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Effects of Chemical Fertilizer Combined with Organic Fertilizer Application on Soil Properties, Citrus Growth Physiology, and Yield

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Abstract: Chemical fertilizer has been excessively used for high yield of citrus around the world, especially in China; meanwhile, it deteriorates the citrus orchard soil environment. To resolve the conflict, the use of organic fertilizer provides a promising solution. However, the data about organic fertilizer used in citrus orchard is rarely available. Here, four treatments including CK (no fertilizer), CF (chemical fertilizer), OF + CF (chemical fertilizer reduction combined with organic fertilizer; application of N, P₂O₅, K₂O fertilizer and organic fertilizer is 0.564, 0.236, 0.336 and 10 kg/plant), and BF + CF (chemical fertilizer reduction combined with bioorganic fertilizer; application of N, P₂O₅, K₂O fertilizer and bioorganic fertilizer is 0.508, 0.320, 0.310 and 10 kg/plant) were performed in a 'Ponkan' (Citrus reticulata Blanco) orchard to evaluate the effect of organic fertilizer on citrus yield, growth, soil properties etc. when nutrients of fertilizer of each treatment were equal except CK. The data obtained in 2019 and 2020 showed that both OF + CF and BF + CF were beneficial to improve soil fertility (soil physicochemical and microbe properties) and citrus growth physiology (growth, nutrient and photosynthesis), alleviate NO3⁻-N leaching, and promote yields. Comprehensive evaluation indicated that BF + CF was more effective than OF + CF. Together, organic fertilizer has the potential to substitute partial chemical fertilizer with improvement in soil properties, growth physiology, and yield of citrus.

Keywords: Ponkan; organic fertilizer; soil properties; photosynthesis; yield

1. Introduction

Chemical fertilizer has been generally overused around the world. Global chemical fertilizer use has been reported to be about 1.9×10^{11} t, with China ranking first in consumption, accounting for 25% of world usage [1]. Due to great importance of chemical fertilizer to crops in China, the use of chemical fertilizer has continuously expanded from 8.8×10^9 t in 1978 to 6.0×10^{10} t in 2015 [2]. In addition, China's per hectare application of chemical fertilizer, 393.2 kg/hm², is higher than the international environmentally safe use limit of 225 kg/hm², and is also about 3.05 times that of the United States and 2.54 times that of the European Union [3,4]. In 2019, China ranked first in citrus production and cultivated area, with about 4.4×10^8 t and 2.9×10^7 ha accounting for 27.9% and 29.1% of world production and area [5], respectively. The yields of China's citrus increased further with more fertilizer being applied, especially chemical fertilizer. According to some estimates, excessive application of nitrogen (N), phosphorus (P), and potassium (K) fertilizers were approximately 3.6×10^6 , 4.3×10^6 , 3.6×10^6 t of citrus main production area in China, respectively [6]. What's more, the utilization rate of N, P, and K fertilizer in China is 35-40%, 8-46%, and 35-50%, respectively, far below the level of developed countries [7,8]. It is well

known that excessive chemical fertilizer application will adversely affect soil physical and chemical properties, resulting in soil hardness and acidification, which eventually lead to a decline in soil organic matter and fertility [9,10]. In addition, chemical fertilizer also negatively impacts crop quality and causes ecological environment damage, such as water pollution, greenhouse gas emission, and N leaching [11,12]. Moreover, excessive chemical fertilizer application leads to a waste of resources, places a financial burden on farmers, and even reduces the international competitiveness of agricultural products [13,14]. In contrast, organic fertilizer can improve the physical and chemical properties of soil, such as structure, water retention, nutrients, and cation exchange capacity, and promotes positive biological soil properties, enhancing yield and quality and even alleviating the risks of ecological environment deterioration [15–17]. Organic fertilizer is a highly abundant resource in China, with approximately 4.0×10^{10} t available; hence the potential for application is enormous [18]. However, compared to the quick nutrient release of chemical fertilizers, organic fertilizers have low nutrient concentrations, and nutrient release is too slow to support crops in a short time [19]. A beneficial approach to overcome this problem is reduction of chemical fertilizers combined with the application of organic fertilizers; this has been shown to better sustain soil fertility compared to applying chemical or organic fertilizers alone [7,15,20].

Several studies have reported that chemical fertilizer combined with organic fertilizer application (CFOF) improves soil conditions and promotes plant growth and even yield in comparison with only chemical fertilizer application. For example, combined application of organic and inorganic fertilizers greatly increases soil organic matter and the total nitrogen content of the soil and improves soil microenvironment in wheat/maize fields [21]. Hazarika et al. [22] found similar results. According to Xiao et al. [19], organic fertilizer combined with compound fertilizer improved soil quality, whereas the utilization of compound fertilizer worsened soil quality and made the soil acidize; this result was similar to that of Song et al. [20] and Pachuau et al. [23]. Qiu et al. [15] reported that chemical fertilizer combined with biofertilizer application significantly promoted root growth, improved the rate of nutrient distribution in citrus, and improved the external and internal qualities of tarocco blood orange; this result was similar to those of previous study of citrus [24–26]. According to Pei et al. [7], organic fertilizer is an alternative to chemical fertilizer with no loss in yield and fruit quality for citrus. In addition, apple orchard with organic-inorganic mixed fertilizer promoted soil microbial activity and increased soil organic matter by 16% and crop production by 67% when compared with chemical fertilizer application alone [26] and those results were consistent with research of Lai et al. [27]. Some experiments [28–30] also show that application of CFOF improves plant physiological indexes and yield compared with inorganic fertilizers on their own. These studies indicate that CFOF improves soil microbial activity, enhances physical and chemical soil properties, and promotes the absorption and utilization of nutrients, thus facilitating high crop yields. Chemical fertilizer reduction combined with organic fertilizer application meets the requirements for green ecology and is gradually popular in China [7]. Recently, research on reducing chemical fertilizer use and applying organic fertilizer has focused on the effects of reducing N fertilizer on crop yield and quality while rarely measuring changes to soil properties, orchard environment, and plant physiology, especially in citrus systematically, in response to a reduction in chemical fertilizer combined with increased organic fertilizer when equal nutrients of N, P, and K fertilizers are supplied. Due to 'Ponkan' (Citrus reticulata Blanco) being one of the most widely grown varieties of Citrus reticulate, which accounted for 55.3% of the total amount of citrus on cultivated area in China [6], we selected it as our material.

