39 c
N ₈₀ BM	21.2 ± 0.48 a	52.5 ± 0.32 a	19.4 ± 0.36 a	6.50 ± 0.40 d

Soil data were obtained in July 2022 after rice harvest. Values are means ± SD ($n = 3$). Different letters within the same column indicate significant differences among treatments (Tukey's HSD, $p < 0.05$).

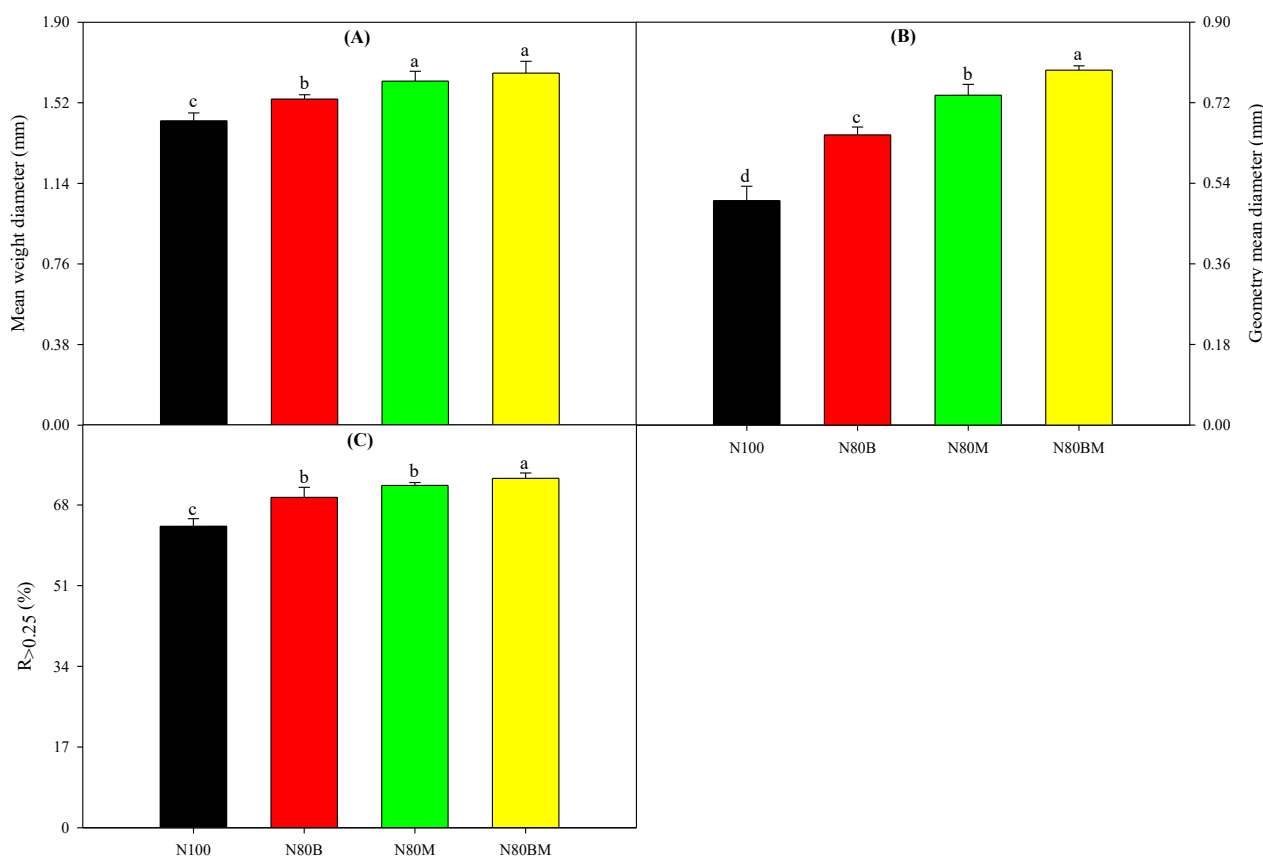


Figure 3. Effects of RSB and MV applied with N reduction on MWD (A), GMD (B), and $WR_{>0.25}$ (C) of soil aggregates of reddish paddy soil. Error bars represent the standard deviation of a measurement ($n = 3$). Different letters indicate significant differences among treatments (Tukey's HSD, $p < 0.05$).

3.5. Contents of Aggregates-Associated C, N, and Their C:N Ratios

RSB and MV, along with reduced N fertilizer, significantly increased SOC, total N, and C:N ratios in mega- and macroaggregates, but decreased these parameters in microaggregates (Figure 4). Compared with N₁₀₀ treatment, N₈₀B, N₈₀M, and N₈₀BM treatments increased SOC in mega-aggregates by 6.76%, 16.1%, and 25.0%, respectively, and total N by 9.89%, 5.40%, and 7.08%, respectively. SOC in macroaggregates also increased by 5.32%, 6.67%, and 9.95%, respectively. However, SOC in microaggregates (0.053–0.25 mm) decreased by 12.7%, 8.64%, and 5.54%, respectively ($p < 0.05$), while no significant difference was observed in aggregates < 0.053 mm ($p > 0.05$). Moreover, N₈₀BM treatment increased the C:N ratio of mega- and macroaggregates by 16.8% and 10.2%, respectively, but reduced the C:N ratio of microaggregates and silt–clay fraction by 6.14% and 7.19%, respectively, compared with N₁₀₀ treatment ($p < 0.05$).

