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In Situ Earthworm Breeding to Improve Soil Aggregation, Chemical Properties, and Enzyme Activity in Papayas

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Abstract: The long-term use of mineral fertilizers has decreased the soil fertility in papaya (*Carica papaya* L.) orchards in South China. In situ earthworm breeding is a new sustainable practice for improving soil fertility. A field experiment was conducted to compare the effects of four treatments consisting of the control (C), chemical fertilizer (F), compost (O), and in situ earthworm breeding (E) on soil physico-chemical properties and soil enzyme activity in a papaya orchard. The results showed that soil chemical properties, such as pH, soil organic matter (SOM), total nitrogen (TN), available nitrogen (AN), and total phosphorus (TP) were significantly improved with the E treatment but declined with the F treatment. On 31 October 2008, the SOM and TN with the O and E treatments were increased by 26.3% and 15.1%, respectively, and by 32.5% and 20.6% compared with the F treatment. Furthermore, the O and E treatments significantly increased the activity of soil urease and sucrase. Over the whole growing season, soil urease activity was 34.4%~40.4% and 51.1%~58.7% higher with the O and E treatments, respectively, than that with the C treatment. Additionally, the activity of soil sucrase with the E treatment was always the greatest of the four treatments, whereas the F treatment decreased soil catalase activity. On 11 June 2008 and 3 July 2008, the activity of soil catalase with the F treatment was decreased by 19.4% and 32.0% compared with C. Soil bulk density with the four treatments was in the order of O $\leq$ E < F < C. The O- and E-treated soil bulk density was significantly lower than that of the F-treated soil. Soil porosity was in the order of C < F < E < O. Soil porosity with the O and E treatments was 6.0% and 4.7% higher, respectively, than that with the F treatment. Meanwhile, the chemical fertilizer applications significantly influenced the mean weight diameter (MWD) of the aggregate and proportion of different size aggregate fractions. The E treatment significantly increased the MWD, but the F treatment decreased it. The MWD with the E treatment was 14.5% higher than that with the F treatment. The proportion of >2 mm size aggregates in the O and E treatments was vastly improved. In conclusion, in situ earthworm breeding in orchards performed better than traditional compost and chemical fertilizer in improving soil aggregation, chemical properties, and enzyme activity. This is a new, organic fertilizer application for improving soil structure, chemical properties, and soil enzymes due to the activities of the earthworms and the production of vermicomposting.

Keywords: in situ earthworm breeding; organic fertilization; soil properties; soil enzyme

1. Introduction

Mineral fertilizers have been used widely to increase crop and fruit yields all over the world for a long time. Numerous studies have shown that chemical fertilizer use may have many adverse effects on soil, such as acidification, soil hardening, the decrease of the soil physical structure and soil fertility, and the reduction of microbial communities and soil enzyme activities [1–3].

Soil amendment with compost is an effective agricultural practice used constantly to improve soil fertility and to manage animal waste. Comprehensive reviews have suggested that the addition of compost not only improves soil structure, aggregate stability, and fertility [3–6], but also influences the soil microflora [7]. Studies have also shown that the addition of good quality compost could increase global microbial biomass and enhance soil enzyme activity [8–11]. Although many efforts have been devoted to investigating the impacts of compost on soil, few studies have been conducted to develop new organic methods for improving soil properties in agriculture.

In situ earthworm breeding is a new, organic fertilization method that breeds earthworms at a distance of 50 cm from plants in orchards. Compared with compost, this method has some important advantages as the assimilation of earthworms can manage a large amount of animal waste, as well as crop residues, so that the environment of rural areas can be improved [12]. In addition, the activities of the earthworms improve the soil physical structure and play an active part in soil aggregate formation. Most importantly, this method produces a large amount of vermicompost. It has been shown that vermicompost may be physically, nutritionally, and biochemically superior when compared with traditional compost [3,13]. It could improve soil fertility, plant growth, and increase crop yield [3,14–17]. An interesting study found that in situ earthworm breeding significantly improved the growth, yield, and quality of papayas [12]. However, less is known about the impacts of in situ earthworm breeding on soil physiochemical properties.

Soil enzyme activities are the direct expression of the soil microbial community to metabolic requirements and available nutrients. It has been related to soil physiochemical properties and microbial community structure [18]. Therefore, investigation should be carried out to evaluate the effect of in situ earthworm breeding on soil physiochemical properties and enzyme activities. The objective of the present study was to examine the effects of inorganic and organic fertilizations on soil physiochemical and biological properties and identify better options for improving soil structure and fertility in papaya orchards.

We hypothesized that (1) in situ earthworm breeding would perform the best in improving soil physical properties due to the activities of the earthworms when compared with traditional compost and mineral fertilizer; and (2) in situ earthworm breeding would improve soil chemical properties and enhance soil enzyme activities.

