

Article

Study on the Effects of Reducing Nitrogen Fertilizer: Stabilizing Yield and Carbon Sequestration by Synergistic Utilization of Chinese Milk Vetch and Rice Straw in Double-Cropping Rice Area

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Abstract: The excessive application of chemical fertilizers in rice fields exacerbates soil degradation and poses a threat to food security. Achieving an increase in rice production and minimizing environmental costs are inevitable requirements for achieving sustainable rice production. The synergistic utilization of rice straw (RS) and Chinese milk vetch (MV) is a sustainable measure to improve soil quality in Southern China. How this management strategy impacts agricultural productivity and soil carbon (C) sequestration under different fertilization conditions is unclear. Several treatments, including only chemical fertilizer (F), F + MV (FM), F + RS (FS), and F + MV + RS (FMS) under a standard rate of nitrogen (N₁₀₀) and 40% reduced nitrogen (N₆₀) levels were designed to explore changes in rice yields and soil organic carbon (SOC) concentrations, stocks, and soil labile organic C fractions (permanganate oxidizable C) during 2018–2020 in a double-rice-cropping system. The results show that the FMS treatment reduced soil bulk density to alleviate soil compaction and improved the soil carbon management index. The synergistic utilization of MV and RS replacing 40% of the chemical N fertilizer could still maintain the rice yield. Compared to the F treatment, the average annual grain yield was significantly increased by 9.82% and 5.84% in the FMS treatment; SOC concentration was increased by 16.05% and 19.98% on average ($p < 0.05$), and SOC stock was increased by 1.78 Mg C ha⁻¹ and 2.37 Mg C ha⁻¹ under the N₆₀ and N₁₀₀ levels, respectively. The random forest regression model and correlation analysis demonstrated that the inputs of chemical N, organic N and C, and appropriate C/N ratio promoted soil C accumulation. Furthermore, the structural equation model analysis exhibited that the C input affects the highly labile organic carbon (HLOC) and total labile organic carbon (LOC); the HLOC had a positive effect on SOC ($p < 0.05$). N input had a significant effect on LOC and yield. Our results suggest that the synergistic utilization of MV and RS plays an important role in ensuring stable grain production, improving soil C sequestration capacity, and maintaining soil environmental health in Southern China.

Keywords: green manure; rice straw; replacement of mineral nitrogen; rice yield; soil organic carbon; labile organic carbon fractions



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1. Introduction

As the main agricultural land-use method in China, paddy fields play a very important role in food security, carbon sequestration, and emission reduction [1]. Chemical fertilizer

application is an important agricultural measure to maintain and improve farmland fertility and ensure rice yield [2]. However, an excessive application of mineral fertilizers will cause a series of environmental issues, such as soil compaction, acidification, and enrichment of heavy metals and toxic elements that may endanger food safety and even personal health. Soil organic carbon (SOC) is critical in maintaining soil sustainable productivity and crop yields [3]. The dynamic changes in SOC also play a crucial role in global climate change by mitigating carbon dioxide emissions. Paddy soil is the most active part of the soil C pool, because it has huge potential to increase the C stock for maintaining crop productivity and mitigating climate change [4,5]. Therefore, it is crucial to enhance SOC storage and reduce chemical fertilizer applications in paddy fields for environment-friendly sustainable development.

The utilization of rice straw (RS) is a common and effective practice to increase SOC for a better nutrient supply to paddy soil. However, RS with a high carbon-to-nitrogen ratio ($C/N > 60$) input will lead to competition between rice plants and soil microorganisms for nitrogen (N), which may reduce the decomposition rate of fresh C and hurt rice growth [6]. In recent years, the practice of combining RS with Chinese milk vetch (MV) has become increasingly popular in Southern China. This method helps to fix nitrogen in the air through biological nitrogen fixation and activates nutrients in the soil, making it a valid strategy for improving soil quality. Moreover, the synergistic utilization of RS and MV with a low C/N (approximately 15) provided a more balanced ecological stoichiometry for microbial metabolism and more balanced nutrients; the co-incorporation extended nutrient availability and made nutrient release synchronize with rice demand, consequently increasing rice yield [7,8]. The application of MV and RS also affected labile organic C, which was readily available to microorganisms to provide available nutrients for crop growth [9] and could be transformed to new C as sequestered and stabilized in soil by microorganisms [10]. Non-labile organic C could provide abundant nutrients for an extended period due to a slow rate of turnover; this represented the soil's potential for soil C accumulation and storage [11]. When fresh organic matter was input, microorganisms quickly decomposed it into labile organic carbon, with only a small fraction being stabilized or sequestered [12]. The report pointed out that half of the "primed" C originated from the soil-stable C pool due to the RS as the fresh C inputs, which caused the slow sequestration rate of non-labile organic carbon [13]. Therefore, labile and non-labile SOC fractions are important indicators of C turnover and accumulation, and it is thus essential to understand their changes in reply to the synergistic utilization of MV and RS. However, the co-incorporation of MV and RS into paddy fields for soil C sequestration and rice yield depends on the combined amounts of chemical fertilizers, especially N application [14,15]. The report pointed out that if full N fertilizer was used together with green manure, it might lead to a decrease in microbial network complexity, reducing the types and quantities of microorganisms. Also, excessive chemical N fertilizer application might cause a decrease in soil pH, further affecting the growth of microorganisms [16]. Therefore, to protect the environment and promote the sustainable development of agriculture, it is necessary to reduce the application of chemical N fertilizer. Researchers have found that N reduction combined with MV and RS return could facilitate the formation of rice yield components to increase rice yield and soil physical, chemical, and biological properties; improve soil quality; and reduce the negative impacts on soil and the environment in the Guangxi and Jiangxi provinces [16–18]. The utilization of MV and RS specifically supplied with different N levels can provide a synergistic effect for the sequestration of organic C in rice soil. The main mechanism of C sequestration in rice soil is the incorporation of carbon-rich soil organic matter into the soil, which preserves and protects it from degradation in the soil [19]. The combination of organic and inorganic nutrient management practices can not only promote the growth of rice and microorganisms to increase C inputs of plant and microbial residual, but also improve soil structure and achieve accumulation and stability of C in soil [20].

In this study, a paddy field was investigated for its synergistic utilization of MV and RS based on traditional chemical fertilizers and a 40% reduction in mineral N fertilizer

application in Southern China. The changes in rice yield, SOC, permanganate oxidizable organic C fractions, and SOC accumulation in the topsoil layer were analyzed. The main objectives of this paper were (I) to confirm the response of rice yield to the synergistic utilization of MV and RS with and without a chemical N fertilizer application reduction; (II) to investigate annual variation trends of SOC and soil labile organic C fractions (permanganate oxidizable C) under the continuous input of MV and RS; and (III) to reveal the interactive effects of N and C inputs on SOC and organic C fractions.

2. Materials and Methods

2.1. Site Description and Experimental Design

A field experiment was established in 2016 at the scientific research experiment station of the Hunan Academy of Agricultural Sciences in the town of Gaoqiao (113°21' E, 28°28' N), Changsha County, Hunan Province, China. The climate is subtropical, with a mean annual temperature of 16.8 °C and precipitation of 1400 mm. The soil type is Fe-accumuli-stagnic anthrosols derived from quaternary red clay [21]. The physicochemical properties of the initial soil (0–20 cm) were as follows: soil organic matter 17.93 g kg⁻¹, N 1.05 g kg⁻¹, alkali-hydrolyzed N 171.00 mg kg⁻¹, available P 11.70 mg kg⁻¹, available K 39.80 mg kg⁻¹, pH 4.81, and bulk density 1.08 g cm⁻³.

