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Substitution of Chemical Fertilizer with Organic Fertilizer Can Affect Soil Labile Organic Carbon Fractions and Garlic Yield by Mediating Soil Aggregate-Associated Organic Carbon

Naijuan Hu [1], Canyu Liu [2], Qian Chen [3], Jide Fan [2], Yutao Wang [3] and Hongwu Sun [1,*]

[1] Institute of Agricultural Economics and Development, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China; hunaijuan@163.com
[2] Xuzhou Institute of Agricultural Sciences in Jiangsu Xuhuai Area, Xuzhou 221131, China; liucanyu@126.com (C.L.); fanjide@163.com (J.F.)
[3] College of Agriculture, Nanjing Agricultural University, Nanjing 210095, China; 2018810098@njau.edu.cn (Q.C.); wyutaoo@163.com (Y.W.)
* Correspondence: sunhwjaas@163.com

Abstract: This study aimed to explore the impact paths on soil organic carbon and crop yield of completely or partially substituting chemical N fertilizer with organic fertilizers. A four-year field experiment was conducted and included four treatments: (i) N0, no N fertilization application; (ii) NF, only synthetic N fertilizer application; (iii) 1/2OF, organic fertilizer substituted for 100% of the synthetic N fertilizer, with the total N application amount being equivalent to half that of NF; and (iv) 1/3OF + 2/3NF, organic fertilizer substituted for 1/3 of the synthetic N fertilizer with the total N application amount from organic and synthetic fertilizer being equivalent to that of NF. Soil total organic carbon (TOC), labile organic-carbon fractions (microbial biomass carbon (MBC), dissolved organic carbon (DOC), particulate organic carbon (POC), and easily oxidized organic carbon (EOC)), the carbon pool management index (CPMI), soil aggregated distribution, and water-stable aggregate-associated organic carbon were determined. Structural equation modeling (SEM) was used to clarify the impact paths of TOC and garlic yield changes under different N fertilizer treatments. Results showed that compared with N0 and NF, 1/2OF and 1/3OF + 2/3NF significantly increased TOC contents by 14.1–20.6%. Soil MBC, DOC, and EOC under 1/2OF were significantly higher than under N0, whereas the 1/3OF + 2/3NF treatment had significantly greater POC. The CPMI was improved by organic fertilizer treatment, with 1/2OF treatment being significantly higher than N0 and NF. The proportion of soil aggregate mass with particle sizes >2 mm was significantly greater under N0, while 1/3OF + 2/3NF significantly increased the proportion of particle sizes of 0.5–2 mm. Soil water-stable aggregate-associated organic carbon showed a trend of first increasing and then decreasing, with the largest particle sizes being 1–2 mm. Moreover, organic fertilizer significantly increased soil water-stable aggregate organic carbon compared with N0 and NF. Similarly, the garlic yield increased with organic fertilizer treatment, while 1/3OF + 2/3NF significantly increased the yield by 37.2% and 15.3%, respectively, compared with N0 and NF. Furthermore, SEM analysis indicated that fertilizer regimes could directly affect TOC and labile organic carbon components by affecting aggregate-associated organic carbon. In particular, aggregates with particle sizes of 0.5–2 mm played an important role, indirectly affecting garlic yield and CPMI. These results indicate that organic fertilizer application has the potential to improve soil organic-carbon content and garlic yield; moreover, fully applying organic fertilizer can reduce N fertilizer input while still maintaining an increase in soil organic carbon and crop yield in the short term. However, caution is still needed regarding of the type and quantity of organic fertilizer added in different cropping systems, and with different soil textures.

Keywords: organic fertilizer; soil organic-carbon fractions; carbon pool management index; soil aggregate-associated organic carbon; impact path; garlic yield

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1. Introduction

The application of chemical nitrogen (N) fertilizers has made a great contribution to the increase in grain yields in the past few decades [1]. However, chemical N fertilizers often improve crop yields in the short and medium term [2], while long-term excessive application of chemical N fertilizers not only reduces the utilization rate of N fertilizers and is not conducive to yield improvement, but also causes serious environmental pollution, including soil acidification [3], non-point source pollution [4], ammonia volatilization [5], and nitrous-oxide emissions [6]. It has been shown that partially or completely replacing chemical nitrogen fertilizers with organic fertilizers can improve soil fertility, reduce leaching N losses, and increase crop yields. Thus, this is considered a reasonable fertilization method and is widely adopted in various agricultural ecosystems [7–9].

Soil total organic carbon (TOC) plays a major role in soil nutrient cycling and soil microbe activities, and it has a significant positive correlation with farmland production [10,11]. Salehi et al. [12] reported that manure plus chemical fertilizer significantly improved SOC by 2.45%. However, due to the stability of TOC and high background carbon content, changes in TOC under short-term or medium-term conditions are generally not easily detected [13]. Soil labile organic-carbon fractions refers to active organic carbon components that are easily decomposed by microorganisms and can quickly respond to land management measures, and include microbial biomass carbon (MBC), dissolved organic carbon (DOC), particulate organic carbon (POC), and easily oxidized organic carbon (EOC) [14]. MBC is primarily affected by soil microbial biomass and plays an important role in the decomposition of soil organic matter [15]. DOC is the main energy source for soil microorganisms, and can serve as an important indicator for evaluating soil microbial decomposition and nutrient availability [16]. EOC is an indicator that reflects the easily decomposable component of soil organic carbon in early stages [17]. Where the particle size of the organic carbon is greater than 53 μm, it is called particulate organic carbon (POC). This is an active intermediate product in the conversion of animal and plant residues into soil humus, and has lower stability and is more susceptible to human management measures than TOC [18]. In addition, the carbon pool management index (CPMI), used to monitor changes in TOC and EOC, is a valuable parameter for evaluating the ability of management practices to improve soil quality, and can assess the state and rate of change in agricultural soil carbon in agricultural ecosystems [13,19]. Zhang et al. [20] reported that, in wheat crops, organic fertilizer partially substituted for synthetic N fertilizer could significantly increase the soil TOC and its fractions' contents, as well as CPMI, compared with N0 and NF. Xu et al. [21] also found that long-term chemical N fertilizer plus manure application could increase TOC stability and POC, DOC, EOC, and MBC concentrations in both 0–10 cm and 10–20 cm soil layers. However, some studies have found that using organic fertilizers increased the TOC content, but did not affect the labile organic components [22].

