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Responses of Soil Macro-Porosity, Nutrient Concentrations and Stoichiometry Following Conversion of Rice–Wheat Rotation to Organic Greenhouse Vegetable System

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Abstract: To investigate the long-term effects of organic manure on soil macro-porosity and nutrient stoichiometry in greenhouse production, we studied the physical and chemical properties of soils under different vegetable systems in Jiangsu Province. These systems included organic greenhouse vegetable (OGV), organic open-field vegetable (OFV), conventional greenhouse vegetable (CGV), and conventional open-field vegetable (CFV), with rice–wheat rotation (RWR) soils used as a reference.The results showed that, compared to conventional systems, organic vegetable production increased soil macro-porosity, soil organic carbon (SOC), and total nitrogen (TN) content, as well as C:N, C:P, and N:P, particularly in the tilled layer. SOC, TN, and total phosphorus (TP) levels increased rapidly during the first 14 years of OGV cultivation, followed by a decline. SOC, TN, and stoichiometric ratios were significantly positively correlated with soil macro-porosity. The study suggests that converting RWR to OGV does not degrade soil aeration, and long-term application of organic manure positively impacts nutrient retention in the tilled layer, although the effects are time- and depth-dependent. The study highlights the potential of long-term organic manure application to improve soil aeration and nutrient balance in OGV, underscoring the importance of optimizing fertilizer management in intensive agriculture to enhance soil quality and crop yield.

Keywords: intensive cropping land-use conversion; long-term organic manure; soil nutrient chemical stoichiometry; soil aeration; different agricultural managements

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1. Introduction

Urbanization has led to the disappearance of many fertile agricultural lands. In response, the government has implemented policies to protect grain fields, making vegetable fields a favored option for urban expansion. As a result, the conversion of rice–wheat rotation (RWR) fields to vegetable cultivation has been widely promoted to meet the increasing demand for higher-quality vegetables [1]. However, the two production systems are fundamentally different. RWR fields typically showed higher bulk density and lower fertilization levels compared to vegetable fields, suggesting that greenhouse vegetable systems converted from RWR may encounter more severe soil quality challenges, particularly relating to soil structure [2]. Additionally, organic manure is widely used by farmers to mitigate soil degradation in greenhouse cultivation [3], nonetheless, its effectiveness in

addressing poor soil aeration and meeting the porosity requirements for optimal vegetable growth has not been thoroughly examined.

Soil macro-pores play a key role in vegetable nutrient absorption and root growth by influencing soil infiltration, and permeability or carbon dioxide (CO_2) emissions [4]. When soil macro-porosity drops below 10%, restricted gas exchange can severely impede vegetable growth, limiting the roots to access oxygen for optimal development [5]. Researchers have increasingly focused on how fertilizer type and cultivation years affect macro-porosity changes. Moreover, the soil porosity decreased greatly in short-term greenhouse cultivation compared to open-field vegetable production using chemical fertilizers [6], whereas the organic greenhouse vegetable systems had higher macro-porosity than organic open-field systems [7]. Further, long-term organic manure application does not lead to a continuous linear increase in soil macro-porosity; instead, macro-porosity reaches equilibrium within a certain period [4,8]. Although some studies [9–11] have explored soil macro-porosities changes in organic greenhouse soils, there are few investigations into soil macro-porosity and the distribution of functional pores in relation to long-term vegetable cultivation, different fertilization practices, and tillage management simultaneously.

Interactions between soil organic carbon (SOC) and soil porosity characteristics have received increasing attention [12]. A strong positive relationship between soil physical properties and SOC was identified by Herencia, Garcia-Galavis, and Maqueda [6]. However, the SOC accumulation rate depends on the complex transfer process among soil nutrients rather than a sole input of organic manure. Soil carbon (C) acts as an energy source, while nitrogen (N) and phosphorous (P) are essential nutrients, all of which are recognized as critical for vegetable growth and development [13,14]. Contradictory results have been reported regarding changes in soil C and nutrient levels following land-use conversion. Some researchers observed significant increases [14,15] in SOC, total N, P, K, and available N, P, and K in greenhouse soils compared to cereal fields, while other studies reported a decrease in SOC, with notable increases in soil N and P following the RWR (rice–wheat rotation) conversion [11,16]. Moreover, some studies [17,18] have shown that the SOC and TN contents in greenhouse soils increased gradually during the initial period but eventually stabilized or slightly decreased. It may be due to the effects of land-use conversion on the coupling of C, N, and P in soils through fertilizer application, agricultural management, and crop types [6,19].

The ratios of soil C, N, and P are indicators of C, N, and P balance, and assist in explaining variations in soil C and nutrient accumulation rates, and are key factors in assessing soil quality. For example, the soil C:N ratio is commonly used as a maker of soil organic matter mineralization. A higher C:N ratio indicates a slower soil organic matter decomposition rate [20]. Globally, the average C:N ratio for croplands at a depth of 0–30 cm is 12.50 [21], while in Chinese soils at a depth of 0–10 cm, the average value is 14.40 [22]. Some studies suggested that the average value of C:N ratios in greenhouse vegetable soils or open fields is lower than average values in Chinese soils and global cropland [19]. Additionally, the soil C:P ratio is an essential indicator to reflect soil P mineralization. Flooded RWR soils provide favorable conditions for P accumulation compared to the drier vegetable fields. Results have suggested the average value of C:P in rice and vegetable fields is only 0.77 and 0.70, respectively [19]. However, other studies have reported C:P ratios of 26.40 in wheat–maize rotation fields and 16.06 in greenhouse vegetable soils [14]. Furthermore, the soil N:P ratio is a sensitive indicator of nutrient availability limitations for plants N, often correlating with higher levels of primary productivity [22]. In greenhouse vegetable soils, the N:P ratio ranges from 1.71 to 7.60, which is greatly lower than the average N:P ratio in Chinese soils, reported to be between 5.2 and 9.3 [22]. While many studies have focused on the concentration and stoichiometric ratios of these elements to assess C cycling, soil fertility, and nutrient availability, most of this research has been conducted in forest and grassland farming systems [13,23]. There has been limited research on soil nutrient stoichiometry and its impact on soil macro-porosity, particularly in the context of long-term land-use changes in greenhouse vegetable production systems.