Therefore, the purpose of this research is to explore the effects of CFOF on soil properties, citrus growth physiology, and yield when nutrients of N, P, and K fertilizers are equal and to evaluate the effects of different fertilization treatments on soil environment. This work could provide a theoretical basis for the scientific reduction of chemical fertilizer and identify the amount of organic fertilizer necessary for sustainable development of the citrus industry.

2. Materials and Methods

2.1. Study Sites and Materials

The study was conducted in two consecutive years, 2019 and 2020, in the citrus orchard of the citrus Research Institute, Southwest University (longitude, 29°76′ N; latitude, 106°38′ E; altitude, 240 m above sea level), using 15-year-old 'Ponkan' (*Citrus reticulata* Blanco) grafted on to *Poncirus trifoliata* (L.) as study material. In this study, we selected four separated plots for experiments in Ponkan orchard and we randomly selected 15 trees (uniform growth size and plant spacing 3 m × 4 m) per treatment in the experimental plots. All measurements were performed on the three central trees, and treatments were distributed in a completely randomized block design with five replications. The mean annual temperature, annual sunshine time, and annual precipitation were 19.26 °C, 1178.70 h, and 1171.60 mm, respectively, with the maximum temperature in July. The orchard soil is loose loam. The pH value of the orchard soil was 5.03 ± 0.29 , while the organic matter and available N, P, and K were $20.71 \pm 2.86 \text{ g}\cdot\text{kg}^{-1}$, 89.15 ± 4.59 , 49.61 ± 7.64 , and $190.28 \pm 10.07 \text{ mg}\cdot\text{kg}^{-1}$, respectively.

Four different treatments, namely, CK (no fertilizer), CF (chemical fertilizer), OF + CF (chemical fertilizer reduction combined with organic fertilizer), and BF + CF(chemical fertilizer reduction combined with bioorganic fertilizer) were performed. Organic fertilizer and bioorganic fertilizer were produced by Sichuan Runzhou Biotechnology Co., Ltd. (Jianyang, China). The N, P, and K nutrient content of each treatment except CK was consistent. Table 1 presents the four treatments of specific fertilization. CK was treated with no fertilizer.CF was treated with chemical fertilizers, including urea (N 46.7%), calcium–magnesia phosphate (P_2O_5 12%), and potassium sulfate (K_2O 51%). OF + CF was treated with chemical fertilizer (urea, calcium-magnesia phosphate, and potassium sulfate) reduction combined with organic fertilizer (N+P₂O₅+K₂O \geq 5.0%; organic matters such as biogas residue, wheat husk, and dregs of beans were made by fermentation). BF + CF was treated with chemical fertilizer (urea, calcium-magnesia phosphate, and potassium sulfate) reduction combined with bioorganic fertilizer (N+P₂O₅+K₂O \geq 5.0%, on the basis of organic matter, and added bacteria spore such as Bacillus subtilis, Brevibacillus laterosporus, Bacillus licheniformis, etc.). Each treatment was performed in five replications. We applied organic or bioorganic fertilizer combined with chemical fertilizer in October (color transition period of Ponkan) and then supplemented with only chemical fertilizer in March (germination period of Ponkan) and July (expansion period of Ponkan). Organic or bioorganic fertilizer was applied firstly in October in 2018, and the study of two consecutive years of study of 2019 and 2020 was conducted. During these three physiological periods of Ponkan growth, the proportion of N, P, and K (chemical fertilizer) was 40%:40%:20%, 20%:30%:50%, and 30%:50%:20%, respectively.

Treatment	Organic/Bioorganic Fertilizer Application (kg/Plant)	Nutrient of Organic Fertilizer (kg/Plant)		Nutrient of Chemical Fertilizer (kg/Plant)			Total Amount of Nutrients (kg/Plant)			
		Ν	P_2O_5	K ₂ O	Ν	P_2O_5	K ₂ O	Ν	P_2O_5	K ₂ O
СК	0	0	0	0	0	0	0	0	0	0
CF	0	0.000	0.000	0.000	0.705	0.480	0.520	0.705	0.480	0.520
OF + CF	10	0.141	0.244	0.188	0.564	0.236	0.332	0.705	0.480	0.520
BF + CF	10	0.197	0.160	0.210	0.508	0.320	0.310	0.705	0.480	0.520

Table 1. Test design of fertilization.

Note: CK: no fertilizer; CF: chemical fertilizer; OF + CF: chemical fertilizer combined with organic fertilizer; BF + CF: chemical fertilizer combined with bioorganic fertilizer. Reduction of chemical fertilizer and addition to organic/bioorganic fertilizer. The amount of organic/bioorganic fertilization is 10 kg/per plant, and the total amounts of nutrients of each treatment are equal except CK by adjustment of chemical fertilization.

2.2. Organ Sampling and Nutrient Measurement

In September, current-year mature branches were sampled, and twigs and leaves of branches were separated. Leaf area was measured by the Wseen LA-S image analysis system (Hangzhou Wseen Testing Technology Co., Ltd., Hangzhou, China). Each treatment included ten branches as our materials, and five replications per treatment. Thickness of a hundred leaves and twig length were measured by Vernier caliper and ruler. The twig and leaf samples were dried in an oven at 105 °C for 30 min, followed by incubation at 75 °C until achieving a constant weight, and then the dry matter of a hundred leaves were measured. Samples were ground with a powder grinder and then passed through a 0.5 mm sieve. After H₂SO₄-H₂O₂ digestion of samples, the N, P, and K concentrations of twigs and leaves were measured by the semi-micro Kjeldahl N determination method, the molybdenum blue colorimetric method, and flame photometry [31], respectively. The roots were sampled from soil pits with a size of 20 cm imes 20 cm imes 40 cm (length imes width \times height) near the drip line of each plant in October, five replications per treatment. The yield per citrus tree was investigated in December, each treatment included three citrus trees and five replications per treatment. Fertilizer contribution rate was calculated by the method in Jiang et al. [32]. The root and fruit measurement of N, P, and K concentration was same as that of twigs and leaves.