Soil aggregates are the fundamental units of soil structure, and aggregates with different particle sizes play diverse roles in the abilities of soil related to nutrient supply, preservation, and conversion [23]. Soil organic carbon is an important cementitious substance that promotes the formation of aggregates, and the two are interdependent and coexist. Soil aggregates have a significant role in retaining soil fertility and structure by protecting soil organic carbon from mineralization. Soil aggregates are divided into large aggregates (particle size > 0.25 mm) and micro aggregates (particle size < 0.25 mm) [24]. Among these, aggregates with a particle size > 0.25 mm are the best structural bodies in soil, and their quantity is significantly positively correlated with soil fertility. Soil water-stable aggregates can better represent the structure and stability of soil, and the higher the content of soil water-stable aggregates, the better the stability of soil structure [25,26]. Organic fertilizers, including straw and manure, can greatly affect soil aggregates by enhancing soil organic binders, thus promoting the connection between soil particles and the stability of aggregates [27]. A previous study showed that applying organic fertilizers alone or combined with chemical fertilizers improved the stability of soil aggregates, especially for large aggregates with a particle size > 0.25 mm [28].

Garlic (*Allium sativum* L.) is a widely consumed spice crop worldwide. Garlic products and their by-products have various medicinal values such as antioxidant, antibacterial, anti-inflammatory, immunomodulatory, antihypertensive, anticancer, and anti-hyperlipidemic [29]. Therefore, around the world, garlic is considered a "medicinal and food homologous" crop that is superior to traditional and alternative drugs [30]. According to the FAO, in 2020, the total area of garlic cultivation world-wide was approximately 1.63 million hectares, with an annual production of approximately 28 million tons [31]. Since 1994, China's garlic production, consumption, and export have ranked first in the world, with about 50% and 70% of the world's production and planting area, respectively [31]. Garlic cultivation in China has the prominent characteristics of high yield and returns, and one of the main reasons for this is the high-intensity application of chemical fertilizers. However, long-term overuse of chemical N fertilizers, coupled with rough fertilization methods and improper irrigation methods, has led to increased soil salinization, decreased organic matter content, increased soil-borne diseases, and reduced fertilizer utilization rates, which, furthermore, seriously affects the yield and quality of garlic, and directly affects its economic benefits [32–34]. Ma et al. [35] found that replacing 40% of chemical N fertilizer with organic fertilizer could improve the garlic productivity and the complexity of the microbial community network in Southwest China, and this can be recommended as a reasonable fertilization practice for garlic. Although some studies have reported the impact of organic fertilizer replacing chemical fertilizers on soil quality and yield in garlic fields [36], there are certain differences in results among different garlic production areas due to differences in cropping systems, climate characteristics, and management practices. Furthermore, the impact paths of fertilizer management methods on the soil labile organic-carbon fractions, CPMI, and yield of garlic soil are still undefined.

We conducted a four-year field experiment in the main garlic-producing areas of Jiangsu Province, China, exploring the effects of reducing N fertilizer and replacing chemical fertilizers with organic fertilizers on soil organic carbon compositions and garlic yield. The research objectives were: (1) clarifying the response of soil organic carbon, labile organic-carbon fractions, CPMI, water-stable aggregate organic-carbon contents, and garlic yield to partial or complete substitution of organic fertilizer treatment; (2) analyzing the significant correlation between different organic-carbon fractions, CPMI, and yield; and (3) determining the direct and indirect pathways of fertilizer management measures affecting soil TOC and garlic yield.

2. Materials and Methods

2.1. Experimental Site

A field experiment was conducted at the Experimental Demonstration Base of Xuzhou Agricultural Science Research Institute, Xu zhou city, Jiangsu province of China (117°41′ E, 34°30′ N), with the mean annual sunshine hours, temperature, and precipitation of 2366 h, 16.5 °C, and 869 mm, respectively. The soil type was a sandy loamy tidal soil. Before this experiment (October 2019), the initial properties of soil samples (0–20 cm) were as follows: soil organic matter 17.3 g kg^{-1}, total nitrogen 0.91 g kg^{-1}, available phosphorus 17.7 mg kg^{-1}, and available potassium 117.7 mg kg^{-1}. The pH (H_2O) was 8.5.

2.2. Experimental Design

The positioning experiment was first carried out in October 2019 and the cropping system of this experiment was a garlic–soybean rotation. The experiment adopted a randomized block design, and included four treatments: (i) N0, no N fertilization application; (ii) NF, only synthetic fertilizer application; (iii) 1/2OF, organic fertilizer substituted for 100% of the synthetic N fertilizer, with the total N application amount being equivalent to half of NF; and (iv) 1/3OF + 2/3NF, organic fertilizer substituted for 1/3 of the synthetic N fertilizer, with the total N application amount from organic and synthetic fertilizer being equivalent to that of NF. Referring to the local garlic fertilization management practices, the total N applied in NF was 354 kg N ha^{-1}. The detailed fertilization management practices are shown in Table 1. Each treatment was repeated three times with an area of 200 m^2 (5.0 m × 40.0 m). The experimental variety of garlic ("Xubai No.1") was provided by the Horticultural Research Office of the Xuzhou Academy of Agricultural Sciences, Jiangsu Province, China. The garlic was sown in mid-September and harvested in mid-May of the following year. Other management processes, including irrigation and pesticide application, were carried out in accordance with local conventional methods.