The experiment was carried out in a randomized complete block design with three replicates, and each plot was 4 m × 5 m. The treatments included only chemical fertilizers (F), chemical fertilizers incorporated with Chinese milk vetch (FM), chemical fertilizers incorporated with rice straw (FS), and chemical fertilizers co-incorporated with Chinese milk vetch and rice straw (FMS) in two nitrogen application levels including the standard rate of nitrogen (N₁₀₀) and 40% reduced nitrogen (N₆₀). The ridge of each plot was covered with plastic film for isolation, draining, and irrigation. The N, P₂O₅, and K₂O were applied with rates of 150 kg ha⁻¹, 75 kg ha⁻¹, and 90 kg ha⁻¹ over the early rice period; 180 kg ha⁻¹, 45 kg ha⁻¹, and 120 kg ha⁻¹ over the late rice period in the N₁₀₀ level, while the application in the N₆₀ level entailed N, P₂O₅, and K₂O being applied at rates of 90, 75, and 90 kg ha⁻¹ in early rice period and 108, 45, and 120 kg ha⁻¹ in late rice period. N was added as urea; the P₂O₅ was calcium magnesium phosphate, and the K₂O was muriate of potash. A total of 50% of the total urease and potassium chloride was applied as basal fertilizer, and another 50% was used for topdressing fertilizer in the full rice tillering stage, and calcium magnesium phosphate was used for basal fertilizer. The varieties of early rice and late rice were 'Xiangzaoxian 32' and 'Shenyou 9586', and the rice harvest time was July and November each year.

2.2. C and N Input Estimation

Chinese milk vetch was sown during winter fallow at a density of 30 kg ha⁻¹ without any fertilizer, and the yields of fresh grass in three replicates were measured and then equally divided into plots before 10 days of the rice transplanting (next year mid-April), with shallow water-wetting decomposition. The return of rice straw was also divided equally among the three replicates. Root and stubble samples from an area of 30 cm × 30 cm to a depth of 30 cm at three points in each plot were randomly collected in early and late rice in 2020, estimating the root and stubble biomass and C concentration. The C concentrations of early and late RS were 386.8 g kg⁻¹ and 362.4 g kg⁻¹; the early and late rice root C concentrations were 360 g kg⁻¹ and 329.7 g kg⁻¹, and the rice stubble C concentrations were 377.3 and 376.3 g kg⁻¹; the C concentration of Chinese milk vetch was 400 g kg⁻¹. The N concentration of rice straw and stubble was 6.17 g kg⁻¹, and the rice root N concentration was 10.2 g kg⁻¹; Chinese milk vetch concentration was 31.6 g kg⁻¹. The dry biomass and C and N inputs of MV, RS, and crop residue are shown in Table 1.

Table 1. The dry biomass of crop residues, rice straw (RS), and Chinese milk vetch (MV) returned into the soil and the amount of C and N input.

N Level	--	N ₆₀				N ₁₀₀			
Treatment	--	F	FM	FS	FMS	F	FM	FS	FMS
Dry biomass (t ha ⁻¹)	Crop residues	3.32	3.46	3.31	3.59	3.67	3.63	3.72	3.86
	RS	--	--	8.78	9.41	--	--	9.94	10.36
	MV	--	1.48	--	1.42	--	1.38	--	1.18
Dry biomass (t ha ⁻¹)	Crop residues	1.51	1.58	1.51	1.64	1.67	1.66	1.7	1.76
	RS	--	--	3.26	3.5	--	--	3.7	3.86
	MV	--	0.59	--	0.57	--	0.55	--	0.47
	Total	1.51	2.17	4.77	5.71	1.67	2.21	5.4	6.09
N input (Mg N ha ⁻¹)	Crop residues	0.026	0.027	0.026	0.028	0.029	0.029	0.029	0.030
	RS	--	--	0.054	0.058	--	--	0.061	0.064
	MV	--	0.047	--	0.045	--	0.044	--	0.037
	CF	0.198	0.198	0.198	0.198	0.33	0.33	0.33	0.33
C/N of organic addition	Total	0.224	0.272	0.278	0.329	0.359	0.403	0.42	0.461
	--	57.71	30.52	59.42	46.27	57.71	29.27	59.4	43.39

Crop residues included rice roots and stubbles that remained after the rice harvest. F: only chemical fertilizer application FM: chemical fertilizer incorporated with Chinese milk vetch; FS: chemical fertilizer incorporated with rice straw; FMS: chemical fertilizer co-incorporated with Chinese milk vetch and rice straw; N₁₀₀: standard rate of nitrogen level; N₆₀: 40% reduced nitrogen level.

2.3. Soil Sampling and Soil Analysis

Soil samples were gathered from the 0–20 cm surface layer of each plot at maturity using a five-point sampling method (6 cm in diameter) and thoroughly mixed to form a sample, and then the soil samples were sieved through a 2 mm sieve, after natural air-drying then passed through a 100-mesh sieve for testing. The SOC concentration was tested by the potassium dichromate method, using 33 and 333 mmol L⁻¹ potassium permanganate oxidation to determine the concentrations of highly labile organic carbon (HLOC) and labile organic carbon (LOC) [22], with non-labile organic carbon (NLOC) being equal to organic carbon concentration minus labile organic carbon [23].

The C stocks were calculated as follows [23]:

$$\text{C stocks (Mg C ha}^{-1}\text{)} = \text{C concentration (g C kg}^{-1}\text{)} \times \text{bulk density (g cm}^{-3}\text{)} \times \text{depth (cm)} \times 0.1$$

where 0.1 indicates the unit conversion factor.

The C sequestration rate was calculated as follows [24]:

$$\text{C sequestration rate (Mg C ha}^{-1}\text{ yr}^{-1}\text{)} = (\text{C stock}_{\text{T2}} - \text{C stock}_{\text{T1}}) / \text{T}$$

where T2 and T1 are the SOC stocks in 2020 and 2016, respectively, and T represents 5 years.

The carbon management index (CMI) was calculated as follows [22]:

$$\text{carbon pool index (CPI)} = \text{SOC concentration of treatment} / \text{SOC concentration of soil before the experiment}$$

$$\text{Lability (L) of LOC} = \text{LOC concentration} / \text{NLOC concentration},$$

$$\text{Lability (L) of HLOC} = \text{HLOC concentration} / (\text{SOC} - \text{HLOC}) \text{ concentration, and}$$

$$\text{Lability Index (LI)} = \text{L of treatment} / \text{L of before experiment}$$

$$\text{CMI} = \text{CPI} \times \text{LI} \times 100$$

2.4. Statistics Analysis

Statistical analysis was performed using IBM SPSS Statistic (Version 20.0, IBM SPSS Inc., Chicago, IL, USA). Duncan's multiple range tests at a 5% confidence interval were used to identify the significant difference between different treatments, and Origin2021 (OriginLab Corporation, Northampton, MA, USA) was used for plotting and analyzing

the correlation among C, N input, and soil C with the fitting analysis method. The relative influence of key factors on SOC and rice yield was evaluated using the “randomForest” package in R. Structural equation model (SEM) analysis was performed to reveal the relationships between different factors on yield and SOC by R with the “piecewiseSEM” package. Microsoft Excel 2016 software was used for data processing.

3. Results

3.1. Incorporation of Chinese Milk Vetch and Rice Straw Expanded or Maintained Rice Yield

The total grain yield significantly increased in the FMS treatment whatever the combination was with the conventional N fertilizer application or a 40% reduction in N fertilizer compared to the F treatment, and the total yield was 14.34, 12.55, and 10.67 t ha⁻¹ in the FMS treatment with the N₆₀ level and 15.47, 13.42, and 11.23 t ha⁻¹ in the FMS treatment with the N₁₀₀ level in 2018, 2019, and 2020. Compared to the F treatment under the N₆₀ and N₁₀₀ levels, the FMS treatment increased the average annual yield of early and late rice by 9.82% and 5.84%, respectively ($p < 0.05$). The yield of the FMS treatment under the N₆₀ level had no significant difference from that in the F treatment under the N₁₀₀ level. The FM and FS treatments could increase significantly compared to the F treatment at the N₆₀ level, but this was not the case at the N₁₀₀ level. At the N₆₀ level, the yield of the FS treatment was lower than the FM treatment, but higher at the N₁₀₀ level (Figure 1).