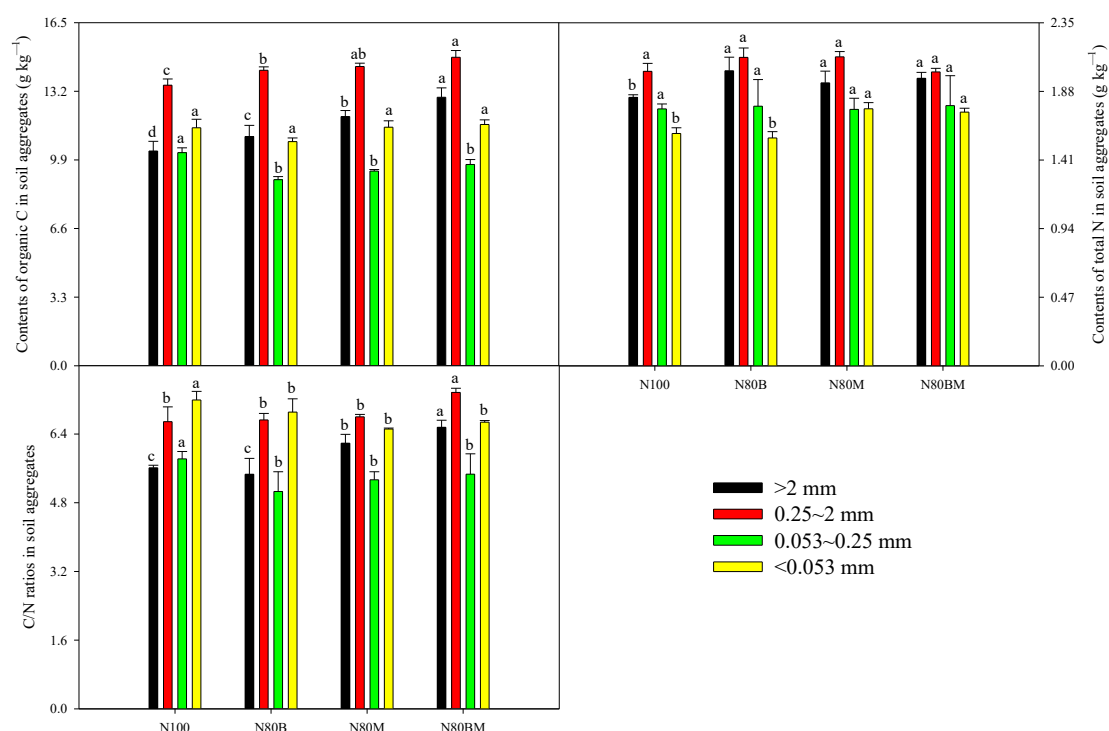


Figure 4. Effects of RSB and MV applied with N reduction on SOC, TN, and C:N ratios in different aggregate-size fractions of water-stable aggregates in reddish paddy soil. Error bars represent the standard deviation of a measurement ($n = 3$). Different letters on columns of the same color indicate significant differences among treatments (Tukey's HSD, $p < 0.05$).

3.6. Contributions of Aggregate-Associated Soil Organic Carbon and Total N to Bulk Soil

Analysis of the contribution rates of aggregate-associated C and N to bulk soil C (CRC) and N (CRN) (Figure 5) showed that macroaggregates contributed the most to bulk SOC and TN (54.2–61.7% and 49.6–55.6%, respectively), followed by mega- and macroaggregates and microaggregates, whereas the silt-clay fraction contributed the least. Relative to N_{100} treatment, N_{80B} , N_{80M} , and N_{80BM} treatments increased the CRC of mega-aggregates by 7.59%, 22.9%, and 35.3%, respectively, and the CRN by 11.6%, 9.35%, and 14.3%, respectively. Similar trends were observed for macroaggregates, with CRC substantially increased by 11.5%, 13.6%, and 13.8%, respectively, and CRN by 12.0%, 9.48%, and 1.84%, respectively ($p < 0.05$). In contrast, N_{80B} , N_{80M} , and N_{80BM} treatments markedly reduced both CRC (by 45.0%, 49.4%, and 64.8%, respectively) and CRN (by 42.1%, 45.2%, and 62.6%, respectively) in the silt-clay fraction compared with N_{100} treatment ($p < 0.05$).

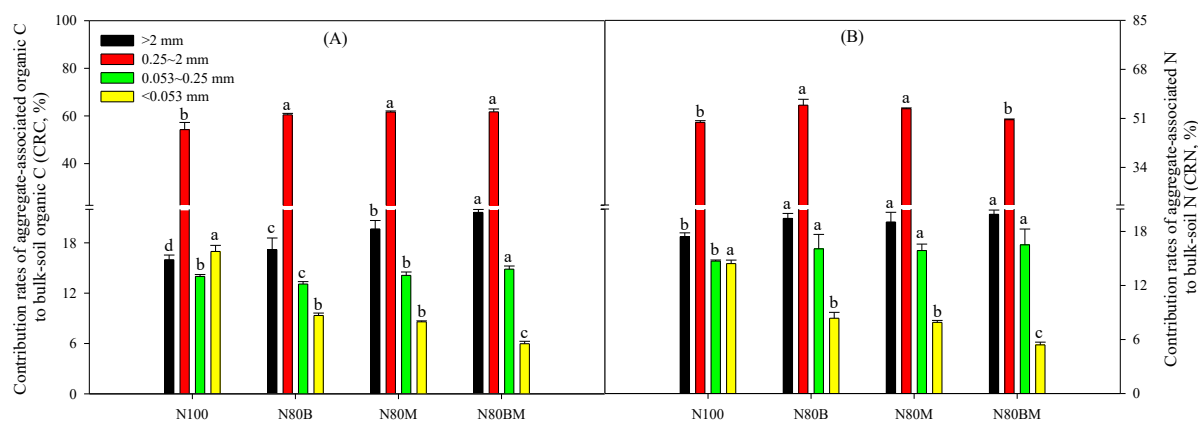


Figure 5. Effects of RSB and MV applied with N reduction on the contribution rate of aggregate-associated organic C to bulk SOC (CRC, (A)) and contribution rate of aggregate-associated TN to

bulk-soil TN (CRN, **(B)**). Error bars represent the standard deviation of a measurement ($n = 3$). Different letters on columns of the same color indicate significant differences among treatments (Tukey's HSD, $p < 0.05$).

