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The Influence of Effective Microorganisms on Microbes and Nutrients in Kiwifruit Planting Soil

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Abstract: To understand the effects of effective microorganisms (EMs) containing multiple strains on microbes and nutrients in kiwifruit planting soil, EMs prepared with four different strains were added to kiwifruit planting soil monthly from April to August. The counts of bacteria, fungi, actinomycetes, and total microbes were determined. The pH, total nitrogen (TN), alkali-hydrolyzable nitrogen (A-N), organic matter (OM), available potassium (A-K), and available phosphorus (A-P) of the soil were measured. Results indicated that the counts of bacteria, fungi, actinomycetes, and total microbes reached 60.33×10^5, 4.00×10^5, 0.92×10^5, and 65.25×10^5 CFU/g, respectively, in August, all of which were higher than those of the control group (CK). The bacterial count of the experimental group (EG) was higher than that of the CK in August. The pH-values of the EG were always lower than those of the CK. In August, the TN content of the EG was 1.52 g/kg, which was higher than that of the CK (1.35 g/kg). A significant negative association between the actinomycetes count and TN ($p < 0.05$) was found. For A-N and OM, the content of the EG (A-N, 125.18 mg/kg; OM, 49.84 mg/kg) was roughly the same as that of the CK (A-N, 112.51 mg/kg; OM, 53.11 mg/kg) in August. However, the A-K and A-P contents of the EG (A-K, 145.25 mg/kg; A-P, 111.25 mg/kg) were lower than those of the CK (A-K, 182.52 mg/kg; A-P, 202.19 mg/kg) in August. Results show that application of EMs in kiwifruit planting soil can increase the counts of soil microbes and might promote the absorption of major nutrients for kiwifruit tree.

Keywords: effective microorganisms; kiwifruit; planting soil; nutrients

1. Introduction

Kiwifruit (*Actinidia deliciosa*) is regarded as a healthy fruit and is becoming more and more popular around the world [1,2]. Kiwifruit is delicious and abundant in vitamin C, catechins, and polyphenolic acids [3–5]. Furthermore, the unique composition of kiwifruit can decrease the risk of cardiovascular disease [6,7]. In China, planting kiwifruit has become a major agricultural economic industry in the Sichuan, Shanxi, Zhejiang, and Jiangxi provinces. Accompanied by the increasing demand of

kiwifruit in recent years, chemical fertilizers were excessively used to increase the yield of kiwifruit. However, excessive use of chemical fertilizer can cause serious environmental problems [8].

To achieve sustainable agricultural development, effective microorganisms (EMs) are widely used in agricultural planting. Chen *et al.* (2007) found that phosphobacterium 9320-SD can enhance soil fertility and promote plant growth [9]. Estiken *et al.* (2010) found that the P, Fe, Zn, K, and Mg availability of strawberry planting soil can be promoted after inoculating *Bacillus* M3 [10]. Yu *et al.* (2014) found that *B. subtilis* BS-15 can increase the overall activity of chestnut soil microbes, maintain soil health, and increase soil fertility [11]. Although the utilization of EMs achieved good effects on planting soil of some crops in previous studies, other initial studies have indicated the limited effect of EMs because of its single component [12]. In view of this, a large member of researchers focused on the application of EMs containing multiple strains to enhance the soil fertility for the planting of various types of crops in recent years [13–17].

However, little is known of the effect of EMs containing multiple strains on the improvement of soil nutrients in kiwifruit planting. Therefore, the objective of the study is to determine the influence of EMs containing four different strains on the microbes and nutrients in kiwifruit planting soil.

2. Material and Methods

2.1. Experimental Area

The experimental area is located in a kiwifruit plantation (31°44′54″–31°02′9″ N, 103°25′42″–103°47′0″ E) in Dujiangyan City, Sichuan, China. The area has a subtropical humid climate, and the average annual rainfall is 1243.80 mm. The altitude of the area is 670 m. In this study, an 80-m^2 plot in the kiwifruit plantation was selected as the experimental area; 15 kiwifruit plants were included in the experimental area. The 15 kiwifruit trees were labeled as No. 1–15, respectively. No. 1–3 were used as control group.