Table 1. Fertilizer management practices in garlic season.

Treatments	**Base Fertilizer ($kg \cdot ha^{-1}$)**					**Topdressing ($kg \cdot ha^{-1}$)**
	Synthetic Fertilizer (N:P:K = 15:15:15)	**Urea ($N \geq 46\%$)**	**Organic Fertilizer ($N \geq 0.79\%$, $P \geq 0.75\%$, $K \geq 0.62\%$)**	**K_2O ($P \geq 51\%$)**	**P_2O_5 ($K \geq 12.0\%$)**	**Urea ($N \geq 46\%$)**
N0	/	/	/	220	937	/
NF	750	300	/	/	/	225
1/2OF	/	/	22,525	/	/	/
1/3OF + 2/3NF	/	286	15,017	38	/	225

N0 represents no N fertilizer application; NF represents synthetic N fertilizer application; 1/2OF represents organic fertilizer substituted for 100% of the synthetic N fertilizer, with the total N application amount being equivalent to half of NF; 1/3OF + 2/3NF represents organic fertilizer substituted for 1/3 of the synthetic N fertilizer, with the total N application amount being equivalent to NF.

2.3. Soil Sampling and Measurement

Before the garlic harvest in May 2023, soil samples of 0–20 cm were obtained from each plot through a five-point sampling method using a soil drill (diameter 5.0 cm). All collected soil samples were divided into two groups after removing plant residues, roots, and stones. A part of the soil samples was stored in a 4 °C refrigerator for measurement of soil MBC and DOC contents. The other part was air-dried, ground, and sieved through 2.00 mm and 0.25 mm screens for measuring soil TOC, EOC, and POC. Additionally, the soil samples for aggregate were obtained in each plot by a cylinder (20 cm height, 15 cm diameter). Before being air-dried, the soil samples were crushed and sieved through a 10 mm sieve, and then a wet sieving method was used to separate soil aggregates [37].

Soil TOC and DOC contents were measured with a total organic carbon analyzer (Multi N/C 3100 TOC/TN, Analytik-Jena, Jena, Germany) [38]. MBC was determined using the chloroform fumigation K_2SO_4 extraction method [39], and EOC was determined using the 333 mmol L^{-1} $KMnO_4$ oxidation method [40]. Soil POC were determined using the methods described by Cambardella and Elliott [41]. The determination of organic-carbon content in water-stable aggregates was first carried out by measuring the weight of each particle size of mechanically stable aggregates using the dry sieve method, and calculating their mass percentage content. Then, the water-stable aggregates were obtained using the wet sieve method, and the content of each particle size of water-stable aggregates was calculated [42]. Lastly, the organic-carbon content of each particle size of water-stable aggregates was measured according to the method of TOC measurement.

CPMI for each treatment was determined according to Blair et al. [13]. In this study, N0 was used as the reference. Based on the variation of TOC between the reference soil and the sample soil, the carbon pool index (CPI) was calculated as follows:

$$\mathrm{CPI} = \frac{\mathrm{TOC_S}}{\mathrm{TOC_r}}$$

where $\mathrm{TOC_S}$ and $\mathrm{TOC_r}$ represent the TOC content of sample and reference soil, respectively. The carbon lability (L) is the ratio of EOC to non-labile carbon, which was calculated as follows:

$$\mathrm{L} = \frac{\mathrm{EOC}}{\mathrm{TOC} - \mathrm{EOC}}$$

Based on the changes in L, the carbon lability index (LI) was determined as follows:

$$\mathrm{LI} = \frac{\mathrm{L_S}}{\mathrm{L_r}}$$

where L_S and L_r represent the L of sample soil and reference soil, respectively. Therefore, CPMI was calculated according to two indices as follows:

$$\mathrm{CPMI} = \mathrm{CPI} \times \mathrm{LI} \times 100$$

2.4. Garlic Yield

At physiological maturity, the garlic bulb in each plot was harvested manually. After removing the stem and leaf parts, and the yield was determined by weighing the head of garlic.

2.5. Statistical Analysis

SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. A one-way variance (ANOVA) was used to examine the differences in soil labile organic-carbon pools, CPMI, soil water-stable aggregated organic carbon, and garlic yield among the four treatments, with the least significant difference (LSD) at $p < 0.05$ [43]. Pearson linear correlations between all the indicators were performed to determine the correlation coefficients. Structural equation modeling (SEM) using AMOS version 21.0 (IBM Corporation Software Group, Somers, NY, USA) showed the impact path of soil labile organic-carbon fractions and water-stable aggregate organic carbon on CPMI, TOC, and garlic yield under different N fertilizer application treatments [44]. The graphics were mapped by Origin 2020 (Origin Lab Corporation, Northampton, MA, USA).

3. Results

3.1. Soil TOC and Labile Organic-Carbon Fractions

After the four-year field experiment, soil TOC and labile organic-carbon fractions were significantly affected by N fertilizer addition modes (Table 2). TOC was significantly higher under the 1/2OF and 1/3OF + 2/3NF treatments than under N0 and NF, with an increase of 14.1–20.6%. Compared with N0, MBC was significantly increased by 37.6–68.4% under N fertilizer treatments, and 1/2OF treatment had the highest MBC, which was significantly higher than that of NF, and NF had significantly higher MBC than that of 1/3OF + 2/3NF. Compared with N0, 1/2OF treatment significantly increased DOC content by 22.7%, but no significant differences were revealed among other treatments. Soil POC content was the highest under 1/3OF + 2/3NF treatment, and there was a significant difference compared to N0 treatment. The EOC content under organic fertilizer treatments (1/2OF and 1/3OF + 2/3NF) was noticeably increased by 34.4–35.9% compared with N0, and 1/2OF treatment had the highest EOC, which was significantly higher than that of NF or N0.

Table 2. Effects of different N fertilizer application treatments on soil TOC and labile organic carbon.