2. Materials and Methods

2.1. Site Description

This study was conducted at Yinghuwan reclamation land, Xinhui district, Jiangmen city (23° N, 113° E), which is located in the southwestern Pearl River Delta in Guangdong Province, China. The area is characterized by a typical subtropical monsoon climate. The average annual precipitation is 1763 mm, of which approximately 80% falls during the wet season between May and September. The mean daily high temperature averaged over the entire year is 23.8 °C, with the lowest and highest monthly mean temperatures in January and July, respectively. The content of soil organic matter (SOM) is 24.3 g kg^{-1}, the total nitrogen content (TN) is 1.2 g kg^{-1}, the total phosphorus content (TP) is 0.7 g kg^{-1}, the content of total potassium (TK) is 22.3 g kg^{-1}, the content of available nitrogen (AN) is 80.9 mg kg^{-1}, the content of available phosphorus is 62.8 mg kg^{-1}, and the content of available potassium is 286.4 mg kg^{-1} [12].

2.2. Experimental Design and Treatments

This experiment was conducted in a Hawaiian papaya orchard for approximately one year. Hawaiian papaya plants were transplanted on 31 March 2008, with a planting space of 3.1 m × 3.1 m. The following four soil treatments were used in our study: the control (C), chemical fertilizer (F), compost (O), and in situ earthworm breeding (E). The experimental design was a randomized complete block with three replicates for each treatment. The area of each plot was 65 m^2, with seven papaya trees per plot. In the beginning of this experiment, 5 g $plant^{-1}$ urea (CH_4N_2O, %N = 46.3%) and 50 g $plant^{-1}$ microelement fertilizer were applied to each treatment. Since then, no chemical fertilizer was applied to the C; however, 45 g $plant^{-1}$ urea (CH_4N_2O, %N = 46.3%), 100 g $plant^{-1}$ phosphate (%P_2O_5, P = 12%), and 500 g $plant^{-1}$ compound fertilizer (%N–%P–%K = 15%–15%–15%) were applied in the F treatment. The O treatment was prepared by using cow manure, and 10 kg $plant^{-1}$ cow manure (divided five times) was applied to the O treatment. Before the fertilizers and amendments were applied, a hole of about 30 cm in depth and 50 cm from the papaya plant was dug. After the hole was prepared, the fertilizers and amendments were put in the hole before the hole was covered with the original soil. The total amounts of nutrients applied to each plant in each treatment are summarized as follows: C (TN 2.3 g, TP 0.0 g, and TK 0.0 g), F (TN 110.9 g, TP 102.0 g, and TK 90.0 g), O (TN 98.1 g, TP 42.3 g, TK 40.3 g, and SOM 1.9 g), and E (TN 2.3 g, TP 0.0 g, and TK 0.0 g) [12].

In the E treatment, an earthworm bed (length: 16 m; above width: 40 cm; below width: 60 cm; and height: 30 cm) was prepared approximately 50 cm from the papaya plant in each block. A total of 4.86 kg m^{-3} organic waste produced in the process of beer making was added to the bottom of the bed. Next, earthworms (*Eisenia fetida*) were placed into the earthworm bed at a density of 8 g per m^2. Then, we put rice straw and sun shading net on the bed, followed by the regular addition of water and organic waste so that we could provide a better environment for the earthworms growth and reproduction [12]. Water was added every morning and evening. Organic waste was added once a month with 4.86 kg m^{-3} in the 10 cm depth of the earthworm bed. The field experiment of E is shown in Figure 1. The nutrient contents of the cow manure and organic waste are listed in Table 1.

Figure 1. Field experiment of in situ earthworm breeding in the papaya orchard.

Table 1. The nutrient content of cow manure and organic wastes.

Items	pH	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	SOM (g kg^{-1})
Cow manure [a]	6.1	9.6	4.2	4.0	193.2
Organic wastes	6.1	9.8	5.1	8.9	150.6

Notes. [a] cited from Xiang [12]; TN = total nitrogen, TP = total phosphorus, TK = total potassium, and SOM = soil organic matter.

2.3. Soil Sampling

Soil samples were collected from the surface soil (0–20 cm) at a distance of 90 cm from the papaya plant on 10 April 2008 (papaya young plant stage), 11 June 2008 (plant rapid growing stage), 3 July 2008 (plant blossoming and fruiting stage), 11 August 2008 (fruit setting period), 8 September 2008 (fruit expanding stage), and 31 October 2008 (mature stage), respectively. In each plot, twelve cores (3.0 cm diameter) were randomly sampled after removing the surface litter and were combined to form one composite sample. The soil samples were kept on ice, immediately transported to the laboratory, and then divided into three sub-samples. One sub-sample was air-dried at 25 °C for chemical analysis, one sub-sample was used for aggregate measurements, and the third sub-sample was stored at 4 °C for enzyme analysis.