This study utilizes the "space-for-time" method to quantify changes in soil macro-porosity and nutrient content following the conversion of RWR fields to greenhouse and open-field vegetable production systems. The study aimed to address three key issues: (1) Compared to chemical fertilizer application, how do soil macro-porosity, C, N, and K content, and ecological stoichiometric ratios respond to organic manure application? What trends emerge after converting from RWR? (2) Following land-use conversion, how do soil macro-porosity, C, N, and K content, and ecological stoichiometric ratios evolve with long-term organic manure application, and how do these changes differ between greenhouse and open-field systems? (3) What are the relationships between soil C, N, and P concentrations, their ratios, and soil macro-porosity?

2. Methods and Materials

2.1. Study Sites and Experimental Design

The study sites were located in the urban areas of Nanjing, Jiangsu Province, China (31°16′ N, 119°54′ E), which has a northern subtropical monsoon climate, an average annual temperature of 15.7 °C, and an average annual rainfall of 1073 mm. Cereal fields in the region were mainly managed with a rice–wheat rotation (RWR) cropping system, and the main soil type is Anthrosols (Inceptisols) [24] produced over long-term rice cultivation. Both conventional and organic vegetables were converted from RWR fields, with identical soil types and texture.

Prior to the soil sampling, the organic vegetable sites had been cultivated for 1–14 years. Farmers typically grow vegetables 3–4 seasons per year. While tillage management was consistent across sites, fertilization varied. The conventional vegetable fields received approximately 2–5 t ha^{-1} of chemical fertilizer (compound fertilizer, 15%N-15%P_2O_5-15%K_2O) per year, whereas the organic vegetable site received approximately 44–55 t ha^{-1} of organic manure. The same tillage practices were applied to both greenhouse and open-field vegetable production systems during the same year of conversion. It was important to note that fertilization rates, whether using organic manure or chemical fertilizers, were consistent across both greenhouse and open-field systems.

2.2. Soil Sampling and Analysis

Soil sampling across all fields was conducted after harvest and before tillage. At each point, two soil layers were sampled: the tilled layer (0–15 cm) and the plow pan layer (15–30 cm). Using a space-for-time approach, we collected 24 soil samples from organic greenhouse vegetable soil, ranging in age from 1 year (new greenhouse) to 18 years, categorized as follows: one-year organic greenhouse vegetable fields (OGV1), nine-year organic greenhouse vegetable soil (OGV9), fourteen-year organic greenhouse vegetable soil (OGV14), and eighteen-year organic greenhouse vegetable soil (OGV18). Similarly, we collected soil samples from organic open vegetable fields with transformation histories ranging from 1 to 14 years (eighteen-year soil samples were unavailable), classified as one-year organic vegetable open-field (OFV1), nine-year organic vegetable open-field (OFV9), and fourteen-year organic vegetable open-field (OFV14). For comparison, six soil samples from nine-year conventional greenhouse vegetable soil (CGV9) and nine-year conventional vegetable open-field (CFV9) were collected. The adjacent RWR fields were also sampled as a baseline for vegetable cultivation, with each site represented by three samples.

The soil bulk density (BD) was measured by drying the soil in an oven at 105 °C until a constant weight was reached. Soil macro-porosity (>30 μm) and pore size distribution were determined based on soil hydraulic properties [25]. Previous studies have found that soil macro-porosity is strongly related to crop growth [26,27], so we determined the soil water content at matric potentials of −6, −30, −60, and −100 cm with a sandbox-pressure chamber, representing the porosity sizes of >500 μm, 500–100 μm, 100–50 μm, and 50–30 μm, respectively [28]. The mean values of three replicate samples were used for statistical analysis.

The soil samples were air-dried and sieved before soil properties chemical measurement. Each treatment had three replicates. Soil pH was measured in water within a 1:2.5 soil-to-water paste mixture, and the electrical conductivity (EC) of the soil was determined using a conductivity meter with a 1:5 soil-to-water ratio in a paste mixture [29]. Soil organic carbon (SOC) was measured using a K_2CrO_7-H_2SO_4 oxidation procedure [30]. Soil total nitrogen (TN) [31] was determined with the Kjeldahl method after the digestion with H_2SO. Soil total phosphorus (TP) was determined after the sulfuric acid–perchloric acid digestion method and analyzed by a continuous flow injection analyzer [32]. Soil total potassium (TK) was measured by flame spectrometry [33]. The soil C, N, and P stoichiometric ratios (C:N, C:P, and N:P) were subsequently calculated to assess changes in soil nutrient stoichiometry across different organic systems [34].

2.3. Statistical Analyses

Treatment differences in soil BD, macro porosity, SOC, pH, EC, TN, TP, C:N, C:P, and N:P were assessed using two-way ANOVAs with cropping systems and sampling depth as the main effects, along with their interactions (IBM SPSS Statistics software, version 23.0). The relationships between BD, macro-porosity, SOC, pH, EC, TN, TP, C:N, C:P, and N:P were tested using Pearson linear regressions (Origin 2021, Correlation Plot).

Correlations between soil properties were determined using Spearman's rank correlations. All figures were created using Origin 2021 software.

3. Results

3.1. Impacts of Land-Use Change on Soil Physical–Chemical Properties

The vegetable cultivation systems converted from RWR exhibited distinct physical-chemical properties (Table 1). During the organic vegetable cultivation phase, both greenhouse and open-field systems showed a significant decrease in mean BD over time ($p < 0.05$; Table 1). However, the average BD for the CGV9 field (1.36 g cm^{-3}) was higher than that for the OGV9 field (1.20 g cm^{-3}), indicating that organic manure enhances soil structure. Additionally, greenhouse soil consistently showed lower BD compared to open-field vegetable soil and RWR soil, regardless of whether organic or chemical fertilizers were used. The soil pH increased with continuous organic manure application, while conventional vegetable soil exhibited significant differences, indicating that organic manure can help mitigate soil acidification. Moreover, soil EC presented an increase in the previous period of organic greenhouse vegetable cultivation but declined later.

Figure 1 showed that SOC, TN, and TP contents increased significantly over time in greenhouse systems following the transition from RWR, before decreasing with continued organic manure application. The SOC of OGV14 was significantly higher than that of OGV1, and the SOC of OGV 18 was significantly lower than that of OGV14. Concerning the impact of fertilizer sources on soil C, N, and P accumulation, our findings indicate that SOC and TN levels were higher in organic vegetable soil compared to conventional vegetable soil. Conversely, soil P concentration was higher in conventional vegetable soil than in organic vegetable soil (Table S2).

Compared to the tilled layer, the plow pan layer showed fewer differences in soil properties, except for pH and TK (Figure 1, Table 1). Soil pH increased significantly under organic manure management but decreased notably with chemical fertilizer application. For soil potassium, the TK level initially increased significantly under organic greenhouse management, but then decreased, following a similar trend to that observed in the tilled layer ($p < 0.05$, Table 1).