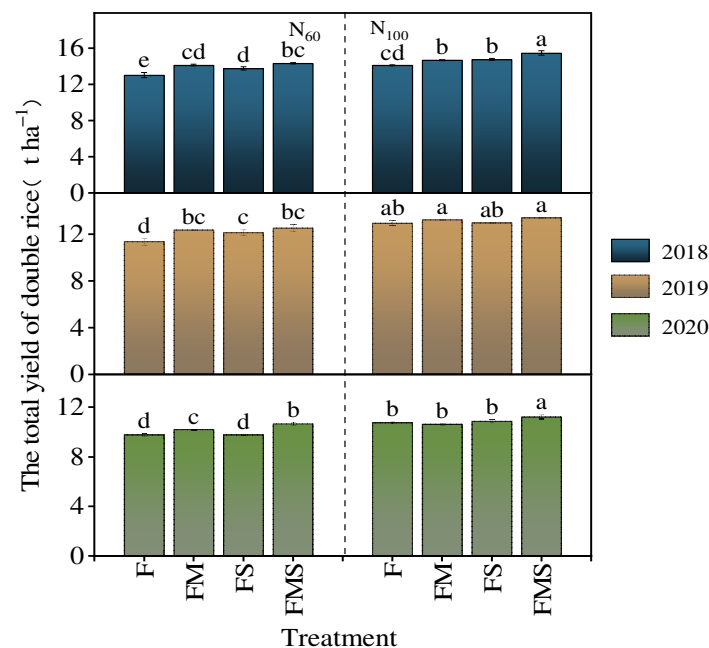


Figure 1. Effects of fertilization regimes on total grain yield of double-rice-cropping system in 2018, 2019, and 2020. (F: only chemical fertilizer; FM: chemical fertilizer incorporated with Chinese milk vetch; FS: chemical fertilizer incorporated with rice straw; FMS: chemical fertilizer co-incorporated with Chinese milk vetch and rice straw; N₁₀₀: standard rate of nitrogen level; N₆₀: 40% reduced nitrogen level). Different lowercase letters in the same column indicate significant differences among all treatments (Duncan, $p < 0.05$).

3.2. Incorporation of Chinese Milk Vetch and Rice Straw Increased SOC Concentrations and Labile Organic C Fractions

The SOC, HLOC, LOC, and NLOC concentrations were significantly affected by different treatments and successive years of cultivation (Table 2). The different N levels had a significant effect on the LOC and NLOC. There was a significant interaction between soil treatments and year on the HLOC and LOC.

Table 2. The multi-factor variance analysis of soil organic carbon and different labile organic carbon fractions.

Source of Variance	<i>p</i> -Value			
	SOC	HLOC	NLOC	LOC
N Level	0.394	0.517	0.024 (*)	0.002 (**)
Treatment	<0.001 (***)	<0.001 (***)	<0.001 (***)	<0.001 (***)
Year	<0.001 (***)	<0.001 (***)	<0.001 (***)	<0.001 (***)
N Level × Treatment	0.631	0.792	0.591	0.307
N Level × Year	0.965	0.896	0.472	0.045 (*)
Treatment × Year	0.853	0.006 (**)	0.120	0.007 (**)
N Level × Treatment × Year	0.981	0.827	0.989	0.856

SOC: soil organic carbon; HLOC: highly labile organic carbon; NLOC: non-labile organic carbon; LOC: labile organic carbon. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

A significant difference in SOC concentrations was not observed between the two N levels in the same organic amendment treatment. Compared to the F treatment, the FMS treatment increased the SOC concentration in the two N levels ($p < 0.05$). The SOC concentration was increased by 16.05% and 19.98% on average during 2018–2020 in the FMS treatment relative to the F treatment under the N₆₀ and N₁₀₀ levels ($p < 0.05$), respectively. There was little difference in the SOC concentration between the FM and FS treatments, regardless of whether the N level was at the N₆₀ or N₁₀₀ level during 2018–2020 (Figure 2).

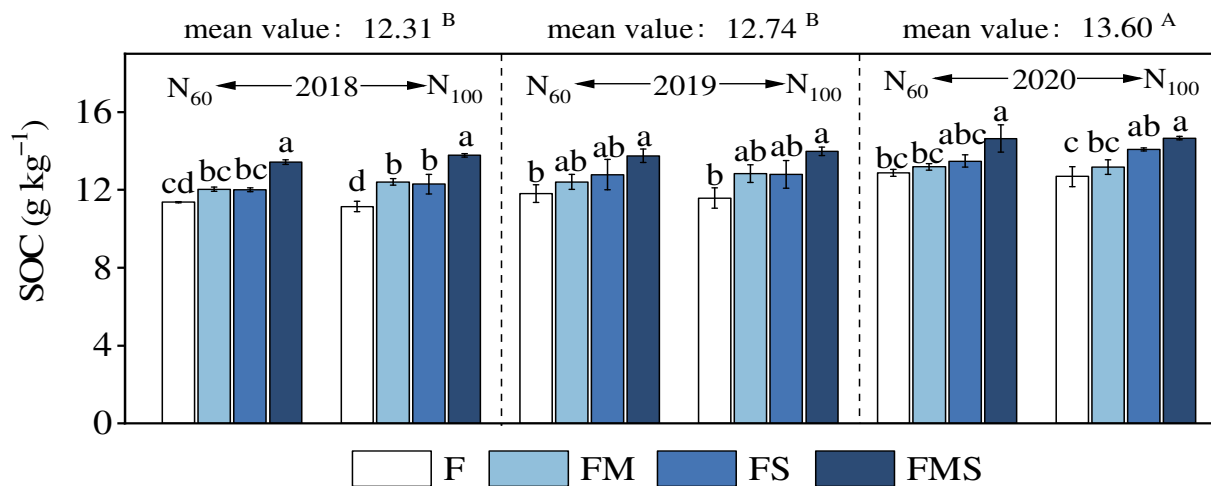


Figure 2. Effects of fertilization regimes on SOC concentration in 2018, 2019, and 2020. (SOC: soil organic carbon. F: only chemical fertilizer; FM: chemical fertilizer incorporated with Chinese milk vetch; FS: chemical fertilizer incorporated with rice straw; FMS: chemical fertilizer co-incorporated with Chinese milk vetch and rice straw; N₁₀₀: standard rate of nitrogen level; N₆₀: 40% reduced nitrogen level). Different lowercase letters indicate significant differences (Duncan, $p < 0.05$) among all treatments, and different uppercase letters after the mean values indicate significant differences (Duncan, $p < 0.05$) between the mean values of all treatments in each year.

The MV and RS return had a significant impact on the concentrations of HLOC, LOC, and NLOC, while the N level had a certain impact on the LOC and NLOC concentrations; the LOC concentrations at the N₆₀ level were higher than at the N₁₀₀ level, but the NLOC concentrations were the opposite (Figure 3). In 2018, the HLOC and LOC concentrations did not show any obvious differences among all treatments. However, compared to the F treatment, the FMS and FS treatments caused an increase in the HLOC concentration by 28.81% and 16.94% at the N₆₀ level and 32.71% and 20.44% at the N₁₀₀ level ($p < 0.05$) in 2019. Additionally, only the FMS treatment led to a significant increase in the LOC concentration. In 2020, the FMS treatment caused an increase of 29.88% and 25.75% in the HLOC concentration relative to the F treatment ($p < 0.05$), under the N₆₀ and N₁₀₀ levels.