2.3. Photosynthesis and Leaf Physiological Parameters

Leaf SPAD values were measured by SPAD–502 (Konica Minolta Co., Ltd., Tokyo, Japan) in April (flowering period), June (fruit drop period), August (fruit expanding period), October (fruit color change period), and December (fruit mature period). Each treatment included ten leaves as our materials, and five replications per treatment. Between 9:00 and 11:00 a.m. on sunny days, Pn(photosynthetic rate)of current-year leaves were measured using a 3051D portable photosynthesis system (Zhejiang Top cloud- agriculture Co., Ltd., Hangzhou, China), and chlorophyll fluorescence parameters, Plabs and Pltotal, of current-year leaves were measured by Handy PEA (Hansatech Instruments Co., Ltd., Norfolk, Britain).

2.4. Soil Physicochemical Properties

Soil at a depth of 0–20 cm, approximately 15 cm away from the fertilization application hole, was sampled in December. Soil porosity was also measured following Haque et al. [33]. Soil was dried naturally after passing through a 2 mm sieve. Soil pH, alkali-hydrolyzable N, available P and K, and soil organic matter were determined following previously published methods [34]. Soil at a depth of 0–20 cm, 20–40 cm, and 60–80 cm from around fertilized hole was sampled, and the NO₃⁻-N contents of the soil samples were measured following Bao [31]. Soil CO₂ emission flux was measured with LI-8100A (LI-COR, Lincoln, NE, USA) in February, April, July, October, and December. Five replications of each treatment were performed.

2.5. Soil Microbe Properties

Rhizospheric soils of citrus were sampled per tree of different treatment, five replicates per treatment, in April, July, and November, respectively. One part of the soil was randomly selected, and urease, sucrase, and acid phosphatase activities were determined using a soil enzyme kit [35] (Solarbio Science & Technology Co., Ltd., Beijing, China). Another part of the soil was cultivated for the number of bacteria, actinomyces, and fungus using soil dilution plating methods [36].

2.6. Statistical Analysis

Data are expressed as mean \pm SD of five replicates per treatment of two consecutive seasons in 2019 and 2020. The SPSS 25.0 software (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses by ANOVA and significance tests. The graphs were plotted

using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). $p \le 0.05$ was considered to indicate statistically significant differences.

3. Results

3.1. *Effect of Different Treatments on Soil Properties and Environment* 3.1.1. Soil Physicochemical Properties

Table 2 presents the effect of CFOF on soil physicochemical properties. In the OF + CF and BF + CF treatments, soil porosity, pH, alkali-hydrolyzable N, available P and K, and soil organic matter were significantly ($p \le 0.05$) higher than that of CF treatments in 2019 and 2020. In addition, soil physicochemical properties of BF + CF were higher than those of OF + CF treatment on the whole, but no significant difference (except available P). These results indicate that CFOF is beneficial to improving soil physicochemical properties, especially when the BF + CF is used.

Table 2. The effect of different treatments on physical and chemical properties.

Year	Treatment	pH Value	Porosity (%)	Organic Matter (g∙kg ⁻¹)	Alkali-Hydrolyzable N (mg∙kg ⁻¹)	Available P (mg∙kg ^{−1})	Available K (mg∙kg ⁻¹)
2019	CK CF OF + CF BF + CF	$\begin{array}{c} 5.01 \pm 0.06 \text{ ab} \\ 4.90 \pm 0.16 \text{ b} \\ 5.11 \pm 0.12 \text{ a} \\ 5.18 \pm 0.13 \text{ a} \end{array}$	$\begin{array}{c} 44.23 \pm 0.50 \text{ b} \\ 44.60 \pm 0.61 \text{ b} \\ 47.82 \pm 1.39 \text{ a} \\ 48.52 \pm 0.59 \text{ a} \end{array}$	$\begin{array}{c} 20.08 \pm 0.41 \text{ b} \\ 21.13 \pm 1.44 \text{ b} \\ 24.20 \pm 1.06 \text{ a} \\ 23.99 \pm 0.72 \text{ a} \end{array}$	$\begin{array}{c} 88.82\pm5.10\ {\rm c}\\ 115.43\pm6.31\ {\rm b}\\ 129.80\pm3.33\ {\rm a}\\ 124.54\pm3.34\ {\rm a} \end{array}$	$\begin{array}{c} 49.45 \pm 2.96 \text{ c} \\ 51.84 \pm 0.72 \text{ c} \\ 69.12 \pm 1.15 \text{ a} \\ 61.97 \pm 4.48 \text{ b} \end{array}$	$\begin{array}{c} 188.50 \pm 1.45 \text{ c} \\ 197.96 \pm 1.63 \text{ b} \\ 218.32 \pm 4.50 \text{ a} \\ 221.15 \pm 5.01 \text{ a} \end{array}$
2020	CK CF OF + CF BF + CF	$\begin{array}{c} 4.97 \pm 0.09 \ \text{b} \\ 4.85 \pm 0.12 \ \text{b} \\ 5.22 \pm 0.10 \ \text{a} \\ 5.20 \pm 0.09 \ \text{a} \end{array}$	$\begin{array}{c} 44.37 \pm 1.40 \text{ b} \\ 44.21 \pm 1.60 \text{ b} \\ 50.95 \pm 1.34 \text{ a} \\ 52.46 \pm 0.73 \text{ a} \end{array}$	$\begin{array}{c} 19.52 \pm 0.81 \text{ c} \\ 20.80 \pm 0.70 \text{ b} \\ 25.36 \pm 0.82 \text{ a} \\ 25.84 \pm 0.43 \text{ a} \end{array}$	$\begin{array}{c} 86.36 \pm 2.96 \text{ c} \\ 123.53 \pm 8.37 \text{ b} \\ 136.20 \pm 3.45 \text{ a} \\ 138.48 \pm 4.58 \text{ a} \end{array}$	$\begin{array}{c} 47.65 \pm 3.50 \text{ c} \\ 54.15 \pm 4.54 \text{ b} \\ 77.33 \pm 4.26 \text{ a} \\ 74.79 \pm 4.86 \text{ a} \end{array}$	$\begin{array}{c} 177.76 \pm 4.71 \text{ c} \\ 196.51 \pm 6.13 \text{ b} \\ 228.44 \pm 7.23 \text{ a} \\ 230.26 \pm 3.69 \text{ a} \end{array}$