2.4. Laboratory Analyses

The soil pH was determined from a soil water suspension (1:2.5 *w*/*v*) by using a pH meter. Soil bulk density was measured gravimetrically. The soil porosity was calculated from the bulk density and particle density according to Huang [19]. The SOM concentration was determined by titration with a Fe^{2+} solution after dichromate oxidation. TN was determined by an Automatic N analyzer (Kjeldhal K06 Full Auto Analyzer, Shanghai Shengsheng Automation Analysis Instruments Co., Ltd., Shanghai, China). The soil TP concentration was determined by molybdate colorimetry [20]. The soil total potassium was quantified using inductively coupled plasma-atomic emission spectrometry (ICPS-7500, Shimadzu, Japan). The soil AN was detected using the alkaline hydrolysis diffusion method [21].

A 100 g sample of air-dried bulk soil (passed through an 8 mm sieve) was placed on the top of a set of nested sieves (2, 1, and 0.25 mm). The soil was dry-sieved and separated into four aggregate classes (>2 mm, 1–2 mm, 0.25–1 mm, and <0.25 mm). The mean weight diameter (MWD) of each (>2 mm, 1–2 mm, 0.25–1 mm, and <0.25 mm) aggregate was determined as reported by Kemper and Chepil [22]:

$$\mathrm{MWD} = \sum_{i=1}^{n} X_i W_i \tag{1}$$

where MWD is the mean weight diameter (mm) of the aggregate; X_i is the mean diameter (mm) of each size fraction; W_i is the proportion of the total aggregate sample in the corresponding size fraction; and n is the number of size fractions.

Urease activity was determined by measuring released ammonium from the soil amended with urea [23]. Catalase activity was analyzed by titration with 0.1 mol L^{-1} KMnO4 [24]. Sucrase activity was determined by 3,5-dinitrosalicylic acid colorimetry using sucrose as the substrate, which was expressed as mg glucose g^{-1} soil [25].

2.5. Data Analysis

The experimental data of soil physical properties (bulk density, soil porosity, MWD, and the proportion of different size class aggregates) were evaluated using one-way analysis of variance (ANOVA). The treatments and experimental data were set as fixed and random variables, respectively. The significant differences among the means of the treatments ($p < 0.05$) were determined by Duncan's multiple range tests using SPSS 13.0 for Windows. The soil chemical properties and enzyme activities over time were also evaluated using ANOVA. At each sampling time, soil samples collected from the same experimental unit were determined every time, and then, each time, three data from the three replicates were obtained in each treatment. Significant differences among the means of treatments at the same sampling time ($p < 0.05$) were then determined by Duncan's multiple range tests. All figures were conducted in Origin version 8.

3. Results and Discussion

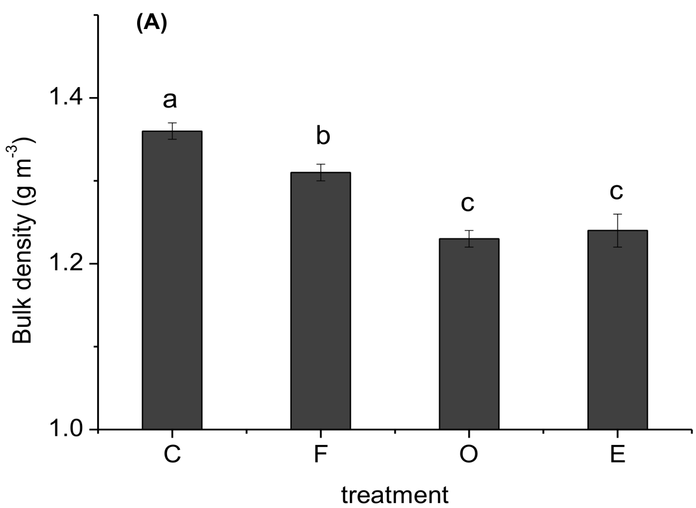
3.1. Soil Physical Properties

The results showed that different fertilizer applications significantly influenced the soil bulk density and porosity (Figure 2A,B). Compared with the F treatment, the O and E treatments significantly decreased the soil bulk density by 6.1% and 5.3%, respectively (Figure 2A). The soil porosity in the four treatments was in the order of C < F < E < O (Figure 2B). The O and E treatments significantly increased soil porosity by 6.0% and 4.7%, respectively, when compared with the F treatment. These results were consistent with previous studies that indicated that organic amendments increased soil porosity and aeration and improved soil structure [2,26].