3.7. Relative Accumulation of Aggregate-Associated Soil Organic Carbon and Total N in Different Particle Sizes

The incorporation of RSB and/or MV with reduced N fertilizer increased organic C accumulation in mega- and macroaggregates, while accelerating organic C depletion in microaggregates and the silt–clay fraction (Figure 6A). Compared with the N₁₀₀ treatment, the N₈₀M and N₈₀BM treatments significantly increased the EF_{SOC} of mega-aggregates by 12.4% and 17.0%, respectively ($p < 0.05$). The EF_{SOC} of microaggregates decreased significantly in all three organic-amended treatments ($p < 0.05$), with the largest decrease observed in the N₈₀B treatment (15.5%) relative to the N₁₀₀ treatment.

Compared with N₁₀₀ treatment, N₈₀B treatment significantly increased the EF_{TN} of megaaggregates by 7.38%, whereas N₈₀BM treatment decreased it by 7.97% and 6.65% in macro- and microaggregates, respectively ($p < 0.05$). No other significant effects on EF_{TN} were observed under RSB and/or MV application with reduced N fertilizer ($p > 0.05$) (Figure 6B).

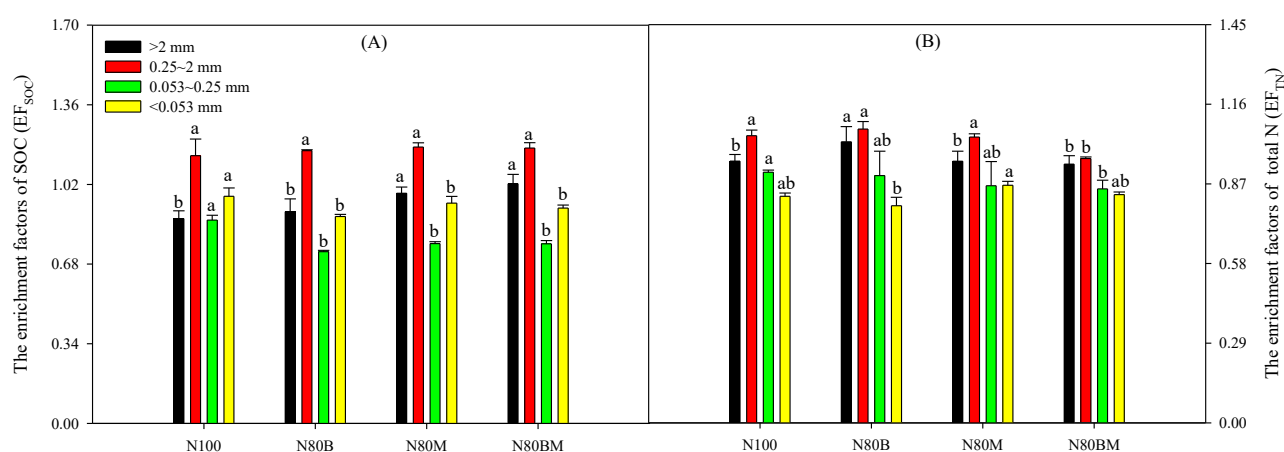


Figure 6. Effects of RSB and MV applied with N reduction on the enrichment factors of SOC (EF_{SOC} , (A)) and total N (EF_{TN} , (B)). Error bars represent the standard deviation of a measurement ($n = 3$). Different letters on columns of the same color indicate significant differences among treatments (Tukey's HSD, $p < 0.05$).

3.8. Mechanisms Associating Rice Yield, Soil Organic Carbon Sequestration, Total N Stocks, and Soil Aggregate Drivers Based on Pearson Correlation and Random Forest Analysis

Pearson's correlation analysis showed strong positive correlations among rice grain yield, SOC_s, TN_s, and water-stable aggregate stability indices (WMD, GMD, and DR_{>0.25}) (Figure 7).

The random forest model (RFM) identified 8, 13, and 14 key predictors for grain yield, TN_s, and SOC_s, respectively (Figure 8). The RFM explained 49.6%, 75.9%, and 55.0% of the variation in grain yield, TN_s, and SOC_s, respectively ($p < 0.05$). For grain yield, the most important predictors were aggregate stability (GMD, WR_{>0.25}, MWD; total MSE = 27.3%) and aggregate distribution (D_3 , D_4 ; total MSE = 27.2%), followed by the contribution rates of aggregate-associated C and N to bulk soil (CRC₃, CRN₄) and SOC content in aggregates >2 mm (SOC₁) (Figure 8a). For TN_s, key predictors included aggregate distribution (D_1 , D_3 , D_4 ; total MSE = 44.5%), aggregate-associated C content in >2 mm and 2–0.25 mm fractions (SOC₁, SOC₂; total MSE = 28.4%), CRC (CRC₁, CRC₂, CRC₄; total MSE =

28.1%), and aggregate stability (GMD , $WR_{>0.25}$; total MSE = 25.0%), followed by the enrichment factor of aggregate-associated C (EFC_1) and the C:N ratio of aggregates >2 mm (R_1) (Figure 8b). For SOC_s, the primary predictors were aggregate distribution (D_1 , D_2 , D_3 , D_4 ; total MSE = 39.5%), aggregate stability (GMD , $WR_{>0.25}$, MWD ; total MSE = 30.3%), CRN (CRN_1 , CRN_4 ; total MSE = 26.2%), and CRC (CRC_1 , CRC_4 ; total MSE = 20.1%), followed by SOC_1 , SOC_2 , TN_1 , and R_1 (Figure 8c).