2.2. Strains and EMs Preparation

The EMs included *B. subtilis*, *B. stearothermophilus*, *B. amyloliquefaciens*, and *Actinobacteria* sp. The *Bacillus* spp. strains were grown at 35 °C in 10 mL of beef extract peptone medium (Hangzhou Microbiology Reagent Co., Ltd., Hangzhou, China) in 100-mL flasks for 16–20 h with vigorous shaking. Next, 5% (*v*/*v*) cells were inoculated into 250 mL of the beef extract peptone medium. Cells were grown at 35 °C for 18–24 h and shaken at 180 rpm to obtain an initial population level of 10^8–10^9 CFU/mL. *Actinobacteria* sp. was grown at 28 °C in 10 mL of actinomycetes culture medium (Hangzhou Microbiology Reagent Co., Ltd.) in 100-mL flasks for 72 h with vigorous shaking. Subsequently, 5% (*v*/*v*) cells were inoculated into 250 mL of the actinomycetes culture medium. Cells were grown at 28 °C for 72 h and shaken at 180 rpm. The level of *B. subtilis*, *B. stearothermophilus*, *B. amyloliquefaciens*, and *Actinobacteria* sp. cultivation reached approximately ~10^8 CFU/mL, respectively. The EMs were prepared by mixing *B. subtilis*, *B. stearothermophilus*, *B. amyloliquefaciens*, and *Actinobacteria* sp. at the ratio of 1:1:1:1.

2.3. Fertilization

The initial level of microbes in the used EMs was approximately 10^6–10^7 CFU/mL. The roots of each plant in the experiment group (EG) were treated with 4 L of the used EMs every month from April to August; 4 L of the diluted medium without microbes was used for the control group (CK). The maintenance and management of kiwifruit trees was performed according to the management standard established by the Sichuan province (Manual for the Production of Green Food "Hongyang" Kiwifruit, DB510824/T 1–2009).

2.4. Collection of Soil Samples

The soil samples were collected in each month (April, May, June, July, and August) before fertilization. The 500-g soil samples from each plant's soil lower layer (approximately 20 cm deep) were collected in sealing sterile plastic bag [18].

2.5. Determination of Soil Microbe Counts

The soil microbe counts were determined by the serial dilution plate count method [19]. Briefly, 10 g of each sample was serially diluted tenfold in a sterile saline solution (0.85% NaCl); 100 µL of each dilution was plated on nutrient agar plates to determine the bacterial count. The plates were incubated at 35 °C overnight. The fungi were cultured in the potato dextrose agar medium, and the actinomycetes were cultured in the actinomycetes culture medium; both cultures were incubated for 72 h at 28 °C. The counts of bacteria, fungi, actinomycetes, and total microbes were recorded as colony-forming units (CFU/g).

2.6. Determination of Soil Nutrients

For soil nutrients, total nitrogen (TN), alkali-hydrolyzable nitrogen (A-N), available phosphorus (A-P), and available potassium (A-K) were measured with Kjeldahl's method, the alkaline hydrolysis diffusion method, the molybdenum-blue method, and the ammonium acetate–flame photometer method, respectively [20]. Organic matter (OM) was measured using Multi N/C 3100 TOC analyzer with HT 1300 Solids module (Analytik Jena AG Multi N/C 3100, Jena, Germany) [21]. Soil pH was measured in a 2.5:1 soil-water suspension using a digital pH meter (Shanghai Rex Instrument Factory PHB-4, Shanghai, China) [22].

3. Results

3.1. Effects of EMs on Microbe Counts

The counts of bacteria, fungi, actinomycetes, and total microbes are shown in Figure 1. The bacterial counts of the EG were similar to those of the CK from April to July (Figure 1a). However, the count of the EG sharply increased in August (60.33×10^5 CFU/g) and was higher than that of the CK (12.00×10^5 CFU/g). For the actinomycetes (Figure 1b), the count of the EG every month was also higher than that of the CK. However, the actinomycetes count of the EG declined after June and reached a minimum value of 0.92×10^5 CFU/g in August. Likewise, the fungal count of the EG was higher than that of the CK for each month (Figure 1c). The fungal count of the EG reached a maximum value of 4.00×10^5 CFU/g in August. For the total microbes (Figure 1d), the count of the EG in August (65.25×10^5 CFU/g) was higher than that of the CK (15.15×10^5 CFU/g).

3.2. Effects of EMs on pH and Soil Nutrients

The pH-value of the EG was lower than that of the CK for each month (Figure 2a). The pH of the EG dropped between May and July, but finally increased in August. The maximum value was 6.98 in May; the minimum value was 6.44 in July. The pH of the CK ranged from 6.87 to 7.22 between April and August.