Furthermore, the MWD of the soil aggregates was significantly decreased by the F treatment but increased by the E treatment (Table 2). Compared with the treatment of the C, the MWD in the E treatment was 14.5% higher than in the F treatment. The reason for the decreased MWD of the aggregates in the F treatment may be due to the chemical fertilizer altering the soil physical structure and preventing soil aggregation. Similar results have also been found in other studies [1].

Furthermore, the O and E treatments significantly increased the proportion of >2 mm size class aggregates, but the F treatment decreased it (Table 2). Compared with the F treatment, the proportion of >2 mm size class aggregates in the O and E treatments was vastly improved by 29.2% and 32.0%, respectively. These results were in accordance with our first hypothesis that soil physical properties were improved by the O and E treatments but reduced by the F treatment. The reason for the E treatment having the best positive effects on the soil physical properties in this study is likely due to the activities of the earthworms. Earthworms are known to have beneficial effects on soil physico-chemical and biological properties: they enhance the incorporation of plant residues into soil aggregates and create soil porosity and stable aggregates through their burrowing, humus formation, and casting activities [27–29].

In contrast, the O and E treatments significantly decreased the proportion of 0.25–1 mm size class aggregates, but the F treatment increased it. No significant difference in the proportion of <0.25 mm size class aggregates was found between the four treatments.



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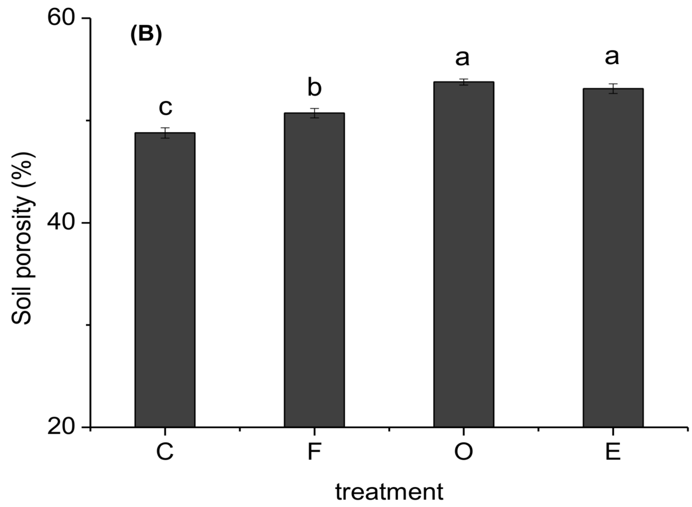


Figure 2. Soil bulk density (**A**) and porosity (**B**) under different fertilizer applications (mean ± standard error, $n = 3$). C = control; O = compost; F = chemical fertilizer; and E = in situ earthworm breeding. Different letters indicate significant differences between treatments ($p < 0.05$).

Table 2. Mean weight diameter (MWD) of aggregate and the proportion of different size class aggregates in dry soil under the four treatments. C = control; O = compost; F = chemical fertilizer; and E = in situ earthworm breeding (mean ± standard error, $n = 3$).

Treatment	MWD (mm)	The Proportion of Different Size Class Aggregates (%)			
		>2 mm	1–2 mm	0.25–1 mm	<0.25 mm
C	1.6 ± 0.0b	50.6 ± 0.6Ab	21.5 ± 0.2Ca	25.5 ± 0.7Bb	2.5 ± 0.2Da
F	1.5 ± 0.0c	44.3 ± 2.4Bc	20.0 ± 0.01Cab	32.9 ± 2.1Aa	2.8 ± 0.6Da
O	1.7 ± 0.0b	57.2 ± 2.0Aa	17.79 ± 0.9Cb	22.8 ± 1.7Bc	2.3 ± 0.4Da
E	1.7 ± 0.0a	58.4 ± 0.2Aa	21.1 ± 0.6Ca	18.9 ± 0.4Bc	1.6 ± 0.3 Da

Notes: Different uppercase letters denote significant differences of different size class aggregates in the same treatment. Different lowercase letters mean a significant difference between treatments ($p < 0.05$).