Cropping systems significantly affected all soil physical–chemical properties (Table 2). Except for pH and TK, significant differences were observed between the tilled layer and the plow pan layer for most properties. In addition, there was an obvious interaction on both the cropping system and soil depth for most soil properties, except for pH and TK.

Table 1. The effects of cropping systems and soil depth (means and standard deviation) on BD, PH, EC, and TK.

Treatment	Depth	BD (g cm^{-3})	pH	EC (mS cm^{-1})	TK (g kg^{-1})
RWR	0–15 cm	1.09 ± 0.07 b	5.48 ± 0.52 cd	0.05 ± 0.01 b	12.54 ± 0.5 b
	15–30 cm	1.56 ± 0.01 a *	5.73 ± 0.21 bcd	0.05 ± 0.01 a	13.46 ± 0.8 b
OGV1	0–15 cm	1.40 ± 0.14 ab	6.97 ± 0.69 a	0.19 ± 0.15 b	17.21 ± 1.31 ab
	15–30 cm	1.59 ± 0.14 a	6.59 ± 0.68 abc	0.12 ± 0.07 a	17.39 ± 0.90 ab
OGV9	0–15 cm	1.20 ± 0.12 b	5.81 ± 0.88 bcd	2.32 ± 0.50 a	16.27 ± 3.45 ab
	15–30 cm	1.58 ± 0.07 a *	6.57 ± 0.63 abc	0.35 ± 0.23 a *	16.20 ± 3.3 b
OGV14	0–15 cm	1.10 ± 0.02 b	5.82 ± 0.18 cd	2.66 ± 0.76 a	20.17 ± 4.84 a
	15–30 cm	1.50 ± 0.04 a *	6.11 ± 0.5 abc	0.47 ± 0.45 a *	22.33 ± 4.03 a
OGV18	0–15 cm	1.07 ± 0.05 b	6.92 ± 0.25 ab	0.63 ± 0.19 b	16.06 ± 0.56 ab
	15–30 cm	1.48 ± 0.04 a *	7.20 ± 0.12 a	0.27 ± 0.08 a*	15.91 ± 0.79 b
OFV1	0–15 cm	1.45 ± 0.11 ab	5.91 ± 0.34 abcd	0.03 ± 0.01 b	18.52 ± 0.83 ab
	15–30 cm	1.42 ± 0.13 a	5.95 ± 0.36 bcd	0.02 ± 0.01 a	18.10 ± 2.01 ab
OFV9	0–15 cm	1.37 ± 0.02 a	6.49 ± 0.55 abc	0.05 ± 0.00 b	16.89 ± 2.27 ab
	15–30 cm	1.54 ± 0.03 a	6.71 ± 0.48 ab	0.07 ± 0.03 a	18.67 ± 0.39 ab
OFV14	0–15 cm	1.27 ± 0.04 ab	6.42 ± 0.57 abc	0.05 ± 0.03 b	17.62 ± 0.8 ab
	15–30 cm	1.52 ± 0.03 a *	6.52 ± 0.47 abc	0.03 ± 0.00 a	17.88 ± 0.3 ab
CGV9	0–15 cm	1.36 ± 0.09 ab	4.67 ± 0.25 d	0.32 ± 0.06 b	16.64 ± 0.52 ab
	15–30 cm	1.54 ± 0.03 a	4.80 ± 0.18 d	0.18 ± 0.1 a	16.73 ± 0.12 b
CFV9	0–15 cm	1.47 ± 0.02 a	4.78 ± 0.39 d	0.15 ± 0.09 b	16.33 ± 1.25 ab
	15–30 cm	1.47 ± 0.11 a	5.36 ± 0.22 cd	0.07 ± 0.02 a	16.91 ± 1.19 b

Note: soil bulk density (BD), pH, electrical conductivity (EC), and total potassium (TK). Different lowercase letters indicate the significant difference in cropping systems using Tukey's HSD post hoc tests in the same depth. * means difference between different depths among the cropping systems is significant at $p < 0.05$.

Table 2. The effects (p values) of cropping systems and soil depth on physicochemical properties.

Properties	Treatment	Depth	Treatment × Depth
BD	0.00	0.00	0.00
Porosities in different sizes			
>500 μm	0.01	0.01	0.02
500–100 μm	0.01	0.01	0.01
100–50 μm	0.01	0.01	0.01
50–30 μm	0.05	0.30	0.63
Macro-porosity	0.01	0.01	0.01
pH	0.01	0.10	0.43
EC	0.01	0.01	0.01
SOC	0.01	0.01	0.01
TN	0.01	0.01	0.01
TP	0.01	0.01	0.01
TK	0.01	0.31	0.98
C:N	0.01	0.03	0.27
C:P	0.01	0.01	0.05
N:P	0.01	0.02	0.55

Note: BD, soil bulk density; EC, electric conductivity; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium. Linear mixed-effects model fit tests were conducted using Satterthwaite approximations for the denominator degrees of freedom (df).

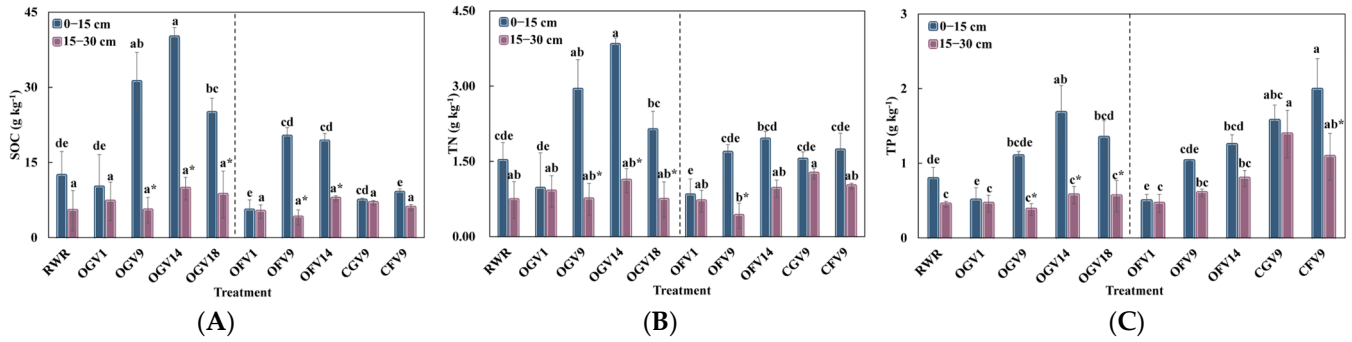


Figure 1. Change in cropping systems on soil nutrients and ratios: (**A**) SOC, (**B**) TN, (**C**) TP. The bar means the standard deviation. Different lowercase letters indicate the significant difference in cropping systems using Tukey's HSD post hoc tests in the same depth. * means differences between different depths among the cropping systems are significant at $p < 0.05$.