Treatments	**TOC** $\mathbf{g{\cdot}kg^{-1}}$	**MBC** $\mathbf{mg{\cdot}kg^{-1}}$	**DOC** $\mathbf{mg{\cdot}kg^{-1}}$	**POC** $\mathbf{mg{\cdot}kg^{-1}}$	**EOC** $\mathbf{mg{\cdot}kg^{-1}}$
N0	12.7 ± 0.56 b	21.7 ± 3.73 d	30.4 ± 2.50 b	39.4 ± 3.99 b	2.59 ± 0.07 c
NF	12.9 ± 0.52 b	53.6 ± 4.07 b	35.7 ± 8.56 ab	42.6 ± 1.02 ab	3.13 ± 0.14 bc
1/2OF	16.0 ± 1.19 a	68.7 ± 7.62 a	39.3 ± 7.10 a	42.8 ± 4.80 ab	4.04 ± 0.39 a
1/3OF + 2/3NF	15.1 ± 0.08 a	34.8 ± 0.35 c	35.2 ± 7.80 ab	48.5 ± 1.57 a	3.95 ± 0.89 ab

Different lowercase letters in the same column indicate significant differences among treatments (LSD, $p < 0.05$). Values are the mean ± SD. N0, NF, 1/2OF, and 1/3OF + 2/3NF explanations are as under Table 1.

3.2. Carbon Pool Management Index

From Table 3, L, LI, CPI, and CPMI were all significantly influenced by the different treatments. Moreover, L, LI, CPI, and CPMI were all highest under 1/2OF treatment, and the 1/2OF treatment significantly increased L, LI, CPI, and CPMI by 23.5%, 24.8%, 20.6%, and 40.1%, respectively, compared with N0. Compared with NF, CPI and CPMI were also significant improved under 1/2OF treatment, with an increase of 15.0% and 24.0%, respectively. In addition, 1/3OF + 2/3NF treatment also had a significantly higher CPI and CPMI than N0.

Table 3. Changes in soil CPMI among different N fertilizer application treatments.

Treatments	L	LI	CPI	CPMI
N0	0.26 ± 0.02 b	1.00 ± 0.00 b	1.00 ± 0.00 b	100 ± 0.00 c
NF	0.32 ± 0.01 a	1.25 ± 0.10 a	1.02 ± 0.02 b	127 ± 11.3 bc
1/2OF	0.34 ± 0.04 a	1.33 ± 0.20 a	1.26 ± 0.14 a	167 ± 20.0 a
1/3OF + 2/3NF	0.31 ± 0.06 ab	1.21 ± 0.23 ab	1.19 ± 0.05 a	143 ± 28.0 ab

Different lowercase letters in the same column indicate significant differences among treatments at $p < 0.05$. Values are the mean ± SD. L, lability; LI, lability index; CPI, carbon pool index; CPMI, carbon pool management index. N0, NF, 1/2OF, and 1/3OF + 2/3NF explanations are as under Table 1.

3.3. Soil Aggregate Mass Distribution and Soil Water-Stable Aggregate Organic Carbon

The particle size composition of water-stable aggregates varied greatly under different treatments (Figure 1). For N0, the proportion of soil aggregate with particle sizes > 5 mm was the highest, reaching 30.6%, followed by that with particle sizes of 2–3 mm, accounting for 18.1%. Moreover, the proportions of large macro-aggregates (>5 mm) in the N0 treatment were significantly greater than those in the other three treatments. The N0 treatment also had significantly higher proportions of aggregate with particle sizes of 3–5 mm than the 1/2OF treatment. There were no significant differences in the proportions of particle sizes of 2–3 mm among different treatments. As the particle size decreased, the proportion of 0.25–2 mm aggregate particles rapidly decreased and was the lowest under the N0 treatment, whereas N0 had the highest proportion of 0.5–1 mm aggregate particles, accounting for 30.4% to 36.3%. For particle sizes of 0.5–1 mm and 1–2 mm, the proportion under 1/3OF + 2/3NF treatment was markedly higher than under other treatments. However, with 1/2OF treatment there was a greater proportion of 0.25–0.5 mm particles than with 1/3OF + 2/3NF and N0 treatments.

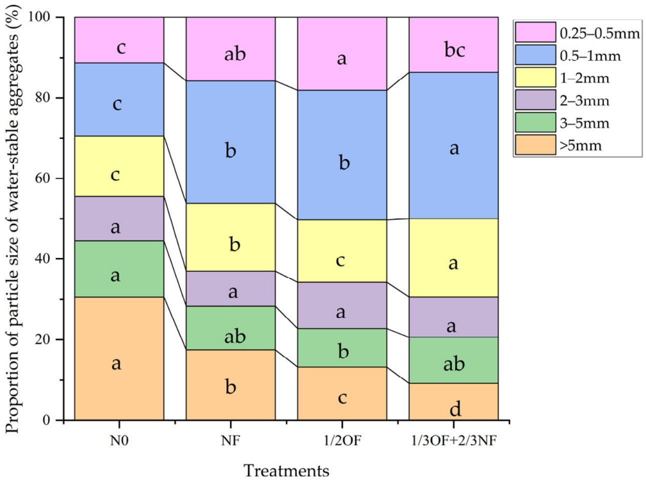


Figure 1. Proportions of mass compositions of water-stable aggregates of different particle sizes under different N fertilizer application treatments. Different lowercase letters indicate the significant differences among treatments (LSD, $p < 0.05$). N0, NF, 1/2OF, and 1/3OF + 2/3NF explanations are as under Table 1.