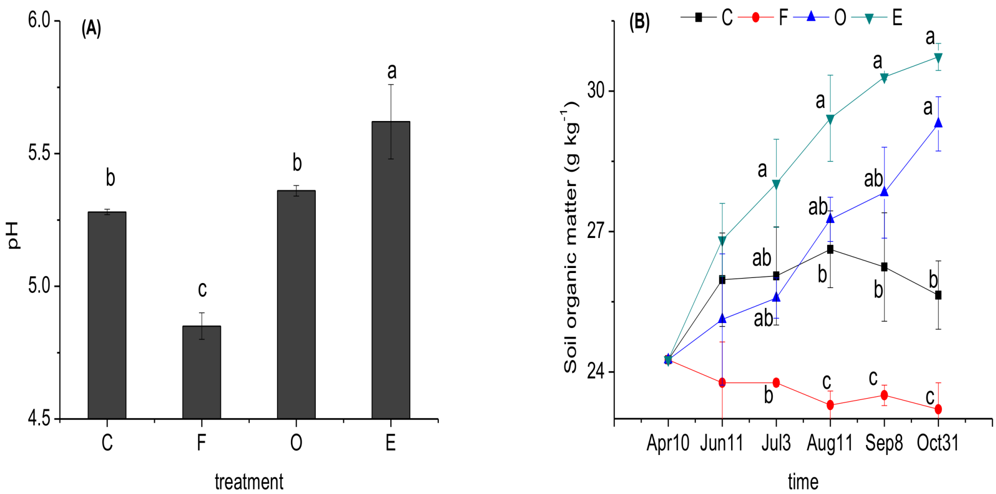
3.2. Soil Chemical Properties

The soil pH, the contents of SOM, TN, AN, and TP were significantly increased with the E treatment, but decreased with the F treatment ($p < 0.05$; Figure 3). From 11 June to 31 October 2008, the content of SOM with the O and E treatments gradually increased with time and reached the maximum on 31 October 2008 (Figure 3B). On 31 October 2008, the SOM content with the O and E treatments was 26.3% and 32.5% higher than that with F treatment, respectively. A significant difference in contents of TN and AN was only found on 31 October 2008 (Figure 3C,D). On 31 October 2008, the TN content with the O and E treatments of was significantly higher than that with the F treatment with percentages of 15.1% and 20.6%, respectively. However, there was no significant difference between the O and E treatments. For TP content, a significant difference occurred after 11 August 2008 (Figure 3E), and the TP content with the E treatment was always higher than that with the other three treatments. However, there was no significant difference in the content of soil total potassium (Figure 3F).

Our results suggest that the O and E treatments significantly improved the soil chemical properties, but the F treatment decreased them, which is consistent with past researches. Oo et al. [30] reported that compost and vermicompost amendments had positive effects on soil organic carbon when compared with unamended treatments because compost and vermicompost are rich in soluble organic carbon unlike conventional fertilizers. However, in comparison to the O treatment, the E treatment had greater effects on the soil chemical properties. This is due to the high-quality castings of the earthworms, which are known as "vermicompost". Frederickson et al. [31] and Singh et al. [32] reported that soils treated with vermicompost had significantly higher contents of TN, extractable phosphorous, and organic carbon when compared with soils treated by traditional compost. Furthermore, the castings egested by earthworms contain a variety of nutrients, such as hormones, enzymes, microorganisms, and inorganic and organic materials from the earthworm gut [33]. The E treatment most greatly improved the soil chemical nutrient properties in our experiment, this result could also be explained by the data of papaya plant growth and yield for all the treatments. Over the whole growing season, the heights of the papaya plant with the F and E treatments were significantly higher than those of the C from 3 July 2008 [12]. In August 2008, the plant heights of the E, F, O and the C treatments were 195.7, 188.7, 172.6, and 156.9 cm, respectively. The yield of the four treatments was in the order of E (10,671.0 kg hm^{-2}) > F (10,477.2 kg hm^{-2}) > O (6927.9 kg hm^{-2}) > C (4835.5 kg hm^{-2}), and there was a significant difference between them [12]. This suggests that most of the soil nutrients in the soil with the F treatment were used to increase the growth and yield of papaya, which resulted in the decrease of the soil chemical properties. However, when compared with the F treatment, the E treatment not only boosted papaya growth and yield, but also improved the soil properties due to the constant high-quality vermicompost.

The soil pH of the C, F, O, and E treatments was 5.3, 4.9, 5.4, and 5.6, respectively (Figure 3A). Compared with the C, the F treatment significantly decreased the soil pH, but the E treatment increased it. The reason for the pH decrease in the soil with the F treatment was the utilization of phosphate. Because phosphate belongs to acid fertilizers, it tends to decrease the soil pH. The decreased pH of the soil with the F treatment probably contributed to the increase in total phosphorus, so that the total phosphorus in the soil with the F treatment was higher than that of the C at several sampling times. The results of the soil pH in this study were in contrast with other studies. Past studies have indicated that the soil pH was decreased by vermicompost amendments [30,34]. The increase in the soil pH in this study was most likely caused by the initial pH of the original soil; in the area of this experiment, the original soil was acidic. However, the increase in the soil pH in this study might be a good thing for the growth of plants, because soil acidification is an important factor that threatens potential productivity and food security in the area. Therefore, in situ earthworm breeding in orchards in subtropical areas not only helps replenish natural soil fertility, but also can contribute to improving the soil pH.

Over the growing season, the content of soil total potassium was drawn down, most likely because the growth of papaya consumed a large amount of potassium.