3.2. Impacts of Land-Use Change on Soil Macro-Porosity and Its Distribution

The cropping system had a significant impact on macro-porosity and pore size distribution. Except for the porosity in the 50–30 µm size class, porosity across different sizes showed significant differences between soil depths ($p < 0.05$, Table S1). Similar results were observed in the interaction between the cropping system and soil depth (Table 2).

Organic manure could raise the soil macro-porosity. In the tilled layer, soil macro-porosity increased significantly from 7.62% to 20.91% over the course of organic greenhouse vegetable cultivation, following the conversion from RWR fields. A similar trend was observed in open-field organic vegetable soil. However, conventional vegetable soil exhibited lower macro-porosity compared to organic vegetable soil in both greenhouse and open-field systems (Figure 2). The distribution of soil pore sizes also increased significantly during organic greenhouse cultivation. Notably, the porosity in the >500 µm size class showed the greatest increase in organic greenhouse systems converted from RWR, followed by increases in the 500–100 µm and 100–50 µm size classes, while the 30–50 µm size class exhibited the least increase (Figure 2). In contrast, no significant changes were observed in the open-filed organic vegetable soil. Additionally, porosity in these size classes was significantly higher in OGV9 soil than in CGV9 soil, except in the 30–50 µm size class. Compared to greenhouse systems, there was no significant difference in porosity between OFV9 soil and CFV9 soil.

The plow pan layer showed a non-significant difference in porosity larger than 500 µm across these cropping systems. However, CGV9 soil had significantly lower porosity in 100–50 µm than that of OGV9 soil, while no significant differences were observed in other pore size classes ($p < 0.05$).

3.3. Effect of Cropping Systems on Soil C, N, and P Ratios

Figure 3 shows the soil C:N, C:P, and N:P ratios in different cropping systems. Similarly, as shown in Table 2, the C:N ratio was strongly affected only by cropping systems rather than the soil depths. Although soil C:P and N:P ratios were significantly affected by soil depths ($p < 0.05$), the interaction of cropping systems and soil depths had minimal effects on N:P ratios (Table 2).

In the tilled layer, significant differences were observed between organic manure and chemical fertilizer management ($p < 0.05$, Table S3). For instance, the average C:N ratio for CGV9 soil (4.88 ± 0.75) was 50% lower than that for OGV9 soil (10.64 ± 0.12) and 39% lower than RWR soil (8.02 ± 1.31). However, there was no significant change in C:N ratios over the long term of organic manure application in both greenhouse and open-field vegetable systems. Additionally, the soil C:P ratios in organic vegetable soil were similar to those in RWR soil but more than 50% higher than in conventional vegetable soil. The soil N:P ratios of OGV9 soil (2.66 ± 0.46) were lower than in CGV9 soil (0.99 ± 0.09), but higher than in RWR soil (2.02 ± 0.88).

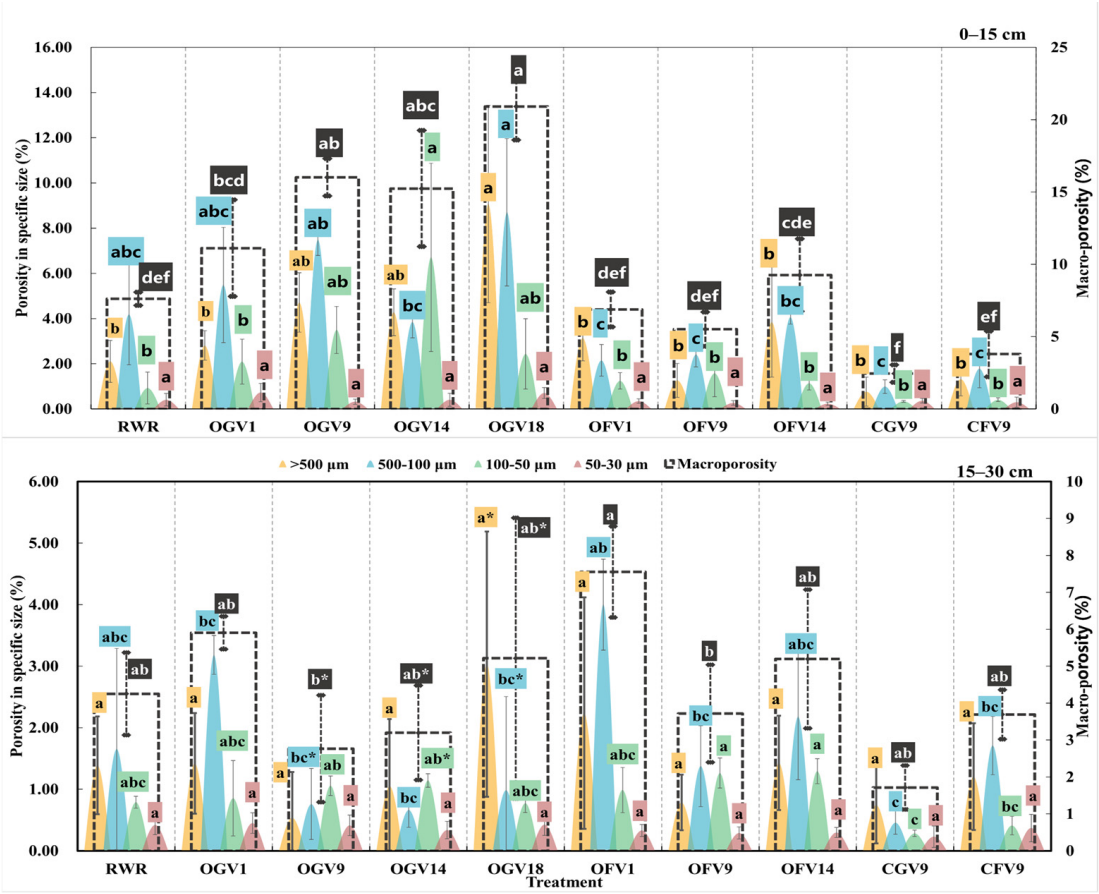


Figure 2. Change in soil macro-porosity (>30 μm) and porosities of different size ranges (>500 μm, 500–100 μm, 100–50 μm, 50–30 μm) across cropping systems. The bars represent standard deviations. Different lowercase letters indicate significant differences between cropping systems, as determined by Tukey's HSD post hoc test at the same depth. An asterisk (*) indicates significant differences between depths within the cropping systems at $p < 0.05$.