Note: CK: no fertilizer; CF: chemical fertilizer; OF + CF: chemical fertilizer combined with organic fertilizer; BF + CF: chemical fertilizer combined with bioorganic fertilizer. Data are mean \pm standard deviation, n = 5. Values in the same row with the same letter(s) are not significantly different per the Duncan analysis at $p \leq 0.05$ between different treatments.

3.1.2. Soil Microbe Properties

Figure 1 shows the effect of CFOF on soil enzyme activity and the number of cultivable microbes. In each case, soil microbe properties were better in July and lower in April and November. In the OF + CF and BF + CF treatments, soil microbe properties were better than the CK and CF treatments on the whole. In particular, in the OF + CF and BF + CF treatments, soil cultivable bacteria and actinomyces were significantly higher than that of CF in July and November. In addition, when comparing soil cultivable fungus measurements to the CF treatment, OF + CF and BF + CF treatments were significantly higher by 48.1% and 12.4% in July, respectively, while they did not differ significantly in November. Measurements were significantly higher than that of CF and BF + CF treatments were significantly higher than that of CF and BF + CF treatments were significantly higher than that of CK and CF in July and November. On the whole, soil microbe properties of BF + CF treatment were better than that of OF + CF. These results showed that CFOF improved soil enzyme activity and the number of cultivable microbes, especially in the BF + CF treatment.

3.1.3. Soil Environment

Figure 2 shows the effect of CFOF on CO₂ emission flux and NO₃⁻-N in soil. As seen in Figure 2a, CO₂ emission flux in OF + CF and BF + CF treatments was higher than that observed under the CK and CF treatments. A rapid growth trend from February to July, with a peak in July, and then and a downward trend were observed. CO₂ emission flux was significantly higher in the OF + CF and BF + CF treatments than in the CF treatment by 57.7% and 60.7%, respectively, in July, and significantly higher in the BF + CF treatment than in the OF + CF and CF treatments by 32.6% and 15.6%, respectively. On the whole, CO₂ emission flux was also higher in the BF + CF treatment than that of OF + CF. As illustrated in Figure 2b, NO₃⁻-N from different soil layers was significantly higher in the CF, OF + CF and BF + CF treatments than that of CK. The 0–20 cm soil layer, when compared between the CF, BF + CF treatments had NO₃⁻-N that was significantly lower by 19.7% in 2019, while the OF + CF and BF + CF treatments were also significantly lower by 21.2% and 25.7%, respectively, in 2020. In the 20–40 cm soil layer, compared with the CF treatment, NO_3^- -N was significantly lower by 16.6% in the BF + CF treatment in 2019, while OF + CF and BF + CF treatments also had significantly lower NO_3^- -N by 21.9% and 25.9%, respectively, in 2020. In the 40–60 cm soil layer, compared with the CF treatment, NO_3^- -N in the OF + CF and BF + CF treatments was also significantly lower by 26.0% and 19.9%, respectively, in 2019, while significantly lower by 33.9% and 46.2%, respectively, in 2020. Additionally, NO_3^- -N in the 40–60 cm soil layer of the CF treatment was larger than that of any other soil layer, while NO_3^- -N in the 20–40 cm soil layer of the OF + CF and BF + CF treatments was larger than that of any other soil layer. Furthermore, NO_3^- -N from each soil layer of the BF + CF treatment was lower than that of those measured from the OF + CF treatment on the whole. Therefore, CFOF is beneficial for slowing down the accumulation and migration of NO_3^- -N in the soil while promoting CO₂ emission flux to some degree, especially in the BF + CF treatment.



Figure 1. The effect of different treatments on microbial properties of soil, including bacteria (a), actinomycetes (b), fungi (c), urease (d), sucrase (e), acid phosphatase (f). CK: no fertilizer; CF: chemical fertilizer; OF + CF: chemical fertilizer combined with organic fertilizer; BF + CF: chemical fertilizer combined with bioorganic fertilizer. The bars were means \pm standard error; the column with different letters indicated significant difference at $p \leq 0.05$.



Figure 2. The effect of different treatments on environments of soil, including carbon flux (**a**), NO₃-N (**b**).CK: no fertilizer; CF: chemical fertilizer; OF + CF: chemical fertilizer combined with organic fertilizer; BF + CF: chemical fertilizer combined with bioorganic fertilizer. The bars were means \pm standard error; the column with different letters indicated significant difference at $p \le 0.05$.

3.2. Effect of Different Treatments on Growth Physiology

3.2.1. Plant Growth

Table 3 presents the effect of CFOF on plant growth index. In comparison with leaf area, thickness of a hundred leaves, dry matter of a hundred leaves of CK and CF, all that of CFOF was promoted in 2019, especially BF + CF significantly. In the OF + CF and BF + CF treatments, twig length was longer than that observed in the CK and CF treatments in 2019. In addition, the plant growth index in 2019 was similar to that of 2020. The plant growth index observed in the BF + CF treatment was higher than that of OF + CF treatment on the whole. These results show that CFOF is beneficial for promoting plant growth, especially the BF + CF treatment.