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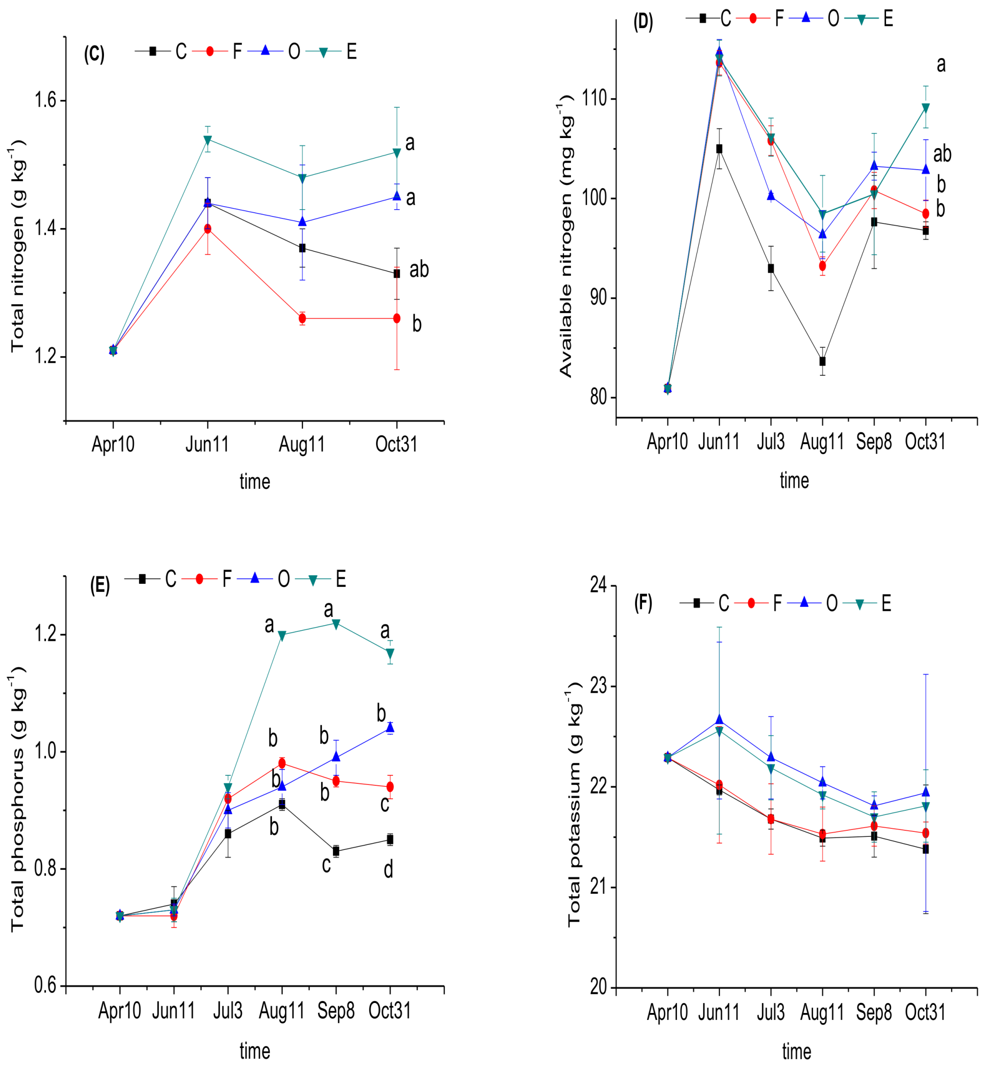


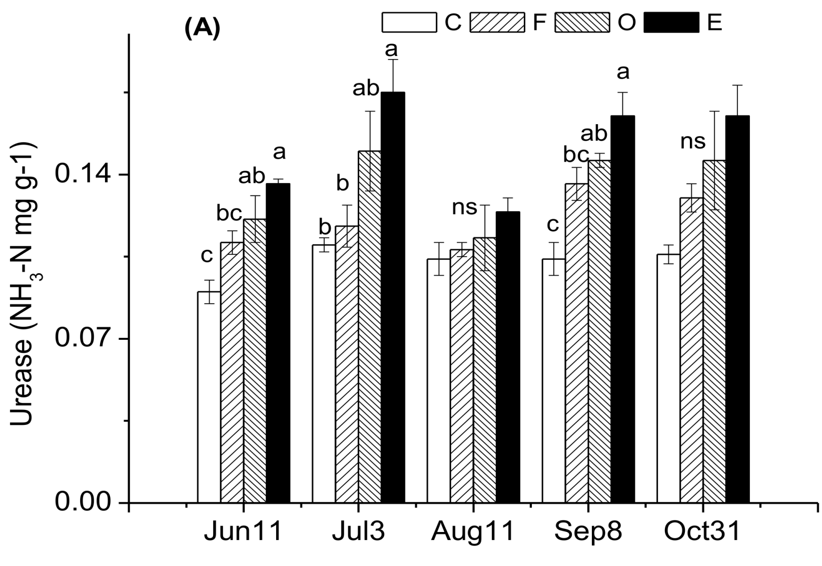
Figure 3. Soil pH (**A**) and the content of soil organic matter (**B**), total nitrogen (**C**), available nitrogen (**D**), total phosphorus (**E**), and total potassium under different fertilizer applications (**F**) (mean ± standard error, *n* = 3). C = control; O = compost; F = chemical fertilizer; and E = in situ earthworm breeding. Different letters indicate significant differences between treatments in individual sampling time tested by one-way analysis of variance (ANOVA, $p < 0.05$).