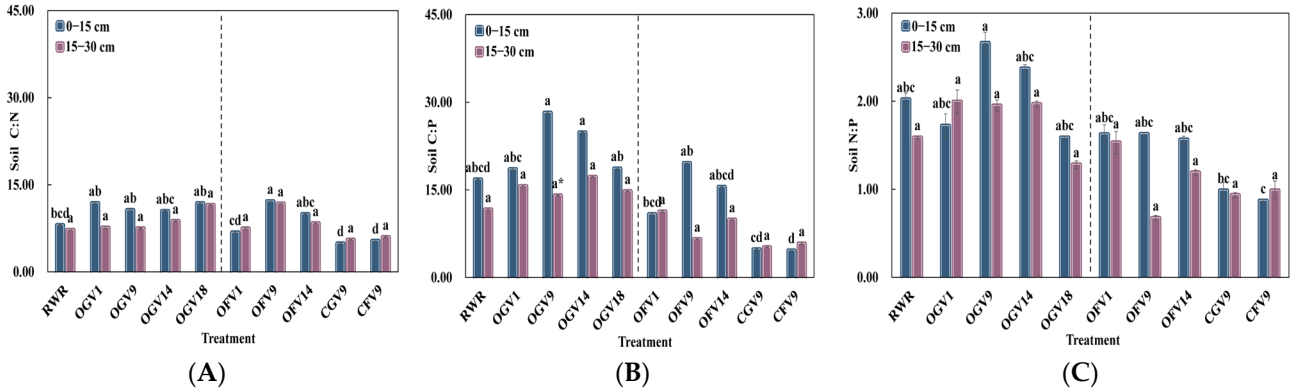


Figure 3. Change in cropping systems on soil nutrients and ratios: (**A**) soil C:N, (**B**) soil C:P, (**C**) soil N:P. The bar means the standard deviation. Different lowercase letters indicate the significant difference in cropping systems using Tukey's HSD post hoc tests in the same depth. * means differences between different depths among the cropping systems are significant at $p < 0.05$.

In the plow pan layer, no significant differences in soil C:N, C:P, and N:P ratios were found among treatments; furthermore, soil C:N, C:P, and N:P ratios in the plow pan layer were not significantly different from those in the tilled layer, except for soil N:P ratio in OGV9 soil.

3.4. Relationship between Soil Physical–Chemical Properties and Soil C, N, and P Ratios

Figure 4 illustrates the relationship among basic soil physical–chemical properties and soil C, N, and P ratios across the two soil depths. In the tilled layer, soil BD exhibited a negative correlation with SOC, TN, and C:N, C:P, and N:P ratios ($p < 0.05$). Soil pH was significantly positively correlated with soil C:N and C:P ratios but significantly negatively correlated with the TP ($p < 0.05$). Soil EC showed a positive correlation with TN, TP, and C:P ratios. Additionally, SOC had a marked positive relationship with TN, and C:N, C:P, and N:P ratios, although there was no significant correlation with TP and TK ($p < 0.05$). Soil TN was significantly positively correlated with C:P and N:P ratios but showed no significant correlation with soil C:N ratios (Figure 4a).

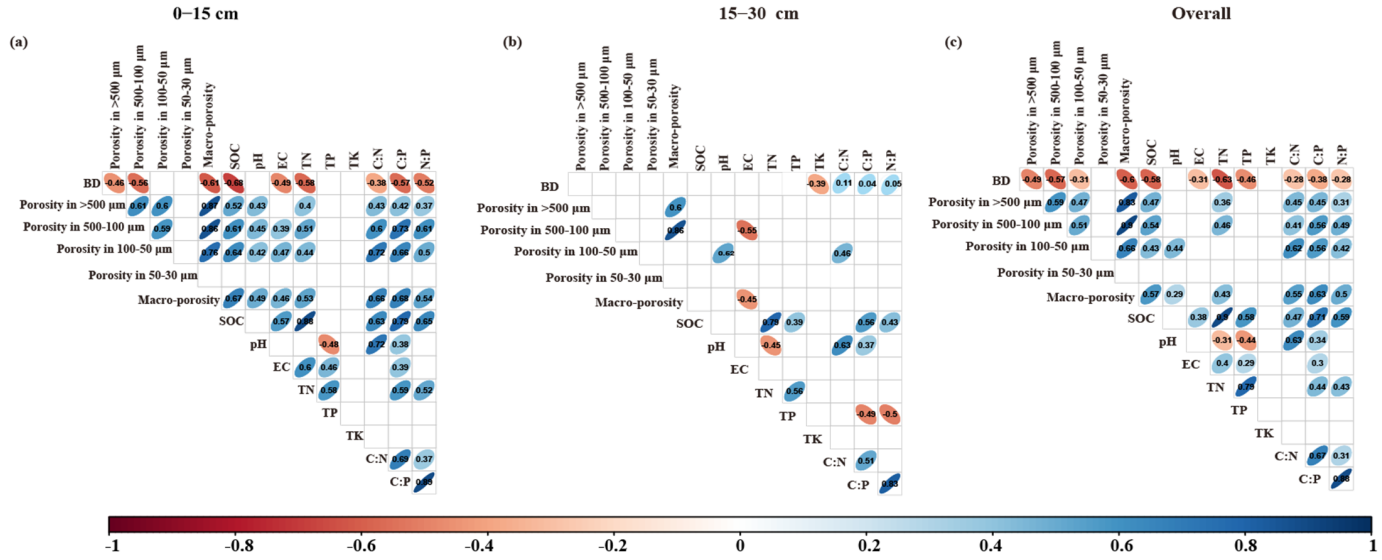


Figure 4. Spearman correlation coefficients between selected soil physicochemical properties and soil carbon and nutrients for each soil depth: (**a**) 0−15 cm, (**b**) 15–30 cm, (**c**) Overall. Note: + (blue bar) represents a positive correlation, and − (red bar) represents a negative correlation. The color is deeper, the correlation is stronger, correlation is significant at the 0.01 level. Abbreviations: BD, bulk density; SOC, soil organic carbon; EC, electrical conductivity; TN, total nitrogen; TP, total phosphorus; TK, total potassium.