As shown in Figure 2, as the particle size decreased, the organic-carbon content of soil water-stable aggregates showed a trend of first increasing and then decreasing, and the particle sizes of 1–2 mm had the highest soil water-stable aggregate organic-carbon content under all N application treatments except N0. For different particle sizes, the organic-carbon contents of water-stable aggregate were the highest under the 1/2OF treatment, which was also significantly higher than under N0 or NF, with an increase of 19.8–39.0%. Furthermore, soil water-stable aggregate organic-carbon contents in particle sizes of 0.5–1 mm and 0.25–0.5 mm were significantly higher under 1/2OF treatment than under 1/3OF + 2/3NF treatment, and those of 1/3OF + 2/3NF were significantly higher than those of NF and N0 treatments.

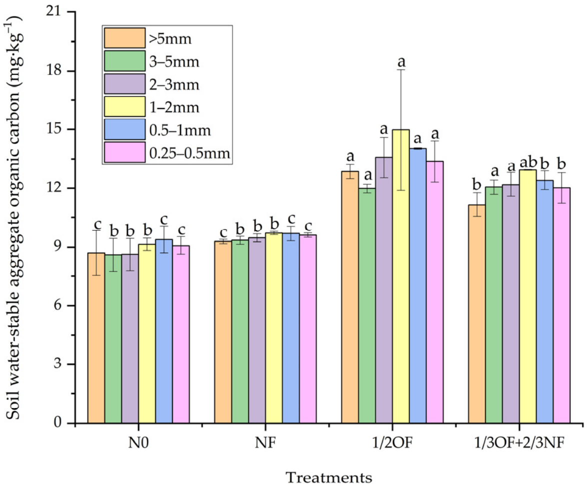


Figure 2. Soil water-stable aggregated organic-carbon contents under different N fertilizer application treatments. Values are means ± SD. Different lowercase letters in bars indicate the significant differences among treatments at $p < 0.05$. N0, NF, 1/2OF, and 1/3OF + 2/3NF explanations are as under Table 1.

3.4. Garlic Yield

From Figure 3, the garlic yield was significantly affected by N application treatments. N0 had the significantly lowest garlic yield at 12.4 × 10^3 kg·hm^{-2}, while the highest yield of 19.8 kg·hm^{-2} was found under 1/3OF + 2/3NF treatment. Moreover, compared with N0 and NF treatments, the garlic yield under 1/3OF + 2/3NF was significantly increased by 37.2% and 15.3%, respectively. There were no significant differences between 1/2OF and 1/3OF + 2/3NF treatments.

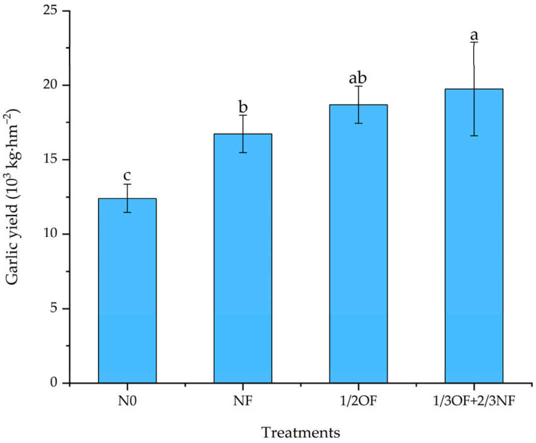
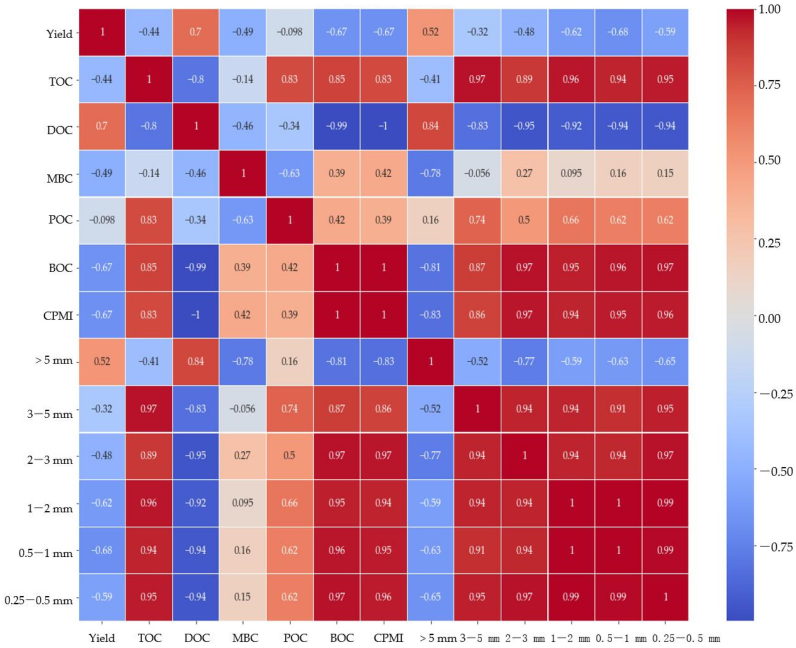


Figure 3. Garlic yield under different N fertilizer application treatments in 2023. Different lowercase letters in bars indicate the significant differences among treatments at $p < 0.05$. Values are means ± SD. N0, NF, 1/2OF, and 1/3OF + 2/3NF explanations are as under Table 1.

3.5. Correlation Analysis

The correlation analysis between garlic yield, soil organic-carbon fractions, CPMI, and soil water-stable aggregate organic carbon is shown in Figure 4. The garlic yield had a significant and positive correlation with TOC, DOC, POC, and soil water-stable aggregate organic carbon with particle sizes of 3–5 mm, 2–3 mm, and 0.25–0.5 mm. There was a significant and positive correlation between TOC and POC, EOC, CPMI, and all soil water-stable aggregate organic carbon with particle sizes > 0.25 mm. However, only POC and water-stable aggregate organic carbon with particle sizes > 0.25 mm had significant correlation with DOC. Soil MBC had a significant positive correlation with EOC, CPMI, and water-stable aggregate organic carbon with particle sizes of 2–3 mm. Significant positive correlations were shown between POC and all other indicators except MBC. EOC had a significant positive correlation with other soil indicators except DOC and water-stable aggregate organic carbon with particle sizes of >5 mm. There was also a significant positive correlation between CPMI and TOC, MBC, POC, EOC, and water-stable aggregate organic carbon except for particle sizes of >5 mm. In addition, a significant positive correlation was found between organic carbon in water-stable aggregates with particle sizes of 0.25–5 mm, but not with particle sizes of >5 mm.