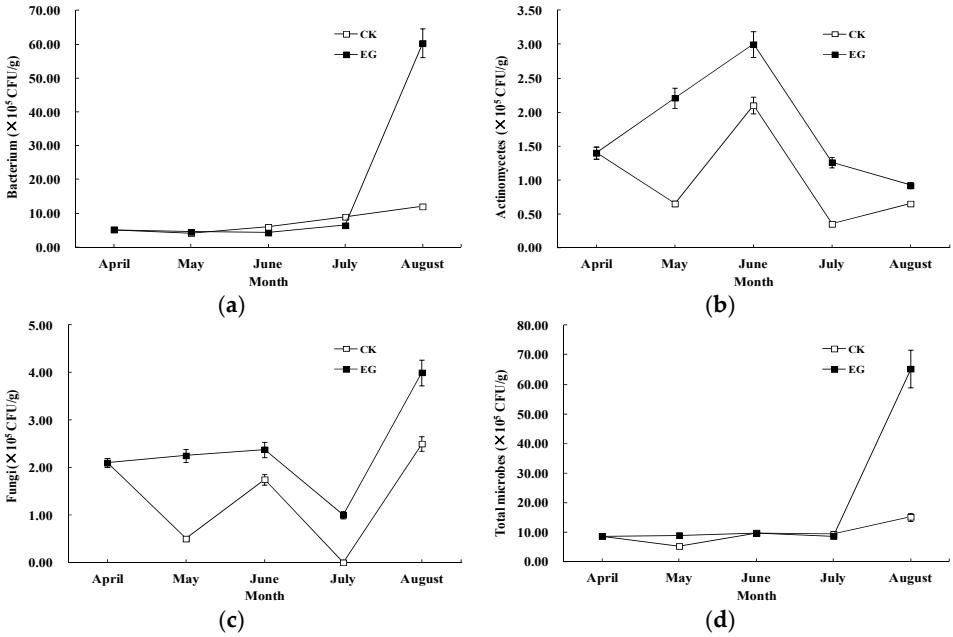

Figure 1. Changes of microbial counts in kiwifruit planting soil: (**a**) bacterium; (**b**) actinomycetes; (**c**) fungi; and (**d**) total microbes.