Compared to the tilled layer, there were fewer significant relationships between soil properties in the plow pan layer. For instance, the correlation coefficients between SOC and soil nutrient stoichiometric characteristics were lower in the plow pan layer than in the tilled layer. Additionally, the Pearson correlation between the BD and C:N ratios was 0.11, while the correlations for the C:P ratio and N:P ratio were 0.04 and 0.05, respectively, in the plow pan layer. These values were significantly different from those observed in the tilled layer, indicating that the effects of soil BD on soil nutrients were primarily concentrated in the surface layer (Figure 4b). Furthermore, the relationship between soil physical–chemical properties and soil nutrient stoichiometric properties based on total sampling points was more aligned with the trends observed in the tilled layer (Figure 4a). This suggests a stronger connection between soil properties in the tilled layer under vegetable cultivation converted from rice–wheat rotation (RWR).

3.5. Correlation between Soil Macro-Porosities and Soil Nutrient Supplements

Compared to other soil properties, soil macro-porosity exhibited a significant positive correlation with SOC, TN, and C:N, C:P, and N:P ratios, while showing an inverse relationship with soil bulk density(BD) ($p < 0.01$; Figure 3A). Analysis of soil pore size distribution revealed significantly positive relationships between soil porosity in the >500 µm, 500–100 µm, and 100–50 µm size classes and soil nutrient stoichiometric characteristics

(C:N, C:P, and N:P ratios), with the exception of the 50–30 μm size class ($p < 0.05$). In the tilled layer, the macro-porosity showed stronger and more significant correlations with soil C:N (r = 0.66), C:P (r = 0.68), and N:P ratios (r = 0.54) compared to the plow pan layer. Conversely, in the plow pan layer, no significant relationships were observed between soil physical porosities, soil nutrient content, and their ratios, except for the 100–50 μm size class.

4. Discussion

4.1. Benefits of Organic Manure in Greenhouse Vegetable Systems on Soil Properties

(1) Soil macro-porosity and its size distribution

Soil macro-porosity is a crucial factor for ensuring proper soil aeration. Large macropores (>500 μm) act as channels for the preferential flow of water and solutes in agricultural soils [32,33]. The 500–50 μm pore size class is typically recognized as "transmission pores", and it plays a key role in facilitating air movement within the soil–water–plant system [35,36]. This improved transmission efficiency of air and water likely contributes to the continued accumulation of soil nutrients in organic greenhouse soil. When soil macro-porosity falls below 10% of the soil volume, plant roots may suffer from oxygen deficiency [5]. In our study, the macro-porosity in the tilled layer of RWR fields significantly increased following long-term application of organic manure in organic greenhouse vegetable cultivation (Figure 2, $p < 0.05$). While greenhouse systems are typically regarded unfavorably for negatively affecting soil structure, our results suggest otherwise in terms of macro-porosity. A previous study [11] demonstrated through X-ray CT analysis that soil macro-porosity (>30 μm) decreased from 11.47% in RWR soils to 8.0% in open-field vegetable and 5.8% in plastic-greenhouse vegetable in the tilled layer. Our research also indicated that CGV9 had significantly lower macro-porosity than CFV9 soil. Conversely, organic greenhouse systems appeared to be more effective in increasing soil macro-porosity than organic open-filed soil. Greenhouse systems operate under controlled, closed conditions, which restrict CO_2 escape and allow it to be reabsorbed by the soil through vegetable photosynthesis and subsequently immobilized by soil microorganisms. In contrast, in open-field systems, SOC decomposes more rapidly, resulting in faster soil carbon loss. Additionally, open-field systems are exposed to climatic factors like rainfall, which can lead to SOC leaching. In our study, the SOC content in the OGV system was significantly higher than in the OFV system. Some previous studies [37–39] have shown that organic manure improves soil structure by altering the SOC content in greenhouse soils. Other studies have demonstrated that SOC accumulation can improve soil macro-porosity [40,41]. Therefore, we speculate that organic manure more readily increases macro-porosity in OGV systems.

(2) Soil C, N, and P accumulation and their ratios

In this study, cropping systems and soil depths significantly impacted soil C, N, and P, as well as their ratios, in vegetable cultivation fields converted from RWR.

We observed a significant increase in SOC in greenhouse vegetable soil with long-term organic manure application, especially in the tilled soil layer. Wang et al. [11] also noted that SOC decreased from 31.64 g kg^{-1} in RWR soil to 14.97 g kg^{-1} in open-field and 13.69 g kg^{-1} in greenhouse vegetable soils over six years with chemical fertilizer. Conventional vegetable soils had lower organic carbon due to reliance on chemical fertilizers, whereas in RWR systems, some biomass, such as post-harvest residues, was returned to the soil, providing an additional source of SOC and TN. However, there was little fresh biomass in the soil after harvest in vegetable systems, which also limited the input source of SOC and TN. Greenhouse environments enhanced SOC decomposition but had lower SOC compared to open-field soils due to limited fresh carbon inputs. SOC and TN were highest in organic greenhouse soils due to substantial organic matter and nutrient retention. There was no significant difference in soil P between organic greenhouse and open-field soils, but conventional vegetable soils had higher P accumulation. This suggests that fertilizer application rates in conventional vegetable soil converted from RWR far exceeded crop

growth requirements. The combination of short vegetable cultivation periods, excessive use of compound fertilizers, and the low mobility of P in soil [14] contributed to the accumulation of residual P [19], as reflected in the higher P content in the soil.

Ratios of soil nutrients provide insights into their cycling patterns [19,23,42]. Our study revealed that SOC, TN, and TP stoichiometry were significantly influenced by both cropping systems and soil depth independently. Compared with the plow pan layer, soil C:N, C:P, and N:P ratios of organic vegetable fields and RWR fields were higher in the tilled layer but not in conventional vegetable fields. Generally, a lower soil C:N indicated a greater capacity for soil C cycling [20]. However, the effects of land-use changes on soil nutrient ratios remain uncertain. Some studies have shown that the soil C:N ratio decreased in vegetable fields converted from paddy fields [19,43]. While others have reported an increase in soil C:N in greenhouse vegetable soil converted from wheat–maize rotation soil [14]. These contrasting results may be attributed to various factors, including differences in fertilizer types, cropping systems (greenhouse versus open-field), and environmental factors (such as soil type, temperature, and precipitation) In our study, the soil C:N ratio in the tilled layer followed the order: OFV9 (12.14) > OGV9 (10.64) > RWR (8.02) > CFV9 (5.33) > CGV9 (4.88) (Table 3). This indicates that organic open-field vegetable soil has a faster rate of SOC decomposition compared to organic greenhouse vegetable soil, which accounts for the observed difference in SOC stocks between the two systems. Additionally, the application of chemical fertilizers decreased the C:N ratio of vegetable soil, likely due to the increase in chemical N addition in the tilled layer, without a corresponding significant change in the soil C pool over the 9-year fertilization.