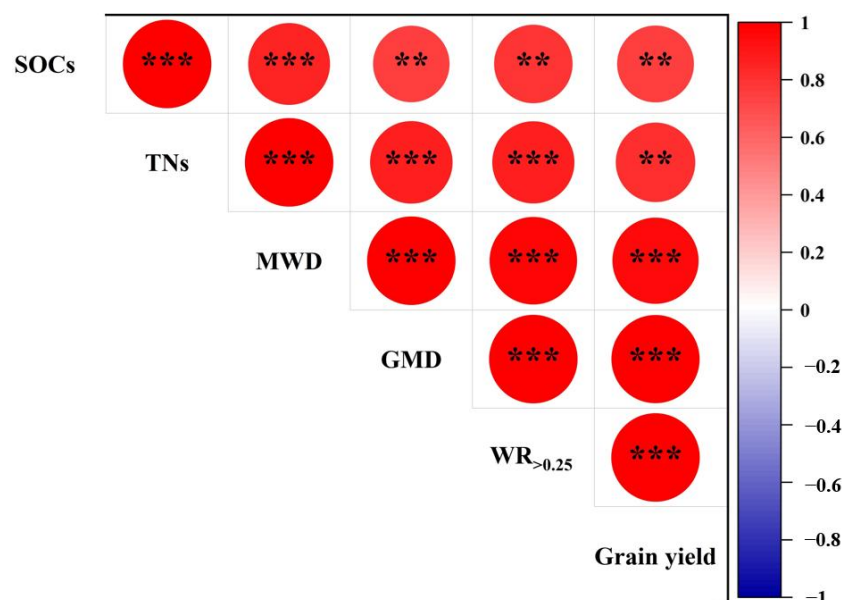


Figure 7. Pearson's correlation coefficients among SOC_s, TN_s, rice grain yield, and soil aggregate stabilities. ** $p < 0.01$, *** $p < 0.001$. SOC_s and TN_s are soil organic C and total N stocks, respectively.

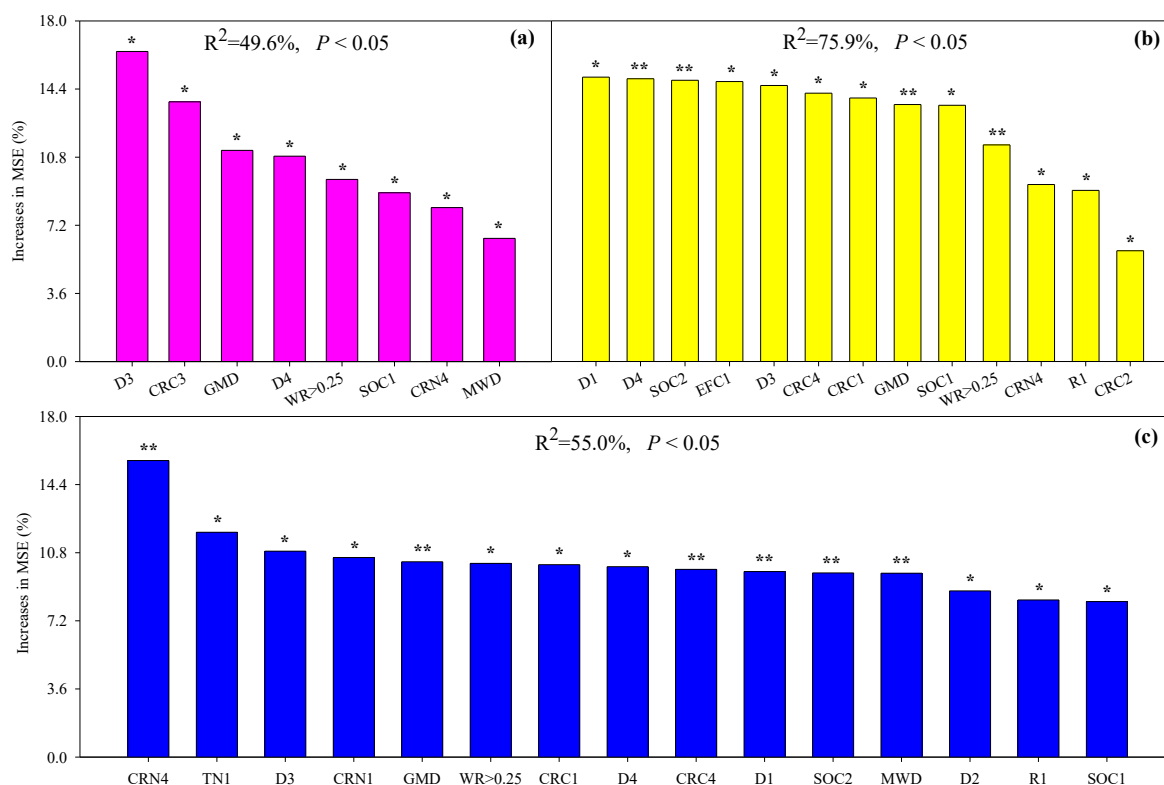


Figure 8. Random forest model indicates the mean predictor importance of aggregates driving the grain yield (a), TN stocks (b), and SOC sequestrations (c). MSE, mean square error. * $p < 0.05$, ** $p < 0.01$. D_1 , D_2 , D_3 , and D_4 are distributions of soil aggregates at >2 mm, 0.25–2 mm, 0.053–0.25 mm, and <0.053 mm, respectively. MWD, GMD, and $WR_{>0.25}$ are the mean weight diameter, geometric