Year	Treatment	Area of Leaf (cm ²)	Thickness of Hundred Leaves (mm)	Dry Matter of Hundred Leaves (g)	Length of Twigs (cm)
2019	СК	$17.53\pm0.77~\mathrm{c}$	$26.74\pm0.38~\mathrm{c}$	$15.63\pm0.52~\mathrm{c}$	$6.98\pm0.54b$
	CF	$18.59\pm1.06\mathrm{bc}$	27.02 ± 0.35 bc	$17.03\pm0.19~\mathrm{b}$	$7.60\pm0.86~\mathrm{ab}$
	OF + CF	$19.71\pm1.05~\mathrm{ab}$	$27.85\pm0.69\mathrm{b}$	$17.75\pm0.69~\mathrm{ab}$	$7.96\pm0.92~\mathrm{ab}$
	BF + CF	$20.78\pm1.42~\mathrm{a}$	$28.88\pm0.74~\mathrm{a}$	$18.06\pm0.78~\mathrm{a}$	$8.50\pm0.83~\mathrm{a}$
2020	СК	$17.38\pm0.86~\mathrm{c}$	$26.00\pm0.33~\mathrm{c}$	$14.86\pm0.62~\mathrm{c}$	$6.80\pm0.30b$
	CF	$18.76\pm0.33\mathrm{b}$	$27.73\pm0.32\mathrm{b}$	$16.52\pm0.56~\mathrm{b}$	$7.95\pm0.10~\mathrm{a}$
	OF + CF	$20.30\pm0.56~\mathrm{a}$	28.50 ± 0.62 a	17.36 ± 0.35 a	$8.13\pm0.39~\mathrm{a}$
	BF + CF	$20.34\pm0.90~\mathrm{a}$	$28.62\pm0.42~\mathrm{a}$	$17.95\pm0.44~\mathrm{a}$	$8.38\pm0.56~\mathrm{a}$
2020	BF + CF CK CF OF + CF BF + CF	$\begin{array}{c} 20.78 \pm 1.42 \text{ a} \\ 17.38 \pm 0.86 \text{ c} \\ 18.76 \pm 0.33 \text{ b} \\ 20.30 \pm 0.56 \text{ a} \\ 20.34 \pm 0.90 \text{ a} \end{array}$	$28.88 \pm 0.74 \text{ a}$ $26.00 \pm 0.33 \text{ c}$ $27.73 \pm 0.32 \text{ b}$ $28.50 \pm 0.62 \text{ a}$ $28.62 \pm 0.42 \text{ a}$	$\begin{array}{c} 18.06 \pm 0.78 \text{ a} \\ 14.86 \pm 0.62 \text{ c} \\ 16.52 \pm 0.56 \text{ b} \\ 17.36 \pm 0.35 \text{ a} \\ 17.95 \pm 0.44 \text{ a} \end{array}$	$8.50 \pm 0.83 \text{ a}$ $6.80 \pm 0.30 \text{ b}$ $7.95 \pm 0.10 \text{ a}$ $8.13 \pm 0.39 \text{ a}$ $8.38 \pm 0.56 \text{ a}$

Table 3. The effect of different treatments on plant growth.

Note: CK: no fertilizer; CF: chemical fertilizer; OF + CF: chemical fertilizer combined with organic fertilizer; BF + CF: chemical fertilizer combined with bioorganic fertilizer. Data are mean \pm standard deviation, n = 5. Values in the same row with the same letter(s) are not significantly different per the Duncan analysis at $p \leq 0.05$ between different treatments.

3.2.2. Nutrient Elements

Table 4 presents the effect of CFOF on leaf nutrient physiology and twigs. The results showed that N, P, and K contents of leaves and twigs were higher in the OF + CF and BF + CF treatments than that of CK and CF treatments, in general. Moreover, N content of leaves in the OF + CF and BF + CF treatments was significantly higher than that observed in the CK treatment by 4.8% and 5.4%, respectively, in 2019, and by 6.2% and 6.3%, respectively, in 2020. Compared with the CK treatment, leaf K content in the OF + CF and BF + CF

CF

OF + CF

BF + CF

2020

 29.40 ± 0.89 a

 $29.62\pm0.16~\mathrm{a}$

 $29.66\pm0.87~a$

treatments was significantly higher by 12.6% and 16.6%, respectively, in 2019, and by 27.9% and 29.5%, respectively, in 2020. N, P, and K contents of twigs measured under the OF + CF and BF + CF treatments were significantly higher than that observed in the CK treatment in 2019 and 2020. Moreover, leaf K content in the BF + CF treatment was significantly higher than that observed in the CF treatment by 7.3% and 7.4% in 2019 and 2020. Furthermore, the nutrient content of leaves and twigs from the BF + CF treatment was higher than that of the OF + CF treatment; however, no significant difference was observed.

Leave Twigs Year Treatment K (g·kg⁻¹) N (g⋅kg⁻¹) $P(g \cdot kg^{-1})$ K (g⋅kg⁻¹) N (g·kg⁻¹) P (g⋅kg⁻¹) CK $27.95\pm0.66\,b$ $1.34\pm0.16~\text{a}$ $13.10\pm0.51~c$ $11.17\pm0.25\,b$ $0.81\pm0.06~b$ $8.17\pm0.40~\mathrm{a}$ 2019 CF 29.15 ± 0.55 a $1.40\pm0.05~\text{a}$ $14.23\pm0.46\,\mathrm{b}$ $11.70 \pm 0.37 \text{ ab}$ $0.92\pm0.07~\mathrm{a}$ 8.46 ± 0.15 a $1.43\pm0.04~\mathrm{a}$ $0.95\pm0.08~\text{a}$ OF + CF $29.30\pm0.15~a$ 14.75 ± 0.52 ab 11.85 ± 0.40 a $8.49\pm0.32~\text{a}$ BF + CF $29.47\pm0.74~\mathrm{a}$ $1.46\pm0.02~\mathrm{a}$ $15.27\pm0.56~\mathrm{a}$ $11.97\pm0.60~\mathrm{a}$ 0.97 ± 0.04 a $8.63\pm0.37~\mathrm{a}$ CK $27.89 \pm 0.53 \text{ b}$ $1.36\pm0.30~a$ $12.38\pm1.05~c$ $11.22 \pm 0.40 \text{ b}$ $0.78\pm0.06\,b$ $8.20\pm0.73\,b$

 $1.43\pm0.07~a$

 $1.41\pm0.07~\mathrm{a}$

 $1.45\pm0.09~\text{a}$

Table 4. The effect of different treatments on nutrients of leave and twigs.