	Yield	TOC	DOC	MBC	POC	BOC	CPMI	> 5 mm	3−5 mm	2−3 mm	1−2 mm	0.5−1 mm	0.25−0.5 mm
Yield	1	-0.44	0.7	-0.49	-0.098	-0.67	-0.67	0.52	-0.32	-0.48	-0.62	-0.68	-0.59
TOC	-0.44	1	-0.8	-0.14	0.83	0.85	0.83	-0.41	0.97	0.89	0.96	0.94	0.95
DOC	0.7	-0.8	1	-0.46	-0.34	-0.99	-1	0.84	-0.83	-0.95	-0.92	-0.94	-0.94
MBC	-0.49	-0.14	-0.46	1	-0.63	0.39	0.42	-0.78	-0.056	0.27	0.095	0.16	0.15
POC	-0.098	0.83	-0.34	-0.63	1	0.42	0.39	0.16	0.74	0.5	0.66	0.62	0.62
BOC	-0.67	0.85	-0.99	0.39	0.42	1	1	-0.81	0.87	0.97	0.95	0.96	0.97
CPMI	-0.67	0.83	-1	0.42	0.39	1	1	-0.83	0.86	0.97	0.94	0.95	0.96
> 5 mm	0.52	-0.41	0.84	-0.78	0.16	-0.81	-0.83	1	-0.52	-0.77	-0.59	-0.63	-0.65
3−5 mm	-0.32	0.97	-0.83	-0.056	0.74	0.87	0.86	-0.52	1	0.94	0.94	0.91	0.95
2−3 mm	-0.48	0.89	-0.95	0.27	0.5	0.97	0.97	-0.77	0.94	1	0.94	0.94	0.97
1−2 mm	-0.62	0.96	-0.92	0.095	0.66	0.95	0.94	-0.59	0.94	0.94	1	1	0.99
0.5−1 mm	-0.68	0.94	-0.94	0.16	0.62	0.96	0.95	-0.63	0.91	0.94	1	1	0.99
0.25−0.5 mm	-0.59	0.95	-0.94	0.15	0.62	0.97	0.96	-0.65	0.95	0.97	0.99	0.99	1

Figure 4. Correlation analysis matrix between different soil and yield indicators. TOC, total organic carbon; DOC, dissolved organic carbon; MBC, microbial biomass carbon; POC, particulate organic carbon; EOC, easy oxidated organic carbon; CPMI, carbon pool management index.

3.6. SEM Analysis

As shown in Figure 5, by using SEM, significant influence paths were determined between soil water-stable aggregate organic carbon, labile organic-carbon fractions, TOC, CPMI, and yield, except for DOC and aggregates with particle sizes > 5 mm. Soil water-stable aggregates with particle sizes of 0.25–5 mm had a direct and significant effect on DOC, POC, and EOC, and an indirect effect on yield and CPMI. In aggregates, the influence coefficient of water-stable aggregates with particle sizes of 0.25–0.5 mm was the highest, reaching 0.986. POC had a direct and positive effect path on TOC and DOC had an indirect and positive effect path on TOC, but a negative effect path was observed between MBC and TOC. TOC had a significant positive and direct effect path to garlic yield. EOC had a direct effect path on CPMI. In addition, an positive influence path was found from soil water-stable aggregate organic carbon to POC via DOC.

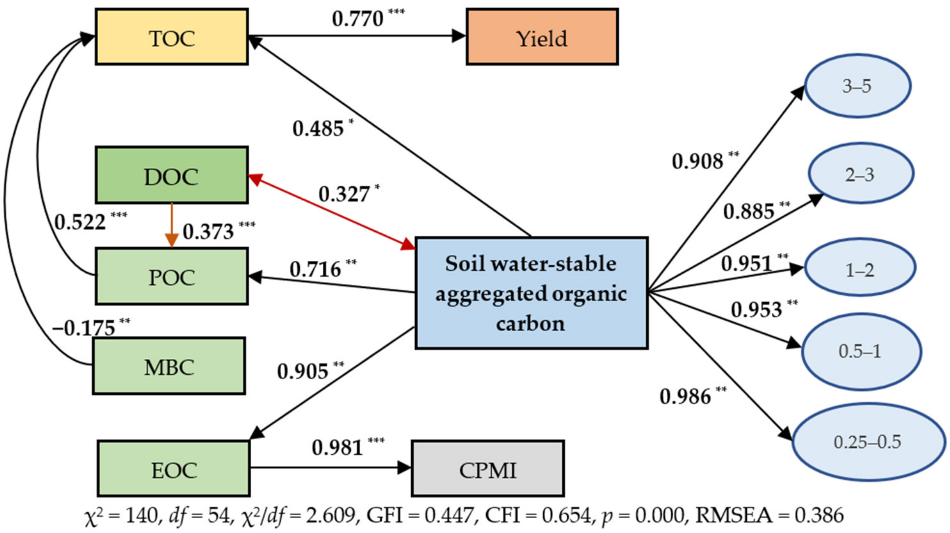


Figure 5. Structural equation modeling of the influence paths of soil water-stable aggregate organic carbon, labile organic-carbon fractions, and TOC on CPMI and yield. χ^2, df, χ^2/df, CFI, GFI, p, and RMSEA are parameters used to evaluate the suitability of the model. *, **, *** represent the significant level at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. TOC, DOC, MBC, EOC, POC, and CPMI explanations are as under Figure 4.