mean diameter, and contents of aggregates with particle sizes > 0.25 mm, respectively. SOC_1 and SOC_2 are aggregate-associated SOC contents at >2 mm and $0.25\text{--}2$ mm, respectively. R_1 is the C:N ratio of soil aggregates at >2 mm. CRC_1 , CRC_2 , CRC_3 , and CRC_4 are contribution rates of aggregate-associated organic C with particle size at >2 mm, $0.25\text{--}2$ mm, $0.053\text{--}0.25$ mm, and <0.053 mm to that of bulk soil, respectively. CRN_1 and CRN_4 are the contribution rates of aggregate-associated TN at >2 mm and <0.053 mm to that of bulk soil, respectively. EFC_1 is the enrichment factor of aggregate-associated organic C with particle size at >2 mm.

4. Discussion

4.1. Combination of RSB with MV Alongside N Reduction Improved Rice Productivity, SOC and TN Stocks in Reddish Paddy Fields

RSB and MV have contrasting chemical properties that can complement each other when co-applied [40,50]. RSB is a high-C, recalcitrant material with a porous structure and abundant functional groups, whereas MV is an N-rich, labile organic input [5,7,37].

Our results showed that applying MV either alone or in combination with RSB under 20% N reduction (N_{80M} or N_{80BM}) significantly increased rice yield compared with N_{100} treatment (Table 1). It may be attributed to the incorporation of these two organic amendments, which enhanced the spatiotemporal synchrony between soil N-supply and crop N-demand within the “soil-rice” system, whilst optimizing the soil micro-environment for root development and accelerating the absorption and utilization of nutrients by rice roots [40]. Accordingly, the primary benefits were enhanced rice productivity and improved soil chemical properties (e.g., pH, nutrient availability) in the short term (Tables 1 and 2; [41]), likely driven by the rapid decomposition and mineralization of MV residue and the immediate physicochemical effects of biochar [12,37]. The sustained and cumulative benefits will be the progressive improvement in soil physical structure [31,49]. Furthermore, the significant year effect on rice biomass (Table 1) provided an opportunity to examine how climatic variability modulates treatment responses. The higher yields in 2021 than in 2022 can be attributed to differences in water availability during critical growth stages. In 2021, above-average precipitation during May ensured sufficient soil moisture during tillering and stem elongation, promoting vegetative growth [51]. In contrast, 2022 experienced below-average precipitation during July and longer sunshine duration, potentially inducing mild water stress during grain filling, which limited grain weight accumulation [52]. Importantly, the treatment $\times$ year interaction was not significant for any yield parameter ($p > 0.05$), indicating that the relative benefits of RSB and/or MV application were consistent across contrasting climatic conditions. This suggests that the soil improvements (e.g., enhanced water-holding capacity through improved aggregation) induced by these organic amendments may buffer against interannual climatic variability, although this hypothesis requires longer-term validation.

In this study, the co-application of RSB + MV along with 20% N reduction (N_{80BM}) significantly increased the soil pH, and contents of SOC and total N in reddish paddy field compared with the N_{100} treatment (Table 2), which aligned with our previous studies [37,40]. Regarding the statistically non-significant soil bulk density (SBD), although a decreasing trend was observed (Table 2), this could be due to the relatively short two-year experimental period, as significant changes in soil physical properties often require longer-term organic matter accumulation [14,15]. The lack of a significant increase in SBD under the N reduction treatments suggested that soil compaction was not exacerbated. However, longer-term monitoring is required to confirm whether soil physical health can be effectively maintained. Moreover, although no significant difference was observed in the bulk-soil C:N ratio (Table 2), this study showed a more sophisticated mechanism at the aggregate scale. The N_{80BM} treatment significantly altered the allocation of C and N among different aggregate fractions, noticeably increasing C:N ratios in macroaggregates

while decreasing them in microaggregates and the silt-clay fraction (Figure 4). This suggests that the combined application of RSB and MV did not simply increase total N and organic C equally, but rather reallocated and stabilized them within the larger and more stable aggregate fractions (Figures 5 and 6). This aggregate-scale C:N enrichment is a key indicator of enhanced soil structure and long-term C sequestration potential, even when the bulk-soil C:N ratio remains constant [14,25].

Our study showed that applying RSB and/or MV with reduced N fertilizer significantly increased SOC_s , TN_s , CSR , and NSR compared with N_{100} treatment, and $N_{80}BM$ treatment exhibited greater soil C and N stock capacity than $N_{80}B$ or $N_{80}M$ treatment (Figures 1 and 2). Jastrow (1996) reported that most of the SOC (>90%) is stored within aggregates >0.25 mm [53]. This study showed that applying RSB and/or MV significantly increased SOC and TN contents in topsoil and the proportions of mega- and macroaggregates compared with N_{100} treatment, with the combined treatment showing the greatest effect (Tables 2 and 3). This may be ascribed to the continuous application promoted macroaggregate formation and stability (Figure 3), which might gradually enhance the physical protection of C and N [53,54]. This, in turn, might lead to a slow but steady increase in SOC and TN stocks and improve the soil resilience to degradation and environmental stress [16,21]. Therefore, this long-term perspective will justify this strategy as a practical and sustainable management approach [55,56].