The soil C:P ratios in OGV and OFV9 topsoils were higher than in RWR soil. Vegetable soil had better aeration and enhanced aerobic microorganism activity, which is beneficial to the decomposition of SOC and TP accumulation [44]. The relatively high levels of C from straw residue return and the chemical fertilizer applied to rice–wheat rotation contributed to maintaining higher C ratios compared to conventional vegetable soil. Studies have shown that the soil C:P ratios can decrease from 26.40 to 16.06 during the transition from wheat–maize rotation to greenhouse vegetable fields, suggesting that organic vegetable soils accumulate high SOC content, while the rate of soil P increase remained steady, leading to an increased soil C:P ratio [14]. In contrast, other research reported the soil C:P ratio was 0.77 and 0.70 in rice and vegetable soils, respectively [19]. This discrepancy may be attributed to climate factors such as temperature and precipitation, which influence SOC loss and P decomposition [45]. The study area in the referenced research experienced higher temperatures and precipitation compared to our study area, resulting in faster decomposition rates, greater nutrient accumulation, and consequently, lower soil C:P ratios [19].

In addition, the soil N:P ratios in vegetable fields converted from RWR showed insignificant changes (Figure 1), which is consistent with the findings by Li J et al. [14]. Fertilizer input into these vegetable soils was not only excessive relative to crop demand but also imbalanced in terms of nutrient proportions. As noted by Li J et al. [14], the P absorption rate in vegetables was typically low, ranging from 10 to 25%. However, the compound fertilizer used in our study contained equal amounts of N, P, and K, and the manure also had high P due to dietary and physiological factors. It suggested that P fertilizer input could be reduced in vegetable cultivation. In contrast, the N:P value was much lower compared to the global cropland average value of 4.40 for 0–30 cm soils [21] and 9.30 reported for 0–10 cm soils, in tropical and subtropical climatic zones of China [22]. This discrepancy is likely due to the higher P accumulation in vegetable fields compared to those on the Chinese and global scales. Additionally, the soil N:P ratios of 7.60–8.80 reported for 0–15 cm depth soil were also significantly higher than those observed in our study [19]. This variation may be attributed to the high temperatures and humidity in the south of China, which accelerates the decomposition of SOC and leads to an increase in the soil N:P ratios.

Table 3. Comparison of soil C, N, and P stoichiometry.

Treatment	Year	Fertilizer Type	C:N	C:P	N:P	Soil Layer (cm)	Location	Reference
Chinese soils	-	-	14.40	136.00	9.30	0–10	China	Tian, Chen, Zhang, Melillo, and Hall [22]
Cropland	-	-	12.50	63.90	4.40	0–30	Globe	Xu, Thornton, and Post [21]
Rice	-	Chemical fertilizer	11.50	0.77	8.80	0–15	Fujian province	Liu, Peuelas, Sardans, Fang, and Wang [19]
Vegetable soils	10	Chemical fertilizer	10.80	0.70	7.60	0–15	Fujian province	
Wheat–maize	-	Chemical fertilizer	9.40	26.40	2.86	0–20	Haiyang	Li, Wan, Liu, Chen, Slaughter, Weindorf, and Dong [14]
Greenhouse vegetable	10	Organic manure/Chemical fertilizer	11.58	16.06	1.71	0–20	Haiyang	
Rice–wheat rotation	-	Chemical fertilizer	8.02	16.78	2.02	0–15	Jiangsu province	This study
Rice–wheat rotation	-	Chemical fertilizer	7.22	11.65	1.59	15–30	Jiangsu province	This study
Greenhouse vegetable	9	Organic manure	10.64	28.21	2.66	0–15	Jiangsu province	This study
Greenhouse vegetable	9	Organic manure	7.48	14.04	1.95	15–30	Jiangsu province	This study
Open-air vegetable	9	Organic manure	12.14	19.59	1.63	0–15	Jiangsu province	This study
Open-air vegetable	9	Organic manure	11.74	6.57	0.67	15–30	Jiangsu province	This study
Greenhouse vegetable	9	Chemical fertilizer	4.88	4.81	0.99	0–15	Jiangsu province	This study
Greenhouse vegetable	9	Chemical fertilizer	5.51	5.17	0.93	15–30	Jiangsu province	This study
Open-field vegetable	9	Chemical fertilizer	5.33	4.62	0.87	0–15	Jiangsu province	This study
Open-field vegetable	9	Chemical fertilizer	5.98	5.81	0.99	15–30	Jiangsu province	This study

Thus, SOC and TN were highest in organic greenhouse vegetable soils except for the soil TP. Soil C:N ratios were highest in open-field organic vegetable soils, while soil C:P ratios peaked in organic greenhouse vegetable. In contrast, conventional vegetable fields had low soil SOC, TN, TP, and C:N and C:P ratios. These findings confirm the benefits of organic manure for nutrient accumulation and immobilization, especially in greenhouse patterns, due to unique conditions and management practices.

4.2. Dynamics of Soil Properties Affected by Organic Manure in Greenhouse Vegetable Cultivation Converted from RWR

(1) Soil macro-porosity and pore size distribution

The increase in soil macro-porosity (>30 μm) at the 0–15 cm depth soil over time, particularly in greenhouse vegetable soil following conversion from RWR, suggests that the enhanced accumulation of SOC and TN promotes soil macro-porosity. Previous studies similarly reported that adding SOC positively impacts soil macro-porosity in greenhouse plots [6,8]. Moreover, the rate of increase in macro-porosity was mainly concentrated

during the initial nine years of cultivation, after which it declined. This trend indicates that SOC may reach saturation after 9–14 years of cultivation, resulting in reduced benefits for macro-porosity. In contrast, the rapid increase in macro-porosity in long-term open-field vegetable soil was observed only after nine years of cultivation, likely due to the unique conditions of greenhouse environments.