Note: CK: no fertilizer; CF: chemical fertilizer; OF + CF: chemical fertilizer combined with organic fertilizer; BF + CF: chemical fertilizer combined with bioorganic fertilizer. Data are mean \pm standard deviation, n = 5. Values in the same row with the same letter(s) are not significantly different per the Duncan analysis at $p \leq 0.05$ between different treatments.

 $14.93\pm0.52\,b$

 15.83 ± 0.14 ab

 $16.03\pm0.64~\mathrm{a}$

Table 5 presents the effect of CFOF on nutrient physiology of fruits and roots. The nutrient content of fruits and roots grown under the OF + CF and BF + CF treatments was higher than that observed for the CK and CF treatments in 2019 and 2020. In 2019, compared with the CF treatment, nutrient content of fruits was significantly higher in the OF + CF and BF + CF treatments, while the nutrient content of roots grown under the BF + CF treatment was also significantly higher. In 2020, N and P contents of fruits were significantly higher in the OF + CF and BF + CF treatment of roots grown in the BF + CF treatment were significantly higher in the CF treatment, and P and K contents of roots grown in the BF + CF treatment were significantly higher than that seen in the CF treatment by 16.7% and 8.9%, respectively. In addition, nutrient content of fruits and roots in the BF + CF treatment was higher than that seen in the OF + CF treatment; however, no statistically significant difference was observed. Therefore, CFOF is beneficial in promoting the absorption of nutrients in the citrus organ, especially the BF + CF treatment.

 $12.57\pm0.36~\mathrm{a}$

 12.11 ± 0.13 a

 12.53 ± 0.71 a

 $0.88\pm0.05~\mathrm{a}$

 0.93 ± 0.06 a

 0.95 ± 0.05 a

 $8.98\pm0.23~\mathrm{a}$

 9.01 ± 0.16 a

 $8.92\pm0.18~\mathrm{a}$

Table 5. The effect of different treatments on nutrients of fruits and roots.

Year	Treatment -		Fruits		Roots			
		N (g·kg $^{-1}$)	P (g·kg $^{-1}$)	K (g \cdot kg $^{-1}$)	N (g \cdot kg $^{-1}$)	P (g⋅kg ⁻¹)	K (g \cdot kg $^{-1}$)	
2019	CK CF OF + CF BF + CF	$\begin{array}{c} 19.17 \pm 0.23 \text{ d} \\ 19.70 \pm 0.48 \text{ c} \\ 20.77 \pm 0.14 \text{ b} \\ 21.87 \pm 0.31 \text{ a} \end{array}$	$\begin{array}{c} 1.26 \pm 0.05 \text{ c} \\ 1.37 \pm 0.07 \text{ b} \\ 1.45 \pm 0.02 \text{ a} \\ 1.48 \pm 0.04 \text{ a} \end{array}$	$\begin{array}{c} 11.23 \pm 0.41 \text{ b} \\ 11.71 \pm 0.13 \text{ b} \\ 12.57 \pm 0.34 \text{ a} \\ 12.73 \pm 0.48 \text{ a} \end{array}$	$\begin{array}{c} 10.82\pm 0.11\ \text{b}\\ 11.03\pm 0.44\ \text{b}\\ 11.46\pm 0.46\ \text{ab}\\ 11.83\pm 0.56\ \text{a} \end{array}$	$\begin{array}{c} 0.36 \pm 0.02 \ \mathrm{c} \\ 0.38 \pm 0.03 \ \mathrm{bc} \\ 0.41 \pm 0.01 \ \mathrm{ab} \\ 0.43 \pm 0.04 \ \mathrm{a} \end{array}$	$\begin{array}{c} 8.56 \pm 0.39 \text{ b} \\ 8.62 \pm 0.67 \text{ b} \\ 8.87 \pm 0.39 \text{ b} \\ 9.72 \pm 0.45 \text{ a} \end{array}$	
2020	CK CF OF + CF BF + CF	$\begin{array}{c} 19.23 \pm 0.23 \text{ d} \\ 20.19 \pm 6.19 \text{ c} \\ 20.94 \pm 5.94 \text{ b} \\ 22.03 \pm 1.03 \text{ a} \end{array}$	$\begin{array}{c} 1.29 \pm 0.05 \text{ c} \\ 1.44 \pm 0.05 \text{ b} \\ 1.51 \pm 0.02 \text{ a} \\ 1.53 \pm 0.01 \text{ a} \end{array}$	$\begin{array}{c} 11.20\pm 0.56 \text{ b} \\ 11.91\pm 0.08 \text{ ab} \\ 12.13\pm 0.23 \text{ a} \\ 12.35\pm 0.78 \text{ a} \end{array}$	10.84 ± 1.07 a 11.10 ± 0.91 a 12.14 ± 1.13 a 12.11 ± 0.17 a	$\begin{array}{c} 0.33 \pm 0.03 \ c \\ 0.36 \pm 0.04 \ bc \\ 0.39 \pm 0.02 \ ab \\ 0.42 \pm 0.01 \ a \end{array}$	$\begin{array}{c} 8.46 \pm 0.32 \text{ c} \\ 8.86 \pm 0.45 \text{ bc} \\ 9.21 \pm 0.33 \text{ ab} \\ 9.65 \pm 0.42 \text{ a} \end{array}$	

Note: CK: no fertilizer; CF: chemical fertilizer; OF + CF: chemical fertilizer combined with organic fertilizer; BF + CF: chemical fertilizer combined with bioorganic fertilizer. Data are mean \pm standard deviation, n = 5. Values in the same row with the same letter (s) are not significantly different per the Duncan analysis at $p \le 0.05$ between different treatments.