This study showed that the co-application of RSB and MV with reduced N fertilizer significantly promoted the formation and stability of macroaggregates and increased aggregate-associated C and N contents (Table 3, Figures 3 and 4). This effect likely arises from exogenous organic inputs with different C:N ratios, which facilitate soil particle aggregation. Huang et al. (2025) reported that low-quality (high C:N ratio; e.g., biochar) exogenous C inputs promoted SOC accumulation, and that greater diversity of exogenous C sources is positively associated with SOC storage [56]. Co-application of low- and high-quality organic inputs (low C:N ratio; e.g., MV) may reduce the priming effect on recalcitrant C and N (e.g., lignin and protein) in native soil organic matter and convert more exogenous C and N into soil C and N pools [57]. Moreover, the recalcitrant biochar may act as a long-term C source and a physical scaffold that could protect this newly formed aggregate structure and slow the decomposition of the labile MV residues [5,37]. This complementary interaction might lead to a more robust and sustained improvement in aggregate stability and C and N retention.

However, we acknowledge that our experimental design lacked an 80% N treatment without organic amendments, which prevents a rigorous partitioning of the additive versus synergistic effects. Nevertheless, we compared the N_{100} treatment with the N_{80} treatments amended with RSB and/or MV. The results showed that a 20% N reduction combined with these organic inputs not only sustained rice yield but also improved soil C and N storage (Table 1 and Figure 2). This suggests that the improvement was primarily linked to enhanced physical protection of organic matter within macroaggregates (Figure 8), even under a 20% reduction in chemical N fertilizer.

4.2. Promoted Soil Aggregation and Aggregates-Associated C, N Under Combining RSB with MV Alongside N Reduction

The SOC and microbial communities strongly influence the size, distribution, and stability of soil aggregates [58]. Increases in MWD and GWD implied that the transformation of fine particles (e.g., silt and clay) and microaggregates into macroaggregates, reflecting improved soil structure [49]. According to the aggregate hierarchy theory, macroaggregates (>0.25 mm) are formed by the cementation of microaggregates (0.053–0.25 mm) and silt-clay particles, mediated by transient binding agents (such as microbial exudates and polysaccharides) and temporary binding agents (such as fungal hyphae and

root hairs). In contrast, microaggregates are held together by persistent binding agents (e.g., humic substances and polyvalent metal cation bridges), which provide higher stability but require a slower formation process [59]. Our results showed that compared with the N_{100} treatment, the $N_{80}B$ and $N_{80}M$ significantly increased the proportions of mega- and macroaggregates, as well as *MWD* and *GWD*, while reducing the silt–clay fraction in reddish paddy soil, and the combined $N_{80}BM$ treatment further amplified these effects (Table 3, Figure 3). These findings indicate that co-application of RSB and MV, along with N reduction, accelerated the conversion of microaggregates and silt–clay particles into macroaggregates, thereby enhancing soil structure and the physical protection of soil C and N, consistent with the previous studies [14,47]. Organic inputs promoted macroaggregate formation through enhanced microbial activity and binding agent production [31,60]. Specifically, the labile C and N from MV likely stimulated microbial growth and exopolysaccharide production, which serve as transient binding agents that bind microaggregates into macroaggregates [32,59]. Meanwhile, the recalcitrant biochar particles may have acted as nuclei for macroaggregate formation, providing a stable physical scaffold around which microaggregates could assemble [61,62]. The increased SOC and TN contents in macroaggregates (Figure 4) further support this interpretation, as the hierarchy theory predicts that newly incorporated organic matter is preferentially stored in macroaggregates before being gradually incorporated into microaggregates over longer time scales [59,63]. The fact that $N_{80}BM$ showed the greatest macroaggregate proportion and stability (Figure 3) suggests that the complementary effects of labile (MV) and recalcitrant (RSB) organic inputs created optimal conditions for hierarchical aggregate formation.

Analyzing the contributions of aggregate-associated C and N in different aggregate-size fractions (*CRC*, *CRN*) provides insight into soil C and N dynamics in response to RSB and MV application. Wang et al. (2025) reported that organic amendments primarily increase SOC in microaggregates [29]. Our results showed higher SOC and TN contents in mega- and macroaggregates under RSB and/or MV treatments (Figure 4). In contrast, microaggregates exhibited lower contribution rates and enrichment factors of C and N compared with N_{100} (Figures 5 and 6). This difference may be attributed to the relatively short (two-year) duration of our experiment, as macro-aggregate formation through physical entanglement of fresh organic matter may precede the eventual stabilization of C within micro-aggregates over longer time scales. These shifts indicate a reallocation of SOC and N among aggregate size fractions. The *CRC* and *CRN* values were jointly determined by the proportion of each aggregate size fraction and the SOC and TN contents in those fractions. RSB and MV application significantly increased both the proportion of macroaggregates and their SOC and TN contents (Table 3 and Figure 4), leading to marked increases in *CRC* and *CRN*.