In addition, different soil pore sizes played varying roles in vegetable cultivation. The porosity of pores larger than 500 μm increased significantly during long-term organic greenhouse cultivation, consistent with the overall trend in soil macro-porosities (>30 μm). It indicated that organic greenhouse vegetable soil may experience increased nutrient leaching after irrigation or rainfall as cultivation progresses. We hypothesize that these pores were formed by penetration, as previous studies have shown that the roots can penetrate the soils with BDs below 1.5 g cm^{-3} [46,47]. Our findings also revealed a marked decrease in soil BD, from 1.40 g cm^{-3} to 1.07 g cm^{-3}, during the transition from RWR to greenhouse vegetable cultivation. However, we observed a significant increase in macro-porosity within the 50–500 μm size class during the initial nine years of cultivation, which then stabilized. In contrast, transmission porosity in open fields increased more gradually. This suggests that the combination of greenhouse systems and organic manure application improves soil water and gas transport capacity more effectively. The effect of organic cultivation on porosity in the 50–30 μm range was minimal, consistent with the findings of Dal Ferro et al. [48]. Overall, organic greenhouse systems were beneficial in increasing macro-porosity, though the effect size diminished over time. Additionally, the peak in soil macro-porosity appeared earlier in organic greenhouse soil than in open-field soil, with the most significant changes occurring in the >500 μm and 500–50 μm size classes, and limited impact on the 50–30 μm size class.

(2) Soil C, N, and P accumulation and their ratios

The increasing concentrations of SOC, TN, and TP in organic greenhouse vegetable fields and organic open-field vegetable soil converted from RWR in our study, can likely be attributed to the substantial application of manure, which accelerates rapid soil organic matter mineralization. Moreover, the C:N ratios of chicken manure (in our study) were relatively low (10–13) [49]. Long-term application of organic chicken manure stimulates soil heterotrophic microorganisms, enhancing organic matter decomposition and leading to nitrate–nitrogen accumulation and an increase in soil TN [50,51]. Furthermore, chicken manure contains phosphorus due to the feed and physiological processes [14]. However, as mentioned earlier, the phosphorus uptake by vegetables is relatively low, resulting in a significant amount of P remaining in the soil. Interestingly, changes in C, N, and P accumulation were primarily observed in the tilled layer, with tillage duration and cultivation management practices having little effect on soil C, N, and P concentrations in the plow pan layer. This suggests that surface application of organic manure alone may significantly enhance nutrient levels in deeper soil layers, even with increased manure inputs. Moreover, the source of fertilizer used may play a more crucial role in influencing soil C, N, and P concentrations in the plow pan layer.

In this study, there were no significant differences in soil C:N, C:P, and N:P ratios at the plow pan layer over time (Figure 3) in organic greenhouse vegetable fields. However, a significant increase in C:N and C:P in the 0–15 cm depth soil was found during the initial nine-year cultivation in organic open-field vegetable soil ($p < 0.05$). This may be due to the different conditions between the two tillage patterns. The greenhouse vegetable soil is always enclosed in a plastic-shed structure with high temperatures and humidity all year round; thus, the soil C:N and C:P were respectively stable over the whole cultivation. In contrast, organic open-field vegetable soil was exposed to the air, experiencing various climate changes. Our study area, prone to frequent rainfall, coupled with the need for regular irrigation in vegetable cultivation, increased the risk of nutrient leaching (e.g., N and P) in open-field soil. Moreover, SOC accumulation increased rapidly during the first nine years of cultivation in open-field vegetable soil, potentially contributing to the rise in soil C:N and C:P ratios. Changes in the soil N:P ratio were not significant in this study,

indirectly suggesting that increased SOC was the dominant factor influencing soil nutrient stoichiometric ratios.

4.3. Correlation between Soil Macro-Porosities and Soil C, N and P Ratios

Previous research has demonstrated a strong positive effect of SOC on soil macro-porosity [6,9]. In our study, we observed a significant effect at 0–30 cm and 0–15 cm depth ($p < 0.05$), but not at 15–30 cm depth. Moreover, the R-value was higher in the 0–15 cm (r = 0.68) than in the 0–30 cm (R = 0.57), indicating that SOC increased macro-porosity only in the tilled layer. This may be because the organic manure application in our study was surface-applied, hardly affecting the deep soil structure. Similarly, the soil TN, and C:N, C:P, and N:P ratios had a notably positive correlation with soil macro-porosity. It showed the soil C and N quantity, as well as their immobilization rate, was important for improving soil aeration. Further, a clear negative relationship was found between soil P and pH. Soil P is difficult for vegetables to absorb and can bind with metals, leading to a decrease in soil pH [52,53]. Higher pH levels corresponded to lower soil P, higher N:P, and C:P ratios, which contributed to increased macro-porosity. Overall, when soil C:N, C:P, and N:P ratios were higher, the decomposition rates of soil C and N decreased, resulting in more SOC and TN remaining in the soil, thereby improving soil aeration.

Regarding the distribution of different size soil macro-pores, the porosity in the 500–50 μm size class in the 0–15 cm depth was most significantly positively affected by SOC, TN, and C:N, and C:P, and N:P ratios. The relation intensity was lessened over the 0–30 cm. This could be attributed to the role of soil transmission pores, which are closely linked to the transport of air, water, and nutrients. Thus, these pores were also the most sensitive to changes in soil nutrients. However, in our study, soil physical–chemical properties had no significant effect on macro-porosity in the 50–30 μm size class. We hypothesize that this may be because these pores function similarly to micro-capillary pores, which are more directly influenced by the activity of soil fungi and root hairs. Overall, the soil C and N accumulation, along with C:N, C:P, and N:P ratios had a significantly positive effect on soil macro-porosity in the tilled layer, especially for the transmission pores.

5. Conclusions

Our study demonstrated that the application of organic manure in greenhouse vegetable production effectively mitigates soil acidification, enhances nutrient accumulation, and improves soil macro-porosity. Soil macro-porosity and SOC and TN levels were significantly higher in organic greenhouse vegetable soils receiving manure compared to open-field organic vegetable soil. The C:N, C:P, and N:P ratios were notably lower in the conventional vegetable soils than in the organic vegetable soils, indicating that organic manure could promote soil nutrient immobilization in vegetable cultivation. Moreover, the impact of organic manure on soil physical–chemical properties tends to reach equilibrium after 9–14 years of greenhouse cultivation, whereas open-field soil may require a longer period to achieve a similar balance. Our findings suggest that applying organic manure is an effective strategy for improving soil aeration, nutrient accumulation, and balance. However, exploring new amendments may be necessary to address the limitations observed in long-term greenhouse vegetable cultivation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14102207/s1, Table S1: Effects of cropping systems and soil depth on soil physical properties; Table S2: Effects of cropping systems and soil depth on soil physical-chemical properties; Table S3: Effects of cropping systems and soil C, N, P ratios.

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