3.2.3. Photosynthesis

Figure 3 shows the effect of CFOF on photosynthetic physiology. Parameters of photosynthetic physiology in the OF + CF and BF + CF treatments were higher than that seen in the CK and CF treatments. As shown in Figure 3a, leaf SPAD values increase to a peak after rapid leaf growth between April and August and then gradually lower and flatten from August to December. Leaf SPAD values measured during the OF + CF and BF + CF treatments were significantly higher than those in the CF treatment, by 6.2% and 7.0% in April, respectively. In addition, leaf SPAD values were significantly higher for the BF + CF treatment than for the CF and OF + CF treatments, by 3.2% and 2.0%, respectively. As shown in Figure 3b, each Pn treatment showed rapid growth to peak from April to August and then rapidly decreased from August to December. In August and December, Pn of the BF + CF treatment was significantly higher than that of the CF treatment by 31.2% and 70.4%, respectively, while significantly higher than that of the OF + CF treatment by 23.6% and 61.7%, respectively. As shown in Figure 3c, d, the trend of Plabs and Pltotal of treatment was similar to Pn. For the OF + CF and BF + CF treatments, Plabs was significantly higher than that of the CF treatment in August and December, while Pltotal of the OF + CF and BF + CF treatments was significantly higher than that of CF in April and August. In addition, photosynthetic physiology for the BF + CF treatment was higher than that of OF + CF on the whole. Consequently, CFOF is beneficial to improving photosynthetic physiology, especially the BF + CF treatment.



Figure 3. The effect of different treatments on photosynthesis physiology of citrus, including SPAD (**a**), Pn (**b**), Plabs (**c**), Pltotal (**d**). SPAD: relative chlorophyll content; Pn: photosynthetic rate; Plabs: light absorption performance parameters; Pltotal: total index of photosynthetic performance. CK: no fertilizer; CF: chemical fertilizer; OF + CF: chemical fertilizer combined with organic fertilizer; BF + CF: chemical fertilizer combined with bioorganic fertilizer. The bars were means \pm standard error.

3.3. Effect of Different Treatments on Yield of Ponkan

Figure 4 shows the effect of CFOF on yield per tree and the contribution rate of fertilizer on Ponkan. As shown in Figure 4a, compared with CF, yield per tree was significantly higher for the OF + CF and BF + CF treatments by 22.9% and 25.2% in 2019 and by 26.5% and 30.6% in 2020, respectively. As illustrated in Figure 4b, compared with CF, the contribution rate of fertilizer was significantly higher for the OF + CF and BF + CF treatments in 2019 and 2020. In addition, yield per tree and the contribution rate of fertilizer for the OF + CF and BF + CF treatments in 2019 and 2020.

BF + CF treatment were higher than that of OF + CF, but the difference was not statistically significant. These results show that CFOF improved yield per tree and the contribution rate of fertilizer of Ponkan, especially the BF + CF treatment.



Figure 4. The effect of different treatments on yield (**a**) and rate of contribution (**b**) of citrus. CK: no fertilizer; CF: chemical fertilizer; OF + CF: chemical fertilizer combined with organic fertilizer; BF + CF: chemical fertilizer combined with bioorganic fertilizer. The bars were means \pm standard error. The column with different letters indicated significant difference at $p \le 0.05$.

4. Discussion

Due to great importance of chemical fertilizer to citrus, chemical fertilizer has been widely used and even overused in order to maintain high citrus yield. This has led to deterioration of soil properties and ecological environment. However, a combination of chemical and organic fertilizer is not only beneficial in improving the properties and environment of soils, but also promotes crop yield and quality [19–30]. Here, our work confirmed that CFOF was helpful in increasing citrus yield and improving soil properties.

It has previously been reported that the content of soil organic matter, which can strengthen the ability of the soil to maintain and supply fertilizer as well as change the structure of soil aggregates and enhance soil fertility, can effectively be improved by increasing the use of organic fertilizer [37]. Additionally, many studies show that organic fertilizer can improve soil physicochemical properties, such as soil organic matter, total porosity, available N, P, and K of soils [38-40]. Thus, the combined application of organic fertilizer and chemical fertilizer could greatly enhance soil properties. In this study, the soil physicochemical properties of the OF + CF and BF + CF treatments were higher than those of the CK and CF treatments in 2019 and 2020. These results indicate that CFOF raised soil fertility and physicochemical properties and that organic fertilizer could contain several active substances, such as humic acid, amino acid, and microbes, among others, which could promote the formation of soil aggregates, making soil more relaxed and breathable, and strengthening the ability to conserve fertilizer and water to a certain degree. These results are similar to those of previous studies [41-43]. Soil microbe content, which is often used as an indicator to evaluate soil quality, plays an important role in the soil ecosystem, not only by assuming the responsibility of decomposer but also by promoting nutrient absorption by roots. In addition, as a crucial member of the soil ecosystem, soil enzymes play an important role in mineralization and decomposition of organic materials, because they can react sensitively to changes in soil environment and reflect soil fertility changes [44,45]. In this study, the soil microbe properties of the OF + CF and BF + CF treatments were higher than those observed in the CK and CF treatments. These findings indicate that CFOF improved the soil microbial community structure in a manner that was relative to the organic matter, humic acid, and amino acids and which could increase soil microbial activity. These results are similar to the research of previous study [46,47]. Moreover, the study showed that soil microbe properties were the largest in July and were related to local temperature and humidity [48].