Previous studies have indicated that prolonged excessive N fertilization accelerates soil acidification, which impairs the formation and stability of macro-aggregates [14]. In contrast, RSB and/or MV combined with reduced N fertilization increased soil pH compared with N_{100} (Table 2), which may enhance microbial immobilization and stabilization of allochthonous N [56]. Biochar's abundant oxygen-containing functional groups strengthen mineral–microbial interactions, thereby enhancing soil particle aggregation [14].

Additionally, biochar may improve soil aeration and oxygen availability, locally increasing redox potential, which could inhibit the reduction of Fe^{3+} to Fe^{2+} and help maintain the cementing capacity of iron oxides [62]. However, the exact electrochemical mechanisms of biochar in such fluctuating paddy systems warrant further investigation. This study showed that the combined application of RSB and MV, along with N reduction, significantly increased SOC and TN contents (Table 2), thereby mitigating soil aggregate

disintegration in paddy fields. GMs also enhanced aggregate formation and stability, as well as aggregate-associated C, by promoting humification, thereby strengthening soil C and N retention capacity and stability [31]. The MV application has been reported to improve clay aggregation and stability by promoting mineral transformation, leading to the production of more kaolinite and metal oxides, as well as the retention of aromatic-C [18]. Additionally, MV may also stimulate rice root growth, activity, and exudation, which could act as binding agents and facilitate the formation of macroaggregates from microaggregates [64]. Finally, MV is known to enhance microbial activity and soil particle aggregation [18], whereas RSB has been reported to adsorb polysaccharides and humic acids released from MV residues, potentially slowing their microbial mineralization and prolonging the persistence of cementing agents [24]. Consequently, RSB provided high-C recalcitrant material and improved physical structure, while MV provided N-rich labile material and enhanced biological N fixation; their combination balances C:N stoichiometry, promotes macroaggregate formation, and enhances physical protection of C and N.

4.3. Associations of Rice Productivity and C, N Stocks Related to Soil Aggregate Dynamics

Aggregate formation and stability are key mechanisms for coordinating soil nutrient consumption and storage (e.g., C and N). They play critical roles in maintaining soil C and N stocks and in creating favorable soil conditions for crop production [17,65]. This study revealed significant positive correlations among rice yield, soil C and N stocks, and aggregate stability indices (Figure 7). The aggregate stability and the proportion of soil aggregates >0.25 mm were primary predictors of SOC and TN (Figure 8). Macroaggregates (>0.25 mm) encapsulate particulate organic matter within their intra-aggregate pore spaces, creating physical barriers that limit the accessibility of decomposer organisms and extracellular enzymes to otherwise labile C and N substrates [6,7]. As documented in previous studies, larger aggregates not only contain more organic matter in absolute terms [17] but also harbor greater microbial diversity and more complex pore networks [7]. Furthermore, stable aggregates resist slaking during wetting events, thereby maintaining the physical integrity of the soil matrix and preventing the sudden release of protected C and N. Moreover, the aggregate-associated C and N allocations were also identified as important predictors, particularly for TNs and SOC (Figure 8). This may indicate that a greater proportion of total soil C and N is stored within physically protected pools, which are generally considered more resistant to mineralization [6]. This provides correlative evidence that the redistribution of C and N toward macroaggregates is associated with enhanced soil C and N storage capacity [9].

Straw biochar significantly increased SOC, TN, and crop yield [24,66]. Incorporating GMs (e.g., MV) into rice-based cropping systems further increased soil N and organic C inputs. This practice recruited beneficial microbiota and promoted soil aggregation, and synergistically enhanced subsequent rice yield, soil C and N stocks, and N management efficiency [16,67]. These factors increased C and N concentrations within soil aggregates, promoted the formation of aggregates >0.25 mm from microaggregates, and improved aggregate stability. Consequently, soil C and N retention was enhanced under RSB and/or MV with reduced N fertilizer input, with the N₈₀BM treatment maximizing these benefits (Table 3, Figures 3, 4 and 6). Sun et al. (2025) reported that higher soil TN promotes greater SOC sequestration, which in turn modifies the soil microbiome and improves N availability, ultimately enhancing rice productivity in reddish paddy soils [68]. Hence, the co-application of RSB and MV promoted macroaggregate formation and enhanced aggregate stability. These stable macroaggregates physically protected the newly incorporated organic C and N, leading to increased aggregate-associated C and N contents and their contribution rates to bulk soil. Ultimately, this resulted in higher bulk-soil SOC and TNs, while simultaneously potentially creating favorable conditions for root proliferation and

yield formation. This finding was consistent with the view that soil aggregation serves as the key mechanistic link connecting combined organic amendment practices with improved agroecosystem performance [9,17]. Although the random forest model used in this study identifies associations and variable importance rather than causal relationships. The mechanistic interpretations are therefore supported by previous studies rather than direct experimental evidence of causality.

The mechanistic link between improved soil aggregation and enhanced rice yield can be understood through multiple pathways. Stable macro-aggregates provide a favourable physical environment for root penetration and proliferation [69]. The increased proportion of >0.25 mm aggregates reduced mechanical impedance, allowing roots to exploit a larger soil volume for nutrient and water uptake. The physical protection of organic C and N within macroaggregates creates a slow-release nutrient reservoir [70]. As aggregates undergo natural turnover, the encapsulated organic matter becomes available to plants at rates that match crop demand, thereby improving nutrient supply synchrony [71]. Stable aggregates enhance soil porosity and aeration, which promotes aerobic microbial activity and facilitates nitrification, potentially increasing N availability for rice plants [72]. The strong positive correlations among aggregate stability indices (MWD, GMD, $WR_{>0.25}$) and grain yield observed in this study (Figure 7) provide empirical support for this mechanistic interpretation.