The FMS, FS, and FM treatments showed a noteworthy increase in the LOC concentration relative to the F treatment at both N levels. The FMS treatment increased most, with a rate of 41.42% at the N_{60} level and 48.58% at the N_{100} level. The FMS treatment brought about an increase in the NLOC concentration by 19.90% and 28.65% under the N_{60} and N_{100} levels in 2018 and 19.59% under the N_{100} level in 2019 ($p < 0.05$). However, the difference was indistinctive in the NLOC concentration among all treatments in 2020.

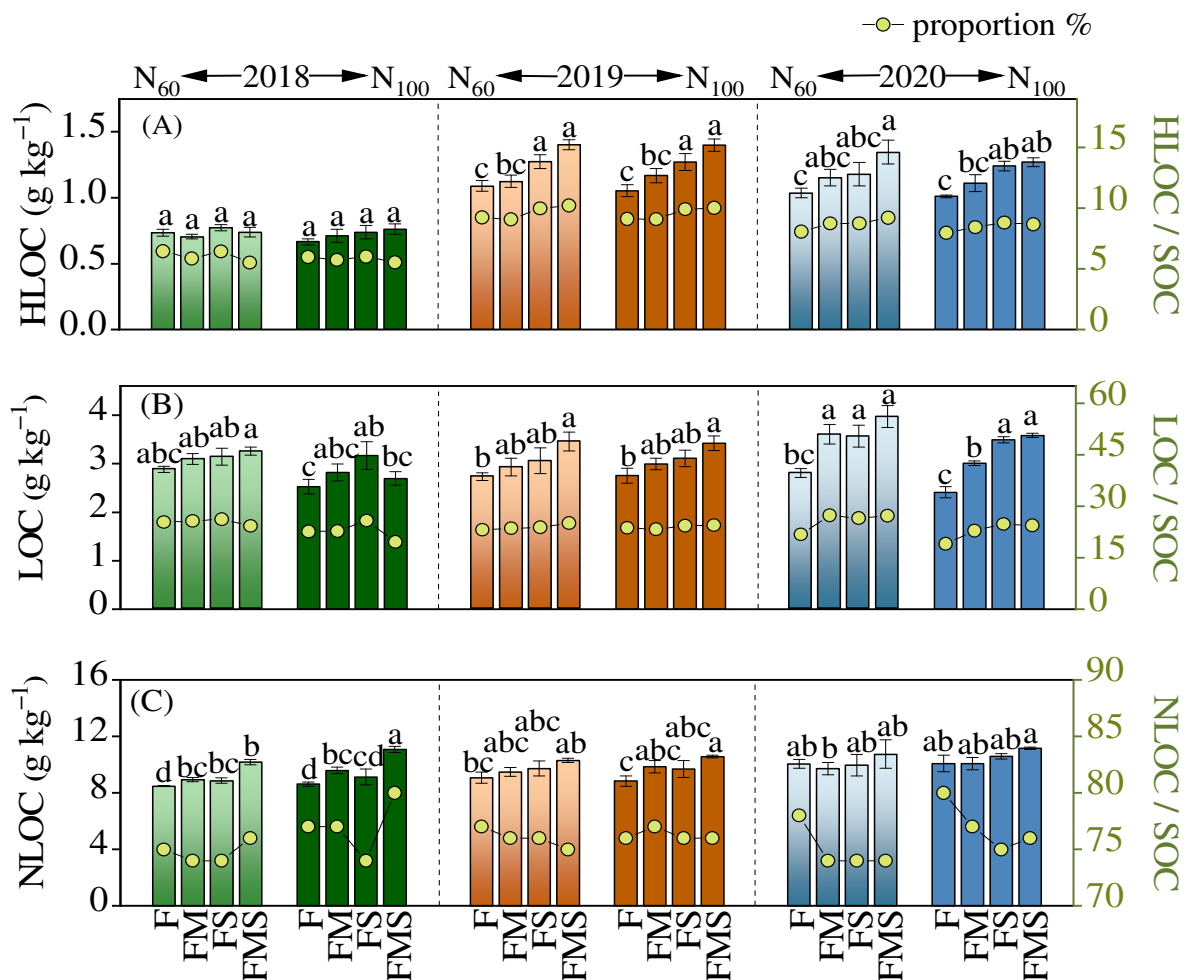


Figure 3. Effects of fertilization regimes on concentrations of soil labile organic carbon fractions. (HLOC (A), highly labile organic carbon; LOC (B), labile organic carbon; NLOC (C), non-labile organic carbon), and their proportions to soil organic carbon (SOC) during 2018–2020. F: only chemical fertilizer; FM: chemical fertilizer incorporated with Chinese milk vetch; FS: chemical fertilizer incorporated with rice straw; FMS: chemical fertilizer co-incorporated with Chinese milk vetch and rice straw; N_{100} : standard rate of nitrogen level; N_{60} : 40% reduced nitrogen level). Different lowercase letters indicate significant differences (Duncan, $p < 0.05$) among all treatments.

From 2019 to 2020, the proportion of HLOC, LOC, and NLOC to SOC was as follows: $HLOC/SOC < LOC/SOC < NLOC/SOC$. The organic addition treatments resulted in higher HLOC/SOC and LOC/SOC compared to the F treatment under the two N levels. NLOC/SOC was lower than in the F treatment. Moreover, NLOC/SOC was higher in the FMS treatment relative to the F treatment only in 2018 under the N_{60} and N_{100} levels.

3.3. Carbon Management Index

The soil carbon management index (CMI) is a momentous index indicating the SOC pool and the carbon dynamic change effects by different treatments [22]. We found that the MV and RS return affected the change in CMI at both the N_{60} and N_{100} levels (Figure 4). Compared to the F treatment, the CMI of LOC increased significantly in the FMS, FS, and FM treatments. At the N_{60} level, the CMI of LOC increased by 52.37%, 35.86%, and 38.72% in the FMS, FS, and FM treatments ($p < 0.05$). At the N_{100} level, the CMI of LOC increased by 58.95%, 55.75%, and 30.93% in the FMS, FS, and FM treatments ($p < 0.05$). It is worth noting that the CMI of LOC in the F treatment at the N_{100} level was less than 100, indicating that the soil quality was deteriorating with the use of chemical fertilization alone.