To some extent, different measures of fertilization application can affect the orchard ecological environment. Zhou et al. [49] and Lv et al. [10] report that long-term and short-term application of organic fertilizer combined with synthetic fertilizer can increase

 N_2O (greenhouse gases) emission. In this study, the CO_2 (greenhouse gases) soil carbon flux seen in the OF + CF and BF + CF treatments was higher than that of CK and CF, leading to the possibility that soil biological properties can be improved. In addition, CO₂ carbon flux for each treatment was largest in July and lowest in December. This could be due to soil microbial activity; the findings were similar to previous studies [50–52]. The long-term, large amount of chemical fertilizer especially nitrogen fertilizer is a main cause of soil N leaching [53]. Nitrogen fertilizer is decomposed by microbes into $NO_3^{-}-N$ and $NO_2^{-}N$, and $NO_3^{-}N$ is soluble in water and can easily leach, often causing groundwater pollution and endangering human and animal health because of its negative charge and strong mobility in solution [54]. According to Liao et al. [55], chemical fertilizer application decreased the diversity of the diazotrophic community, while chemical fertilizer combined with organic manure improved not only the diversity of the diazotrophic community but also their abundance and nitrogen fixation rate. It has also been reported that chemical fertilizer reduction combined with organic fertilizer application is considered an effective measure to reduce the risk of N leaching in farmland [56,57]. In this study, $NO_3^{-}-N$ from the 0-60 cm soil layer of the OF + CF and BF + CF treatments was lower than that of CF, while NO_3^{-} -N from the 40–60 cm soil layer of the OF + CF and BF + CF treatment was lower than that of the 20–40 cm soil layer. This illustrates that chemical fertilizer combined with organic fertilizer can alleviate the risk of N leaching and N migration to deep soil, because organic fertilizer contains organic carbon and humic acid and other relative matter, which adsorbs shallow soil NO₃⁻-N and inhibits its downward migration [58]. These results are consistent with previous studies [59,60].

Nutrition is a key factor affecting quantity and metabolites in plant growth. Citrus is a green plant in all seasons and germinates branches many times a year; consequently, it consumes a several nutrients [61]. Many studies have shown that the use of organic matter like manure, compost, and straw can improve and increase the nutrients of crop organs [62–64]. Thus, combined application of organic matter and chemical fertilizer could promote the nutrients of the crop. In this study, N, P and K found in the branches, roots, and fruits of trees treated with OF + CF and BF + CF were higher than that of CK and CF. These findings indicate that CFOF facilitates absorption and distribution of nutrients in citrus. This may be due to organic fertilizer improving soil physicochemical properties and enhancing the ability of absorption and transportation of nutrients by roots, as previously suggested [65,66]. Ample nutrition plays an indispensable role in plant growth. In this study, the index of plant growth from the OF + CF and BF + CF treatments was better than that of CK and CF, and it was relative to nutrient balance. These results are similar to previous studies [28,67]. Photosynthesis is a key physiological activity of plants and plays an important role in growth and development. It has been reported that more than 90% of crop biomass is derived from photosynthesis [68]. Chlorophyll fluoresce is an ideal parameter for studying the physiological condition of plant photosynthesis in multiple settings, because it reflects various aspects of photosynthesis, such as light energy absorption, transmission, and photoreaction. In addition, PI of the chlorophyll fluorescence parameter can reflect the optical system performance index of a whole leaf, and Plabs and Pltotal comprehensively reflect the light absorption and photosynthetic performance of a leaf [69,70]. In this study, SPAD, Pn, Plabs, and Pltotal measurements in the OF + CF and BF + CF treatments were higher than those of CK and CF. These results indicate that CFOF greatly improved leaf photosynthesis. The promotion of nutrient absorption, leaf growth, and the enzyme activity of leaf-related physiological metabolism could bring about improvement in leaf photosynthesis [50,71]. These findings are similar to previous studies [26,72,73]. In addition, the results showed that photosynthetic physiology was best in August, which may be due to leaf growth, light intensity, and physiological metabolism [74,75].

As discussed above, CFOF could increase soil microbial activity, improve soil's physical and chemical properties, enhance nutrient availability in citrus tree organs, and promote citrus growth and photosynthesis. Yield is also an important parameter to measure because of its economic benefits, which are determined by many factors, such as the condition of growth and photosynthesis. Previous studies have shown that yield can be maintained or even significantly increased relative to application of synthetic fertilizer alone [4,15,21,28]. In this study, yield per citrus tree and the contribution rate of fertilizer in the OF + CF and BF + CF treatments were significantly higher than that of CF. This illustrates that CFOF is beneficial for boosting citrus yield. This result might be explained by the fact that chemical fertilizer combined with organic fertilizer could improve soil fertility and enhance the ability of photosynthesis and growth with the above research findings. These results were consistent with previous studies [76–78]. In addition, nutrient availability of citrus organs, photosynthesis, soil properties, and orchard environment parameters as measured in the BF + CF treatment were better than that of OF + CF on the whole, perhaps because bioorganic fertilizer contains abundant active substances, such as microorganisms, amino acids, and humic acids, which could benefit root growth, water and nutrient absorption, and photosynthesis. These results are similar to previous studies [79–81]. Consequently, CFOF is beneficial to promoting citrus physiology, improving soil characteristics, and increasing orchard yield, which could meet the requirements of green ecological development.

5. Conclusions

The results of this study show that CFOF is beneficial to improving the physicochemical and microbe properties of soil, promoting nutrient content of citrus plants, enhancing photosynthesis and growth of citrus, and thus promoting orchard yield. In addition, CFOF notably alleviates NO_3^- -N leaching and NO_3^- -N migration to deep soil. In summary, CFOF can obtain high yields and ensure good citrus orchard and tree conditions and ecological environment, especially when the BF + CF treatment is used. The application of N, P₂O₅, K₂O fertilizer and bioorganic fertilizer of BF + CF treatment is 0.508, 0.320, 0.310 and 10 kg/plant, respectively.

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