3.3. Soil Enzyme

Soil enzymes are very important due to their role in nutrient cycling and are considered as early indicators for soil biochemical reactions because of their relationship to the soil biology [35,36]. The results of this study showed that different fertilizer applications significantly affected soil enzyme activity. The order of soil urease activity in these treatments was C < F < O < E (Figure 4A). The O and E treatments significantly increased the activity of soil urease (Figure 4A). However, there was no significant difference between the F and the C treatments, and the O and E treatments. On 11 June 2008, 3 July 2008, and 8 September 2008, when compared with the C, soil urease in the soil with the O and E treatments was improved by 34.4% and 51.1%, 36.4% and 59.1%, and 40.4% and 58.7%, respectively. For soil catalase, the F treatment significantly decreased its activity (Figure 4B). On 11 June and 3 July, the activity of soil catalase in the soil with the F treatment was decreased by 19.4% and 32.0%, respectively, when compared with the C. On 11 August 2008, soil catalase was also reduced by the O treatment. However, there was no significant difference between the treatment E and the C. For soil sucrase, the F, O, and E treatments all significantly raised its activity (Figure 4C), while the activity of soil sucrase in the soil with the E treatment was the greatest, that in the soil with the O and F treatments was medium, and that in the C was the least. No significant difference occurred between the O and F treatments. While on 11 July 2008 and 8 September 2008, soil sucrase activity in the soil with the E treatment was significantly higher than that in the soil with the F treatment with percentages of 13.7% and 24.9%, respectively.

These results indicated that soil urease and sucrase activity were significantly improved by the O and E treatments. This has also been demonstrated by other studies. Several studies have pointed out higher microbial biomass and diversity in soils amended with compost [37]. Microbial respiration, enzymatic activities such as dehydrogenase and phosphatase activities, and N mineralization rates are also usually enhanced after amendment with organic substrates [38–40]. Doan et al. [41] reported that soils amended with compost and vermicompost were characterized by higher bacterial, catabolic diversity indices, and higher enzymatic activities than control soils that only received mineral fertilizers. The utilization of organic amendments usually leads to important modifications of both soil microbiological and biochemical properties [42,43]. Increased soil enzyme activities might be due to increased SOM and TN in the soil with the E treatment as past studies have shown that soil enzyme activities can be affected by soil organic carbon and SOM [44–46]. The SOM is not only a pool of plant nutrients but can also serve as an energy source for a diverse population of microorganisms [47]. Furthermore, the earthworms in the E treatment secreted polysaccharides, proteins, and other nitrogenous compounds from their bodies, thereby providing an additional energy source for microorganisms [48]. Several studies have indicated that the bacterial community structure in vermicompost considerably differs from that in analogous composts. Vermicomposts are usually more stable than composts, with a higher availability of nutrients and improved microbiological properties [49–51]. This may be the main reason why the E treatment had a better effect on soil enzyme activity than the compost amendment.

In addition, when compared with the O treatment, the E treatment had three other very important advantages: (1) the E treatment could dispose of some complicated organic waste, which could create a better environment for rural areas; (2) the E treatment guaranteed good quality and provided safer fruit products to consumers [12]; and (3) the E treatment could produce earthworms, which can also be used as Chinese medicine and bring a higher economic return to the farmer. Thus, in situ earthworm breeding in orchards is more sustainable than other treatments as it not only improves soil properties, but also promotes the environment and fruit quality and has other economic benefits.