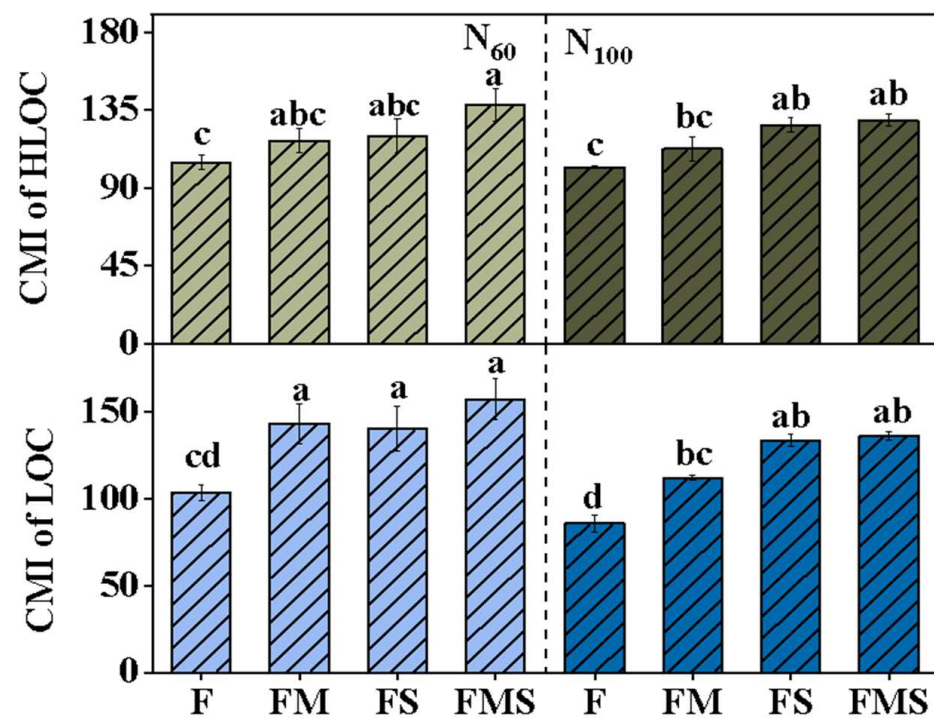


Figure 4. Effects of fertilization regimes on the CMI of HLOC and LOC under N_{60} and N_{100} levels in 2020. (CMI: carbon management index; HLOC: highly labile organic carbon; LOC: labile organic carbon; F: only chemical fertilizer; FM: chemical fertilizer incorporated with Chinese milk vetch; FS: chemical fertilizer incorporated with rice straw; FMS: chemical fertilizer co-incorporated with Chinese milk vetch and rice straw; N_{100} : standard rate of nitrogen level; N_{60} : 40% reduced nitrogen level). Different lowercase letters indicate significant differences (Duncan, $p < 0.05$) among all treatments.

3.4. Incorporation of Chinese Milk Vetch and Rice Straw Affected C Sequestration Rates and C Stocks

The FMS treatment led to a distinct reduction in bulk density ($p < 0.05$) compared to the F treatment, regardless of whether the N_{60} or N_{100} level was applied. The bulk density in the FM treatment had a little change under the two N levels. The indistinctive difference in the NLOC sequestration rate and the NLOC stock in all treatments was observed. However, there was a significant difference in the SOC sequestration rate only between the F and FMS treatments under the N_{100} level but not the N_{60} level (Table 3). Specifically, the FMS treatment increased the SOC stock and the SOC sequestration rate by 11.32% compared to the F treatment under the N_{100} level ($p < 0.05$). It was observed that reducing the chemical N fertilizer application by 40% did not significantly decrease the SOC and NLOC stocks in the same additional organic treatment.

Table 3. Effect of fertilization regimes on soil bulk density, soil C sequestration rates, and stocks in topsoil layer (0–20 cm).

N Level	Treatments	Bulk Density (g cm ^{−3})	C Sequestration Rates (Mg C ha ^{−1} yr ^{−1})		C Stocks (Mg C ha ^{−1})	
			SOC	NLOC	SOC	NLOC
N ₆₀	F	1.09 ± 0.03 ^{abc}	0.84 ± 0.06 ^b	0.77 ± 0.07 ^a	21.05 ± 0.28 ^b	16.46 ± 0.35 ^a
	FM	1.10 ± 0.01 ^{ab}	0.98 ± 0.06 ^{ab}	0.64 ± 0.12 ^a	21.76 ± 0.28 ^{ab}	15.81 ± 0.61 ^a
	FS	1.05 ± 0.02 ^{cd}	0.88 ± 0.10 ^b	0.60 ± 0.17 ^a	21.23 ± 0.50 ^b	15.61 ± 0.85 ^a
	FMS	1.04 ± 0.01 ^d	1.20 ± 0.22 ^{ab}	0.80 ± 0.23 ^a	22.83 ± 1.08 ^{ab}	16.64 ± 1.13 ^a
N ₁₀₀	F	1.10 ± 0.02 ^a	0.82 ± 0.17 ^b	0.86 ± 0.17 ^a	20.93 ± 0.84 ^b	16.95 ± 0.86 ^a
	FM	1.09 ± 0.02 ^{abc}	0.94 ± 0.12 ^{ab}	0.80 ± 0.11 ^a	21.53 ± 0.62 ^{ab}	16.60 ± 0.56 ^a
	FS	1.07 ± 0.02 ^{abcd}	1.15 ± 0.03 ^{ab}	0.87 ± 0.05 ^a	22.59 ± 0.14 ^{ab}	16.99 ± 0.23 ^a
	FMS	1.06 ± 0.01 ^{bcd}	1.29 ± 0.03 ^a	1.00 ± 0.04 ^a	23.30 ± 0.13 ^a	17.64 ± 0.20 ^a

The averages (mean ± SE) followed by different lowercase letters indicate significant differences (Duncan, $p < 0.05$) among all treatments. SOC: soil organic carbon; NLOC: non-labile organic carbon. C sequestration rate is the annual rate during 2016–2020; C stocks are the values from 2016 to 2020. F: only chemical fertilizer; FM: chemical fertilizer incorporated with Chinese milk vetch; FS: chemical fertilizer incorporated with rice straw; FMS: chemical fertilizer co-incorporated with Chinese milk vetch and rice straw. N₁₀₀: standard rate of nitrogen level; N₆₀: 40% reduced nitrogen level.

3.5. The Relationship among C Input, N Input, Organic C/N, and Carbon

The C input had positive linear correlations with the concentrations of SOC and its fraction and SOC stock, but there were no linear correlations between the C input with the NLOC stock (Figure 5). The organic N input (which was the N input from Chinese milk vetch and rice straw) also had the same positive linear correlation with the concentration of SOC and its labile fraction as the C input, but the total N input was different. The total N input had positive linear correlations with the concentrations of SOC and NLOC ($p < 0.05$), except the SOC stock, namely, a positive linear correlation between the total N input and the NLOC stock ($p < 0.05$) (Figure 6). The organic C/N (which was the ratio of C to N from Chinese milk vetch and rice straw) had significant nonlinear correlations (quadratic term fitting) with the concentrations of the SOC, NLOC, and SOC stocks (Figure 7).

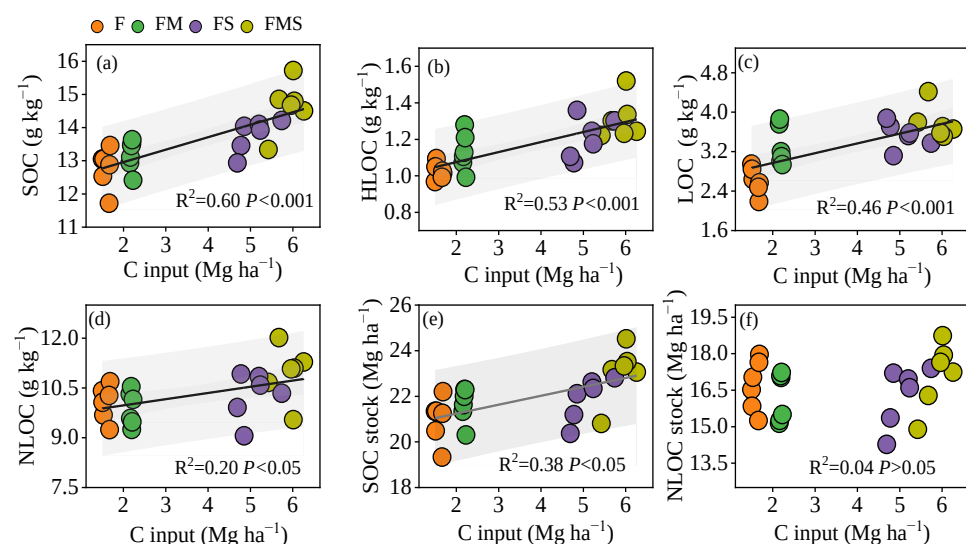


Figure 5. Relationships between the C input and the concentration and stock of carbon. (a) SOC concentration; (b) HLOC concentration; (c) LOC concentration; (d) NLOC concentration; (e) SOC stock; (f) NLOC stock. (SOC: soil organic carbon; HLOC: highly labile organic carbon; LOC: labile organic carbon; NLOC: non-labile organic carbon. F: only chemical fertilizer; FM: chemical fertilizer incorporated with Chinese milk vetch; FS: chemical fertilizer incorporated with rice straw; FMS: chemical fertilizer co-incorporated with Chinese milk vetch and rice straw).