4. Discussion

4.1. Effects of Different N Fertilizer Applications on TOC and Labile Organic-Carbon Fractions

Organic fertilizer is a vital source of soil organic carbon, and it has been widely reported that a reasonable application of organic fertilizer can increase TOC and labile organic-carbon fractions in farmland [45,46]. In this study, both 1/2OF and 1/3OF + 2/3NF treatments had significantly higher TOC than N0 and NF (Table 1), which was consistent with previous research [47,48]. This may have been due to: (i) soil TOC mainly coming from plant litter, root exudates, and plant residue decomposition [49], so that organic fertilizer treatments could promote garlic growth and increase garlic yield (Figure 3) and root and litter, thereby improving carbon input and TOC content [48]; (ii) organic fertilizer application increasing the input of exogenous carbon into the soil, and directly enhancing EOC content, which could promote the conversion of TOC [50]; and (iii) 1/2OF and 1/3OF + 2/3NF treatments also improving the soil aggregate and increasing the water-stable aggregate organic-carbon content (Figure 2), which indicated that organic fertilizer could prevent TOC loss and increase TOC content and stability [51]. In addition, this study found that although the amount of N applied in the 1/2OF treatment was decreased by half, the TOC content was still significantly higher than that in the NF and OF treatments. This may be related to the N provided by soybean plants returning to the field as green manure, which also indicated that excessive N fertilizer input in a garlic field cannot improve the fertilizer utilization efficiency under the conditions of this research. In addition, a study found that organic fertilizer can maintain soil TOC by improving fungal necromass carbon [52]. However, some researchers considered that applying organic fertilizer alone could not meet the requirements of intensive vegetable production and that long-term application could lead to soil acidification [53]. Li et al. [43] suggested that chemical fertilizer could be fully replaced by manure without affecting maize yield and TOC when soil carbon reached the critical value in the Northeast Plain. From this, it can be seen that the background TOC content has a significant impact on N input and utilization efficiency. For low-fertility farmland, the amount of fertilization can be appropriately increased, while for high-level soil fertility, appropriately reducing N fertilizer application will not have a significant impact on soil fertility.

It has been widely reported that applying organic fertilizer alone, or in combination with chemical fertilizer, could significantly increase the soil labile organic-carbon fractions [32]. Soil MBC and DOC are closely related to the activity of soil microorganisms, and organic fertilizers partially or completely replacing chemical fertilizers can improve soil DOC and MBC by providing readily available carbon matrix sources for soil microorganism [54]. In this study, 1/2OF treatment had significantly higher MBC than other treatments, and significantly higher DOC than N0, which also indicated that organic fertilizer was the main carbon source for microorganisms, and an adequate and reasonable amount of organic fertilizer application was more conducive to an increase in MBC and DOC. Additionally, MBC in this study was significantly lower under 1/3OF + 2/3NF treatment than that under NF, and no significant difference was shown in DOC between 1/3OF + 2/3NF and NF. These results were not consistent with previous studies [55,56]. For example, Xu et al. [21] and Lou et al. [54] proposed that MBC and DOC in chemical fertilizer combined with organic fertilizer were significantly higher than those in NPK treatment. This discrepancy in results may be related to the amount of organic fertilizer applied, the years in which it was applied, and the cropping system. Compared with N0, 1/3OF + 2/3NF significantly increased POC content, whereas no significant differences were found between chemical or organic fertilizer application alone and N0. Villarino et al. [57] indicated that plant residual roots, animal manure, stubble biomass, and microbial biomass fragments were the main sources of POC, and relatively complex compounds added to soil are more conducive to the formation of POC, which explains this result of our study. Compared with N0, 1/2OF and 1/3OF + 2/3NF treatments both increased EOC significantly; moreover, 1/2OF also had significantly higher EOC than NF. This could be attributed to the higher exogenous carbon input by organic fertilizer, and the promotion of more carbon decomposition and conversion into TOC [58].

4.2. Effects of Different N Fertilizer Applications on CPMI

Soil CPMI is considered to be a more sensitive index than single soil carbon when measuring the impact of soil management practices [13,19]. Previous studies confirmed that, in other systems, CPMI was significantly increased by long-term organic fertilizer application or organic fertilizer plus chemical organic fertilizer treatment compared with only chemical fertilizer [59,60]. In this study, CPI and CPMI were both significant higher under 1/2OF than N0 or NF. This result was closely related to the increase in TOC and EOC under organic fertilizer treatment. Figure 4 shows that for the CPMI there was a significant correlation between TOC and EOC. A similar correlation was also seen in other studies [32,61]. Tirol-Padre and Ladha [62] attributed the changes in the CPMI to an increased in carbon input and an improvement in organic matter quality. In our study, the SEM indicated that there was a direct influence path between EOC with CPMI, but with no impact path between TOC and CPMI (Figure 5). This discovery suggests that changes in EOC might be more important than TOC for the CPMI. He et al. [47] also showed that a high-activity CPMI and middle-activity CPMI under organic fertilizer regime had greater improvement than a low-activity CPMI.

4.3. Effects of Different N Fertilizer Applications on Aggregate Mass Distribution and Water-Stable Aggregate Organic Carbon

According to literature reports, the formation of soil aggregates is influenced by many factors, among which stubble, cultivation, and fertilization are some important factors [46,63]. A previous study showed that N fertilizer application could promote the formation of soil micro-aggregates (0.25–2 mm) into macro-aggregates (>2 mm), while reducing the content of smaller aggregates, and the application of organic fertilizers resulted in an even more significant influence [64]. Li et al. [65] found that micro-aggregate (0.25–2 mm) mass was significantly decreased under organic fertilizer treatments compared with N0 and NF, which was inconsistent with our research. In this study, >2 mm aggregates were significantly higher under N0 and NF, while 0.25–2 mm aggregates were increased by organic fertilizer treatments (1/2OF and 1/3OF + 2/3NF). This diversity of results might be attributed to the initial soil properties, cropping system, and organic fertilizer types.