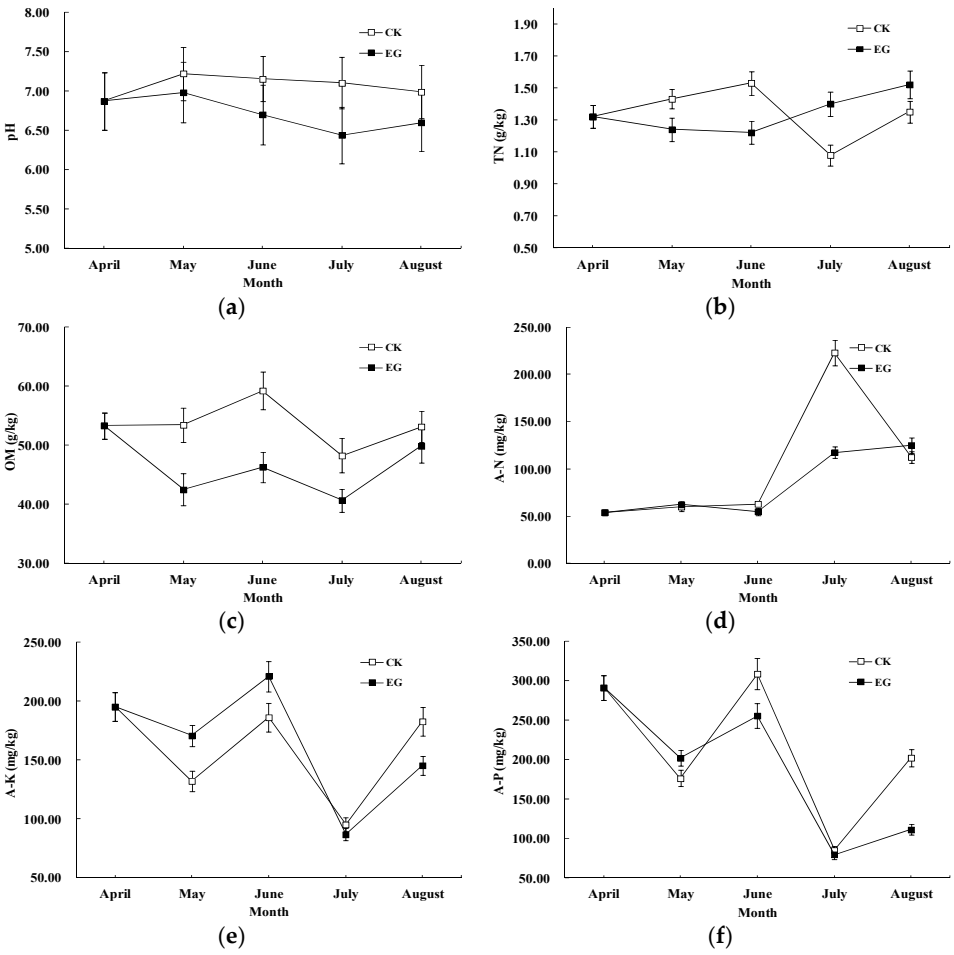

Figure 2. Changes of pH and major nutrients in kiwifruit planting soil: (**a**) pH; (**b**) TN; (**c**) OM; (**d**) A-N; (**e**) A-K; (**f**) A-P.

As shown in Figure 2b, TN was extremely different between the EG and the CK. From April to June, the TN-values of the EG dropped but gradually increased to 1.52 g/kg in August. The OM of the EG was similar with that of the CK (Figure 2c). The maximum values were 59.20 and 53.30 g/kg, and the minimum values were 48.22 and 40.68 g/kg for the CK and the EG, respectively. Figure 2d indicates that the A-N of the EG increased during the whole experimental period and reached 125.18 mg/kg in August. The A-N of the CK followed an increasing trend until July and exceeded that of the EG at 105.50 mg/kg in July, but sharply decreased in August. The value of the EG was higher than that of the CK in August. For A-K (Figure 2e), the change of the EG was consistent with that of the CK. In the EG, the maximum value was 221.00 mg/kg, and the minimum value was 86.73 mg/kg. In the CK, the maximum value was 195.00 mg/kg, and the minimum value was 95.16 mg/kg. The value of A-P is shown in Figure 2f. In the EG, the trend of A-P was similar to that of the CK. The maximum value of the CK was 308.50 mg/kg, and the minimum value was 85.18 mg/kg. The maximum value of the EG was 291.00 mg/kg, and the minimum value was 79.80 mg/kg.

3.3. Association between Microbes and Soil Nutrients

The association between bacteria, actinomycetes, fungi, and major soil nutrients in the EG is shown in Table 1. The absolute values of correlation coefficient (r) of A-N, OM, A-K, and A-P with bacterium were less than 0.70, thereby indicating the low degree of linear correlation. For TN, $r > 0.80$ with bacterium, but $p > 0.05$. Therefore, no significant association was found between TN, A-N, OM, A-K, A-P, and the bacterial count ($p > 0.05$). For actinomycetes, the absolute values (r) of OM, A-K, and A-P were also less than 0.70, thereby indicating their weak linear correlation with actinomycetes. Nevertheless, the absolute value (r) of TN and A-N was 0.887 and 0.724, respectively, but only TN had $p < 0.05$. Therefore, no significant association between A-N, OM, A-K, A-P, or the actinomycetes count ($p > 0.05$) was observed, although actinomycetes had a negative significant association with TN ($p < 0.05$). For fungi, the absolute values (r) of all five indexes were less than 0.70 with $p > 0.05$. Therefore, fungi had no significant associations with TN, A-N, OM, A-K, and A-P.

Table 1. Correlation analysis of bacteria, actinomycetes, and fungi between major soil nutrients.

Microbe (y)	Soil Nutrients (x)	Correlation Equation	r	p
Bacteria	TN	$y = 0.004x + 1.272$	0.835	0.078
	A-N	$y = 0.988x + 66.93$	0.690	0.197
	OM	$y = 0.073x + 45.33$	0.349	0.565
	A-K	$y = -0.477x + 171.3$	−0.230	0.710
	A-P	$y = -1.812x + 217.1$	−0.494	0.398
Actinomycetes	TN	$y = -0.130x + 1.568$	−0.887 *	0.045
	A-N	$y = -30.41x + 136.3$	−0.724	0.166
	OM	$y = -1.570x + 49.27$	−0.253	0.682
	A-K	$y = 41.18x + 91.25$	0.676	0.210
	A-P	$y = 60.62x + 81.30$	0.562	0.325
Fungi	TN	$y = 0.049x + 1.224$	0.429	0.471
	A-N	$y = 6.794x + 66.93$	0.206	0.740
	OM	$y = 2.612x + 40.38$	0.542	0.346
	A-K	$y = 14.42x + 129.8$	0.302	0.622
	A-P	$y = -1.234x + 190.7$	−0.015	0.981

r: correlation coefficient; * $p < 0.05$, significant correlation.