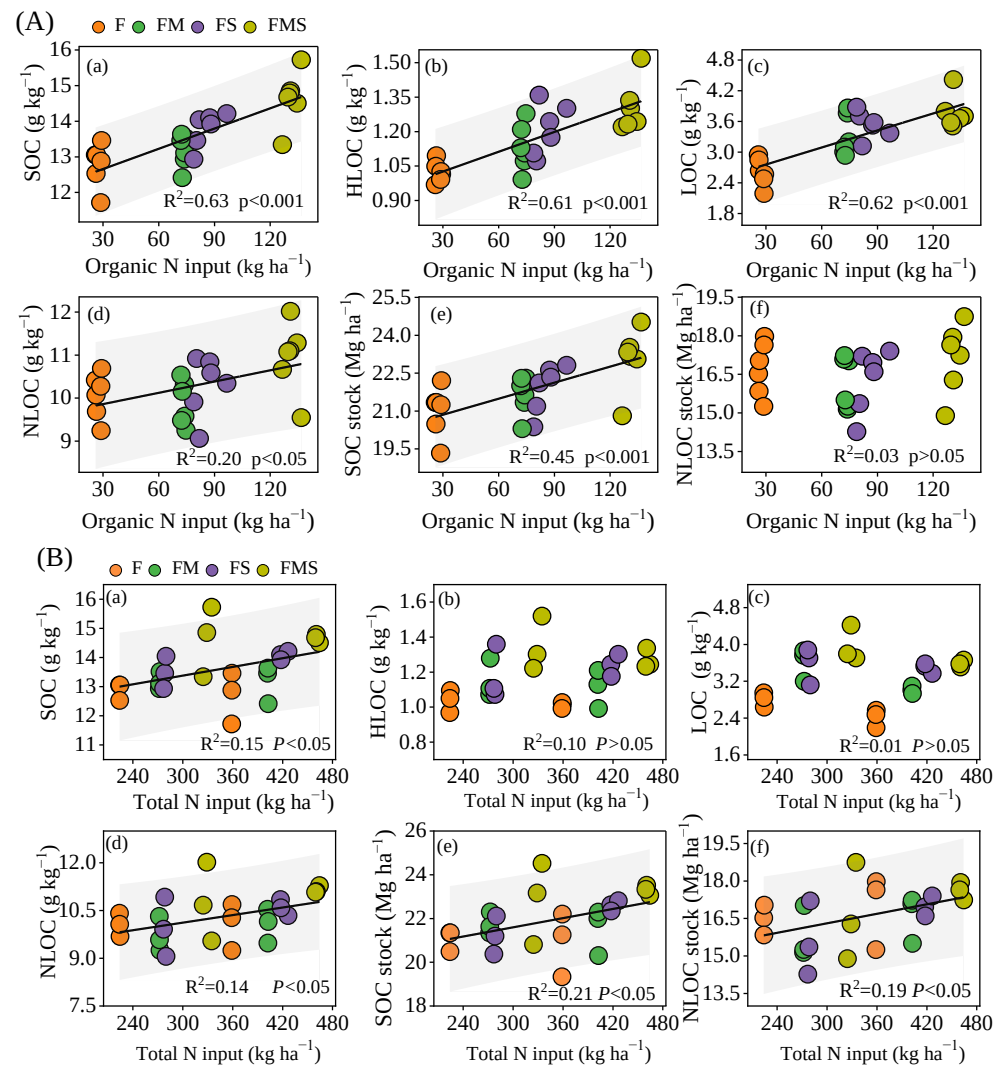


Figure 6. Relationships among the organic N input (A), total N input (B), and the concentration and stock of carbon. (a) SOC concentration; (b) HLOC concentration; (c) LOC concentration; (d) NLOC concentration; (e) SOC stock; (f) NLOC stock. (The total N input including organic N from the Chinese milk vetch and rice straw and chemical N fertilizer input; SOC: soil organic carbon; HLOC: highly labile organic carbon; LOC: labile organic carbon; NLOC: non-labile organic carbon. F: only chemical fertilizer; FM: chemical fertilizer incorporated with Chinese milk vetch; FS: chemical fertilizer incorporated with rice straw; FMS: chemical fertilizer co-incorporated with Chinese milk vetch and rice straw).

3.6. Effects of Key Factors on Yield and SOC

The random forest regression models (Figure 8) and the structural equation model (Figure 9) calculated the importance and effect of relevant parameters on SOC and yield. The random forest regression models showed that HLOC, C, and organic N input played an important role in increasing SOC. The total N input played a crucial role in rice yield, followed by the organic N and C inputs. The SEM showed a direct negative effect from the C input on the yield, but the total effect indicated the influence of the C input on the yield was positive. The HLOC and LOC had a direct positive effect by the C input, and the HLOC and NLOC had a direct positive effect on the SOC. The N input had a negative effect on the LOC but a positive effect on the NLOC.

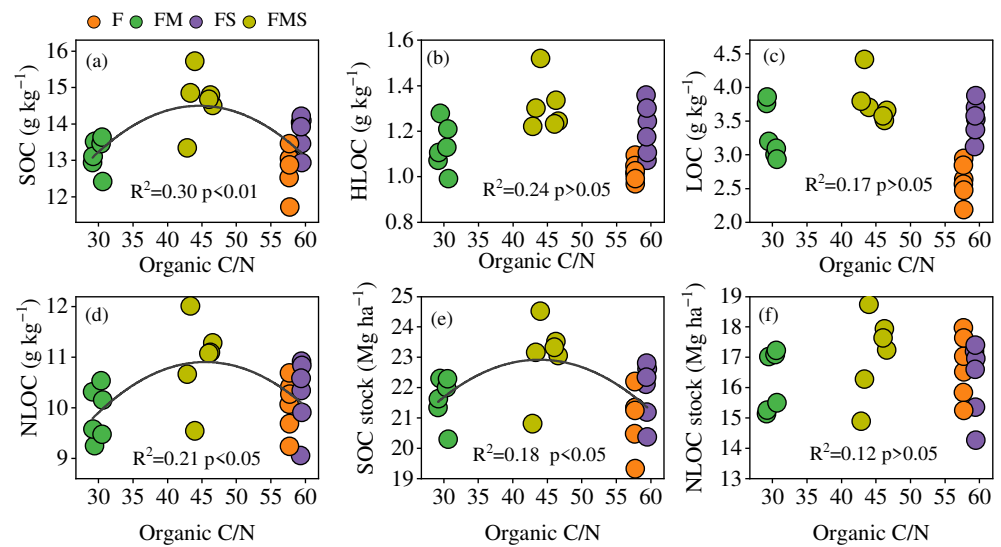


Figure 7. Relationships between the organic C/N and the concentration and stock of carbon. (a) SOC concentration; (b) HLOC concentration; (c) LOC concentration; (d) NLOC concentration; (e) SOC stock; (f) NLOC stock. (The organic C/N was the ratio of C to N from Chinese milk vetch and rice straw. SOC: soil organic carbon; HLOC: highly labile organic carbon; LOC: labile organic carbon; NLOC: non-labile organic carbon. F: only chemical fertilizer; FM: chemical fertilizer incorporated with Chinese milk vetch; FS: chemical fertilizer incorporated with rice straw; FMS: chemical fertilizer co-incorporated with Chinese milk vetch and rice straw).