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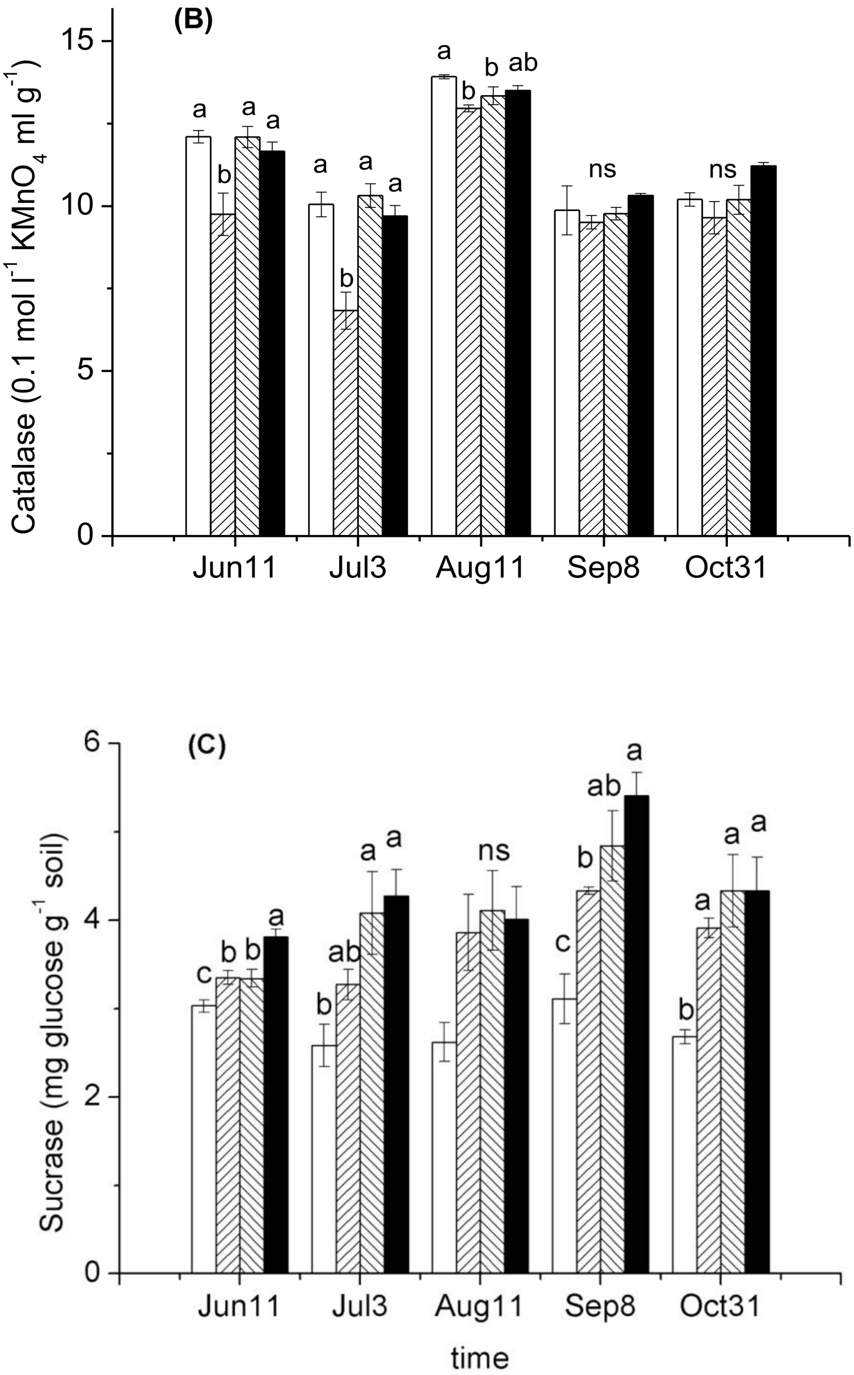

Figure 4. Soil urease (**A**), catalase (**B**), and sucrase (**C**) activities under different fertilizer applications. C = control; O = compost; F = chemical fertilizer; and E = in situ earthworm breeding. Bars indicate means with error bars being standard errors (n = 3). Different lowercase letters on the bars indicate significant differences at the $p < 0.05$ level among treatments in each sampling time.

4. Conclusions

The results of this study showed the beneficial effects of compost and in situ earthworm breeding in orchards in increasing soil properties. The results of the soil analysis showed that the application of compost and in situ earthworm breeding significantly increased soil porosity, MWD, the proportion of >2 mm aggregate fraction, pH, and the contents of SOM, TN, AN, and TP. Thus, compost and in situ earthworm breeding not only served as a source of essential nutrients for plants, but also improved the soil properties. Furthermore, soil enzyme activities were also promoted by the application of compost and in situ earthworm breeding. These results suggest that the utilization of compost and in situ earthworm breeding can contribute to improving soil physical, chemical, and biological properties. However, when compared with compost, in situ earthworm breeding is a better, sustainable option for improving soil fertility, agriculture environment, and fruit productivity and quality and also has other multiple economic benefits.

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Author Contributions: Jiaen Zhang conceived and designed the experiments; Lei Guo and Huimin Xiang performed the experiments; Hui Wei and Benliang Zhao analyzed the data; Huimin Xiang wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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