Xue et al. [66] revealed that, compared to no fertilizer, both NPK and NPK + manure treatments significantly reduced the mass proportion of soil macro-aggregates (>2 mm) and significantly increased the proportion of soil micro-aggregates (0.25–2 mm) under dry-land conditions, and this might be related to the iron-oxide concentration in the aggregates. Additionally, Gao et al. [67] found that straw returning with controlled-release N fertilizer treatments significantly improved proportions of 0.25–2 mm aggregates compared to straw returning with chemical N fertilizer and straw returning with no additional treatments. Thus, it can be seen that the type of organic fertilizer has a large impact on the particle size distribution of soil aggregates.

Improving the content of water-stable organic carbon in soil aggregates is beneficial for the formation and stability of soil structure [68]. This research indicated that with the increase in aggregate particle size, the organic-carbon content of water-stable aggregates showed a trend of first increasing and then decreasing, and the organic-carbon content of water-stable aggregates with a particle size of 1–2 mm was the highest. The main reason for this might be that the organic carbon in macro-aggregates (>2 mm) was easily affected by the environment and its turnover was fast. The macro-aggregates could, therefore, not provide physical protection for the formation and growth of organic carbon, whereas micro-aggregates had a strong protective and cumulative effect, which was beneficial for carbon sequestration [69]. Zhao et al. [70] found that macro-aggregates (>2 mm) contained more organic carbon than micro-aggregates. Different results may be related to the duration of the experiment, as it takes a longer time for stable micro-aggregates to convert into macro-aggregates [71]. In this study, the organic-carbon content of water-stable aggregates with different particle sizes under 1/2OF and 1/3OF + 2/3NF was typically significantly higher than that of N0 and NF. This was possibly because organic fertilizer can directly supplement a large amount of active organic matter into the soil, and some of these organic compounds have hydrophobicity, and the increase in hydrophobic groups in the aggregates leads to enhanced water stability of the aggregates [72], while chemical fertilization can lead to changes in soil pH, electrolyte concentration, and other properties, resulting in adverse effects on soil aggregate structure, reduced stability, and increased mineralization of organic carbon [73]. Wang et al. [74] indicated that applying organic fertilizer could significantly increase the organic-carbon content of aggregates with particle sizes > 2 mm. Based on SEM analysis, it was evident that DOC has a positive effect on water-stable aggregated soil organic carbon (Figures 4 and 5). Applying organic fertilizer could improve DOC through increasing crop root exudates, root system secretion, and microorganisms, thus, increasing the soil organic-carbon content in aggregates.

4.4. Effects of Different N Fertilizer Applications on Garlic Yield

Crop yield is a direct reflection of agricultural soil productivity, and is also the most powerful indicator of changes in agricultural soil fertility. Our study indicated that compared to N0, N fertilizer input significantly increased garlic yield, and, in particular, 1/3OF + 2/3NF resulted in the highest garlic yield. This result was consistent with Liu et al. [75]. Garlic is a N high-demand crop, and N fertilizer application can provide more nutrients for crop growth, thus increasing garlic yield. Some studies have reported that organic fertilizer might have more advantages in improving the soil environment, regulating soil C/N, and increasing soil labile organic-carbon fractions [35,76]. Correlation analysis showed that garlic yield had a positive correlation with TOC, DOC, and POC; therefore, the 1/3OF + 2/3NF treatment could promote the increase in garlic production by increasing TOC, POC, and DOC contents. This finding was similar to those of other studies [77,78]. On the other hand, the combined application of organic and chemical fertilizers could improve soil structure and promote the aggregation of more soil particles [70]. The SEM results further demonstrated the direct impact pathway of TOC on yield and the indirect impact pathway of soil water-stable aggregate organic carbon on garlic yield in this study (Figure 5). Furthermore, garlic yield was also significantly higher under 1/2OF treatment than N0, with no significant difference compared to NF or 1/3OF + 2/3NF. Lou et al. [54]

and Li et al. [44] found that organic fertilizer fully replacing chemical fertilizer might be the best choice for increasing soil carbon sequestration and maize yield based on long-term (>20 years) positioning experiments. However, due to the fact that N in organic fertilizers mainly exists in the form of organic N, the release of organic N and the provision of effective N to plants are slow, and cannot meet crop production needs [79,80]. Therefore, some studies have shown that replacing synthetic fertilizers with organic fertilizers in excess of a certain proportion might reduce crop yield in the first few years, but in later years, the yield recovers or even increases [81–83]. As a consequence, whether organic fertilizers can completely replace chemical fertilizers depends on comprehensive evaluation including different cropping systems, initial soil nutrient content, and management measures.

5. Conclusions

A comprehensive analysis was carried out to quantify the effect of replacing chemical fertilizer with organic fertilizer on soil organic carbon, CPMI, aggregate-associated organic carbon, and garlic yield based on a four-year garlic field experiment. The results indicated that compared to NF, organic fertilizers treatments significantly increased soil TOC, and 1/2OF had obvious positive effects on soil labile organic-carbon fractions and CPMI, whereas garlic yield was significant increased by 15.3% under 1/3OF + 2/3NF treatment compared with NF. Organic fertilizer treatments could improve the soil TOC, DOC, POC, and EOC by increasing the water-stable aggregate-associated organic-carbon content, and indirectly influencing crop yield and CPMI through altering soil organic carbon. Notably, there was a major effect on aggregates with particle sizes of 0.25–2 mm. Consequently, compared with chemical fertilizer application alone, replacing chemical fertilizers with organic fertilizers in garlic fields had a significant and positive influence on improving soil organic carbon and yield, and reducing N fertilizer application still maintained an increase in organic carbon and garlic yield under short-term conditions. However, further verification is needed to determine whether organic fertilizer treatments can completely replace chemical fertilizers under long-term conditions.

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