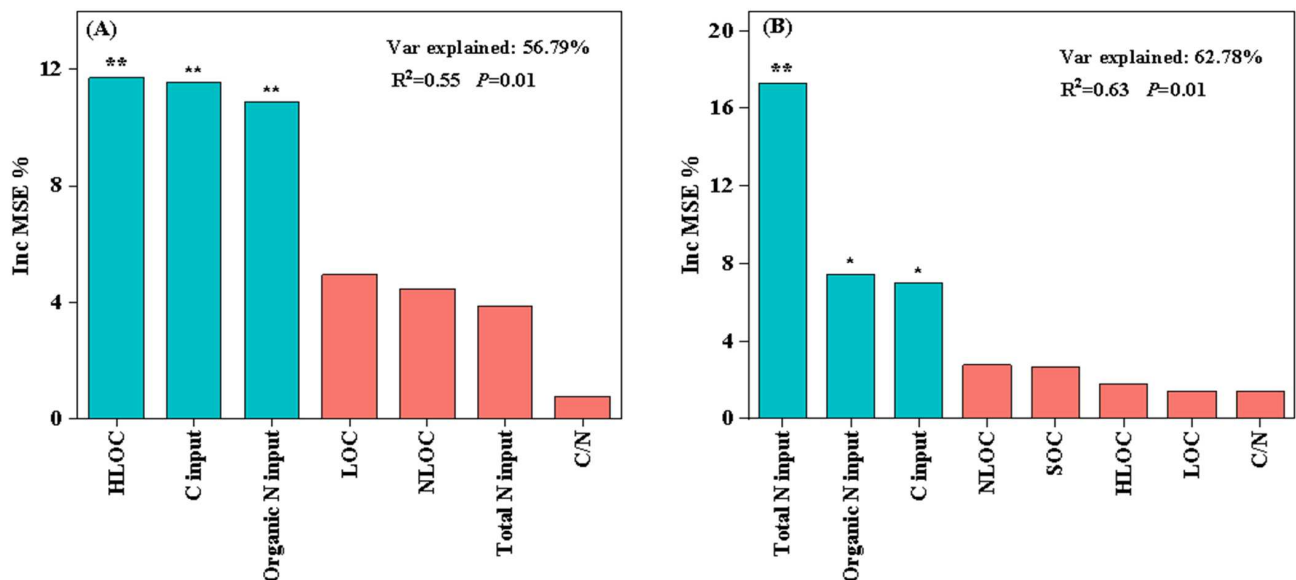


Figure 8. The random forest regression model, (A) SOC and (B) yield. (Inc. MSE means an increased rate of mean square error. The total N input including organic N from Chinese milk vetch and rice straw and chemical N fertilizer input, and the C/N was organic C/N, which was the ratio of C to N from Chinese milk vetch and rice straw. SOC: soil organic carbon; HLOC: highly labile organic carbon; LOC: labile organic carbon; NLOC: non-labile organic carbon; blue and red represent significance and non-significance. *, $p < 0.05$, **, $p < 0.01$).

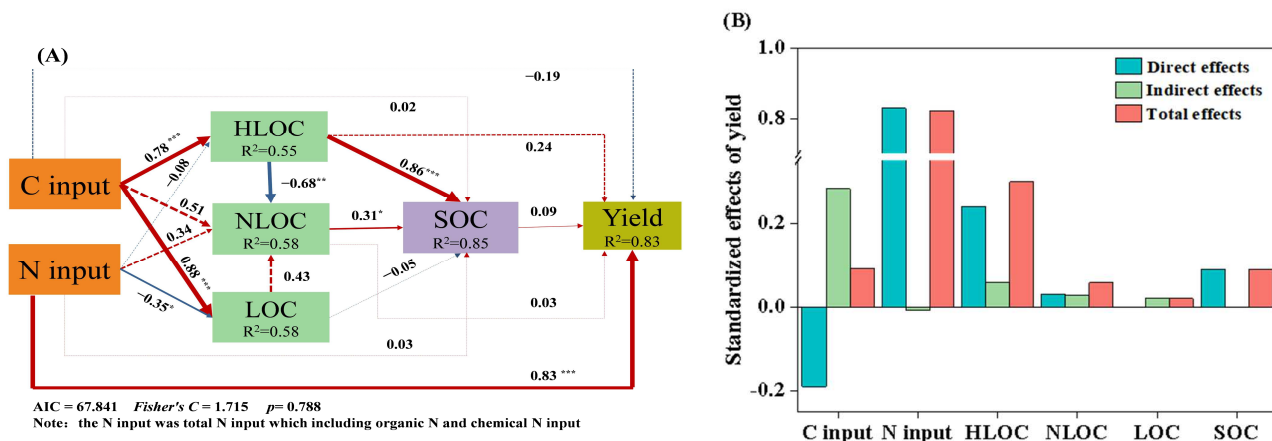


Figure 9. The structural equation model (A) described the effects of the key factors on yield and the standardized effects of yield (B). R^2 denotes the proportion of variance explained. Numbers near the arrows are the standardized path coefficients. The solid-line path indicates that the effect is significant, and a dashed-line path indicates that the effect has no significance. Blue and red represent negative and positive correlations, respectively. *, $p < 0.05$, **, $p < 0.01$, and ***, $p < 0.001$. (The N input includes organic N from the Chinese milk vetch and rice straw and chemical N fertilizer input. SOC: soil organic carbon; HLOC: highly labile organic carbon; LOC: labile organic carbon; NLOC: non-labile organic carbon).

4. Discussion

4.1. Co-Incorporation of Chinese Milk Vetch and Rice Straw Increases the Rice Yield

Rice yield could be improved by several conditions, such as soil fertility, scientifically utilizing fertilization, and management practices. The soil's nutrient concentrations, enzyme activities, and microbial biomass, which are related to grain yield, were improved by the application of fertilizer-containing organic materials. In this study, a noticeable difference in rice yield between the FMS with a N_{60} level and CF with a N_{100} level was undiscovered. This suggests that the use of MV and RS could decrease the amount of chemical fertilizer required by 40% while maintaining high yields, and the yield of double rice cropping was higher in the MV and RS treatments than in the CF treatment, which was similar to another study [24]. This was because both high biomass C and N inputs increase the organic matter and nutrients in the soil, improve soil structure, and promote microbial activity, thereby favoring the growth and development of rice which leads to enhanced rice productivity [4,16]. The mineral N fertilizers were able to quickly provide nutrients for crop growth, and we found that the yield under the N_{60} level was lower than that under the N_{100} level under the same organic addition treatment. Planting Chinese milk vetch could fix C and N, and once returned to the field, it could release nutrients, optimize N provision, and increase plant and grain N uptake in later stages [25]. The reports [26,27] showed that the treatment of MV return could cultivate the soil N pool and supply N better than the mineral N, but the MV with low C/N could decompose quickly, causing the release of nitrogen to not be better matched to the absorption of latter rice. RS return would result in a slow nutrient release rates because of its high C/N ratio. Therefore, the MV and RS joint return could adjust the C/N, which could optimize the N supply for the latter rice, further increasing the rice yield. A synergistic utilization of MV and RS improved the yield components of rice, including panicle length, effective panicle number, effective tiller number, and thousand kernel weight [17,28]. Moreover, MV and RS return could expand the soil C and N pools to promote the nutrient storage capacity. The above might be why the yield of FMS on the N_{100} level was the highest, while the FMS on reduced 40% mineral nitrogen fertilizer produced a comparable yield to traditional conventional fertilization. Reducing the chemical N fertilizer application while ensuring rice productivity was highly beneficial for the green and sustainable development of agriculture, which was also in line with the policy of green transformation in agricultural production to achieve a harmonious

coexistence between agriculture and the environment. MV or RS return would cause the differences in soil properties thus leading to the discrepancy of yield in all treatments. Gao et al. [24] found that the main factors influencing yields included SOM, microbial biomass N, and NP. We found MV and RS could improve the SOC concentration and bring about a positive correlation between the yield with the concentration of SOC. The mineralization of SOC together with a higher decomposition rate of fresh carbon could provide more available nutrients [3], which might be beneficial to grain yield. The co-utilization of MV and RS not only increases the soil's available mineral nutrients but could also optimize the C/N, and the release rate of nutrients could be adjusted reasonably [8,29]. In summary, the synergistic utilization of MV and RS was a good measure to increase yield and promote soil productivity.