Our previous study indicated that co-applying RSB and MV markedly enhanced the enzyme activities and abundances of functional genes related to N-cycling, thereby facilitating N absorption [40]. Although nitrogen-use efficiency (NUE) was not directly quantified in this study, the observed improvements in rice yield under N reduction strongly suggest enhanced NUE in the N₈₀BM treatment. The significant increase in total N content and TNs in the N₈₀BM treatment (Figure 1B, Table 2) suggests that more N was retained in the soil rather than lost to the environment. Additionally, MV enhances biological N fixation through symbiotic rhizobium bacteria, which can contribute 30–60 kg N ha⁻¹ annually [32,33]. Finally, biochar's high surface area and functional groups can adsorb NH₄⁺ and reduce N leaching and volatilization losses [73]. The enhanced aggregate stability under N₈₀BM likely further reduced N losses by protecting organic N within physically inaccessible pore spaces [12,74]. Collectively, these mechanisms suggest that N was conserved through physical protection and biological fixation and supplied to rice plants at rates that met crop demand under the combination of RSB and MV, thereby improving apparent NUE. Future studies should directly measure NUE using ¹⁵N isotope tracing techniques to confirm this inference.

4.4. Limitations of This Study and Prospects for Future Outlook

This study showed that combining RSB with MV alongside 20% N reduction significantly improved rice productivity and increased soil C and N stocks by enhancing the formation and stability of soil aggregates (especially mega- and macroaggregates) and by promoting aggregate-associated C and N allocation. From an agronomic perspective, the combined application of RSB and MV appears practically feasible within existing farming systems. MV is cultivated during the winter fallow period without occupying additional land that could otherwise be used for cash crops. The seeding rate is modest and requires minimal management. RSB is produced from self-rice-straw collected from the same plots after rice harvest, creating a near-closed nutrient loop that reduces reliance on external inputs and addresses the challenge of straw disposal. Although the initial investment in biochar production and MV seeding may be higher than conventional fertilization, the reduced chemical N input and the maintained or increased rice yield may help offset these costs over time, although formal economic analysis is needed to confirm this. Long-term economic benefits of improved soil quality and ecological environment, e.g., enhanced

nutrient retention, reduced soil erosion and greenhouse gas emissions, are difficult to quantify in a two-year study but may contribute substantially to overall profitability. Site-specific economic assessments are needed to validate feasibility across different farm scales and regional contexts.

However, soil properties were measured only after the second year of the experiment. Therefore, the lack of intermediate measurements precludes assessment of the trajectory and rate of change, and our results represent only a static comparison of equilibrium or near-equilibrium states after two cycles of amendment application. Additionally, the experimental design lacked an 80% N treatment without amendments, which prevents a rigorous distinction between additive and synergistic effects of RSB and MV. Future studies with a full factorial design are needed to definitively quantify the nature of these interactions. Second, soil properties were measured only in the second year after rice harvest, the lack of intermediate data precludes inference on the trajectory or rate of change. The results of this study just represented a static comparison of equilibrium or near-equilibrium states after two cycles of amendment application. Third, this two-year study did not capture long-term (e.g., decadal) C and N turnover processes. In addition, given that humus fractions (humic acid, fulvic acid, and humin) were not separately quantified in this study, future investigations should explore how the distribution of humic substances across aggregate size fractions responds to combined RSB and MV application, as humus quality plays a critical role in long-term C stabilization. Finally, in paddy fields, periodic drying–rewetting cycles cause redox fluctuations that alter the dynamics of metal oxides (e.g., Fe, Mn) and microbial processing of organic matter. Consequently, the mechanisms underlying persistent organo–metal complex stabilization and necromass formation were not elucidated.

Therefore, future studies should further focus on: (i) long-term (≥ 10 years) and multi-site field experiments across different paddy soils with diverse physiochemical properties (e.g., texture and pH) to validate the generalizability of SOC and TN enrichment in macroaggregates under combined RSB and MV application; (ii) investigating the role of metal plaques (e.g., Fe, Mn) on C and N storage and the underlying mechanisms in paddy soils; (iii) using stable isotope techniques (e.g., ^{13}C and ^{15}N) to trace soil C and N partitioning between anabolism and catabolism to clarify metabolic efficiency.

5. Conclusions

This study demonstrated that a 20% N reduction combined with RSB and MV sustained rice yield and enhanced soil C and N stocks in reddish paddy soil. Notably, the co-application of these organic amendments promoted macroaggregate formation and stability, redirecting C and N toward larger aggregates where they were physically protected. Random forest analysis confirmed aggregate distribution and stability as primary predictors of both rice yield and soil C, N stocks, highlighting soil aggregation as a central mechanism. In summary, all three hypotheses were supported by our experimental evidence. The co-application of RSB and MV alongside 20% N reduction enhanced rice productivity while improving soil aggregation and C, N sequestration in reddish paddy soil, providing a scientifically grounded strategy for sustainable N management in rice production. Practically, this strategy created a near-closed nutrient loop using winter-grown MV and self-rice straw-derived biochar, partially substituting for synthetic N fertilizers and offering a sustainable pathway for nutrient management in rice production. However, further long-term validation across diverse sites is needed to confirm the broader applicability of these findings.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16141351/s1>, Table S1: Meteorological data during

the rice growing seasons in 2021 and 2022; Table S2: Initial physicochemical properties of the initial topsoil (0–20 cm) in 2021; Table S3: Physiochemical properties of MV and RSB; Table S4: Application rates and schedule of RSB, MV, and chemical fertilizers across treatments.

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