4.2. Effects of Chinese Milk Vetch and Rice Straw on SOC and Its Labile Fractions

MV and RS as important organic fertilizers could increase SOC, especially labile carbon [3]. The labile pool was the primary one that promoted microbial activity and nutrient cycling in the soil C pools [30]. On the contrary, the non-labile organic carbon was slowly changed by microbial activity; therefore, it plays a vital role in accruing the organic carbon stocks [23]. Although a single MV return was conducive to the C input, it mineralized rapidly and might induce a positive priming effect of the soil C because of its low C/N ratio [8]; co-incorporation of RS with a high C/N together into the field could adjust the C/N ratio into the field to decrease the soil C priming effect. In our study, the FMS treatment had the most significant enhancement of the SOC concentration because of the extensive and various C inputs [3]. The available nutrients released from the decomposition of MV and RS could also promote rice root system growth, increasing a large amount of total biomass, root residues, and secretion plunged into the soil, which also benefited SOC accumulation [23]. Organic material addition would influence microbial communities, which could directly or indirectly control the soil C storage through microbial biomass, and the increase in microbial metabolism and debris would lead to an increase in SOC concentration. Those were indirect or direct ways to accumulate and stabilize C in soil [31]. By combining MV and RS, it was possible to augment the advantages of RS or MV return individually to the paddy field to improve soil fertility, further affecting microbial communities and enzyme activities and ensuring a suitable C/N ratio, which could improve bacterial abundance and hydrolytic enzyme activities [32,33]. Additionally, the utilization of MV and RS improved the soil structure to promote the formation of small macroaggregates from silt-clay particles, benefiting SOC protection and sequestration [34]. In addition, in flooded rice fields where anaerobic conditions prevail, a low activity of microorganisms and slower decomposition rate lead to the C sequestration in the soil, but the relative balance between SOC sequestration and CH₄ emissions determines the net C accumulation in agroecosystems [19].

The labile organic carbon fractions increased by the utilization of organic materials [4], which was also confirmed in this study. The HLOC and LOC concentrations increased with increasing C input. Moreover, the C input could directly affect the soil's labile organic C pool, and organic materials combined with chemical fertilizers enhanced microbial activities by providing an available source of carbon substrate by the C input, thereby transforming more plant residual carbon into unstable forms of organic carbon [23]. It was found that the C input could directly affect the soil carbon storage and the soil's labile carbon fractions, but had little effect on non-labile organic carbon [4]. The difference was that we found that FMS had an increasing effect on the NLOC concentrations, but the improvement effect was less and less significant from 2018 to 2020. Xu et al. [15] noted the combined utilization of CF with MV and RS largely promoted organic carbon stabilization because carbonyl C and aromatic C were associated with Fe/Al oxides. The higher NLOC concentrations and proportions of LOC were found in the organic material treatments than in the F treatments, which was confirmed by the CMI. The CMI of HLOC and LOC was increased significantly by the MV and RS return, which was consistent with another study [23]. The

CMI significantly increased with organic material addition, which suggested that the MV and/or RS return could improve soil quality and impede soil degradation.

Some researchers pointed out that MV and RS could increase labile organic carbon, but soil organisms might rapidly mineralize the additional part of labile pools, which has an adverse effect on C sequestration [35]. These might be the reasons why there were no measurable differences in the NLOC stock and SOC stock in the other treatments relative to the F treatment, except for the FMS treatment at the N₁₀₀ level. Except for the addition of organic matter, environmental factors, such as soil properties, temperature, pH, Eh, and water status, as well as agricultural fertilization management all affect the C balance in agroecosystems. At the same time, soil C sequestration potential, turnover rate, and dynamics are also affected by soil type and climate [19,20]. The SOC and NLOC stocks of all treatments had a little change in our work, which could be due to a lower decomposition rate of SOC and higher stabilization efficiency of the organic residues in soil with lower SOC levels [5]. However, the C stocks had an increasing trend when both MV and RS were integrated, which indicated that the MV and RS return together was a strategy to heighten C fixation and stock in the long term. Because of the short term of the experimental period, long-term monitoring is needed to evaluate the stability of the synergistic utilization of MV and RS for soil C sequestration in the future.

4.3. N Affects the Accumulation of SOC and Its Labile Fractions

The co-incorporation of MV, RS, and chemical N allowed both organic and inorganic N to improve the N availability and increase the contribution of plant-derived carbon to SOC. The finding also suggests that the long-term chemical N addition could increase the plant-derived C plunge into soils; moreover, a weakened C loss by decomposition, which largely guaranteed SOC increases. The improvement of N effectiveness could decrease the soil priming effect, which resulted in a declined soil C loss, because N effectiveness affect the activity of soil microorganisms, alter microbial metabolic efficiency [36]. We found that the concentration and stock of SOC were not significantly different in the treatment at two N levels after five years, but there was more SOC concentration at the N₁₀₀ level than at the N₆₀ level in the treatment of organic return. The FMS treatment increased the SOC stock and sequestration rates significantly at the N₁₀₀ level, which may be because the application of MV and RS with enough mineral N fertilizer supplied adequate organic N and C. When the supply of N was abundant, microorganisms quickly invested more C for biomass synthesis and not respiration, which was beneficial for increasing C accumulation [37]. The reports observed that the chemical N addition induced a higher priming effect when fresh C was input; the input of MV and RS might not improve SOC stock, because the chemical N addition might also enhance soil respiration and reduce microbial biomass, leading to a counteraction to the increased C inputs [38–40]. We found a little increase in the stock of SOC at the N₆₀ level in the organic return treatments relative to the F treatment. Other studies found that labile C was affected by the chemical N addition, but did not affect C stability [38,40]. Liu et al. [40] reported the organic C pool was not affected by the mineral N addition. This indicates that the N addition was able to influence the SOC levels but also depended on management practices. In our study, the N input had a positive effect on the NLOC and a negative effect on the LOC and HLOC, the linear correlation between the N input with the stock of SOC and NLOC. This indicated that the combination of organic N and chemical N was beneficial for the carbon accumulation.

5. Conclusions

Our study shows that the synergistic utilization of MV and RS increased the rice yield based on traditional chemical fertilizers, and it could replace 40% of chemical N fertilizer applications without sacrificing rice yield in double cropping. The co-incorporation of MV and RS was a good measure to increase SOC and its labile fractions and meliorate soil environment. The C input affected the SOC by directly affecting the HLOC and LOC. Moreover, a suitable C/N ratio and the combination of organic N and mineral N were

beneficial for C accumulation, and reducing the application of chemical N fertilizers would not affect the C storage. Overall, co-incorporating Chinese milk vetch and rice straw was a suitable management practice for organic resources to ensure the sustainable development of paddy fields in Southern China. In addition, to better explore the mechanisms underlying soil C sequestration, investigating the effect of the synergistic utilization of MV and RS on soil C in different geographical regions is necessary in the long-term future. Also, how these treatments specifically influence yield formation needs to be explored further for improved sustainable agricultural development.

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Data Availability Statement: Data available on request due to restrictions, e.g., privacy or ethical restrictions. The data presented in this study are available on request from the corresponding author. The data are not publicly available because the first author is still performing their PhD research.

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