4. Discussion

Interestingly, the counts of bacteria and actinomycetes of the EG (Figure 1a,b) were higher than those of the CK because the used EMs contain bacteria and actinomycetes. Likewise, the fungal counts of the EG (Figure 1c) were also higher than those of the CK, which is probably attributed to the decreased soil pH (Figure 2a). Fungi prefer to live in slightly acid soil environments [23]. In general,

the application of the EMs can eventually increase the counts of bacteria, actinomycetes, and fungi in kiwifruit soil. Based on the observed counts of bacteria, fungi, and actinomycetes, the percentages of all three microbes were calculated (see Table 2). Bacteria is one of major members of soil microbes and plays an important role in promoting organic matter decomposition, accelerating mineral nutrition cycle, maintaining and improving soil fertility [24,25]. As shown in Table 2, the proportion of bacteria was always the highest in the EG and the CK for each month, which indicate that the application of EMs still maintains an important position of bacteria in the kiwifruit planting soil. In addition, as compared with the CK, the proportions of bacteria in the EG were reduced 28.24%, 16.90%, and 22.29% from May to July, respectively. However, the proportions of fungi and actinomycetes in the EG increased 15.54%, 6.70%, and 11.53% and 12.70%, 10.20%, and 10.76% from May to July, respectively. The results show that the application of the EMs can change the biological diversity of the kiwifruit planting soil ecosystem.

Table 2. Percentage of bacteria, fungi, and actinomycetes counts in kiwifruit planting soil.

Month	Bacteria (%)	Fungi (%)	Actinomycetes (%)
April	59.76	24.17	16.07
May (CK)	78.24	9.46	12.30
May (EG)	50.00	25.00	25.00
June (CK)	61.00	18.00	21.00
June (EG)	44.10	24.70	31.20
July (CK)	96.24	0.00	3.76
July (EG)	73.95	11.53	14.52
August (CK)	79.21	16.50	4.29
August (EG)	92.46	6.13	1.41

CK: control group; EG: experimental group.

In this study, the soil of the EG appeared to have slight pH variation (pH ranged from 6.44 to 6.98; Figure 2a), which indicated that the microbe treatments of planting soil can decrease the soil pH. The decreased pH may be explained by the production of organic acids, as reported in previous studies [26,27]. However, the extent of the pH reduction of the EG was not obvious, and the pH-values of the EG were still near neutrality.

The addition of EMs can promote the activity of urease and invertase in planting soil, thereby increasing the decomposition of OM and the release of available soil nutrients [28]. Simultaneously, the fruit expanding stage for kiwifruit occurs from April to June in the study area. In this stage, the demand of nitrogen is relatively larger than that of other nutrient elements. Thus, the TN and OM contents of the EG decreased from April to June (Figure 2b,c, respectively). In addition, the A-N content of the EG was slightly lower than that of the CK from April to June. *B. subtilis* can promote planting upon the absorption of nitrogen and lead to the increase of nitrogen content in plants [29–31]. This result implies that more A-N might be absorbed by the kiwifruit tree after the EMs are added to the planting soil. According to the results, it can be deduced that the EMs can improve the A-N utilization rate in kiwifruit planting soil. The fruit maturity stage for kiwifruit occurs in July and August in the study area. In this stage, more various organic substances are needed to support the growth of kiwifruit. The demand of nitrogen relatively decreases in this stage. Thus, the A-N contents of the EG and the CK increased in July and August (Figure 2d). However, the A-N content of the EG was still obviously lower than that of the CK in July. The result may also be related to the promoting effect of A-N utilization of the EMs. In addition, the previous study reported that the overabundance of soil water content caused by rainfall could have decreased the A-N content [32]. In the experimental period, the rainy season occurred in July; thus, rainwater may also have washed away much A-N in the soil of the EG.

In the study, the A-K-values of the EG were higher than those of the CK during May to June (Figure 2e), which is mainly caused by the function of potassium-dissolving through *Bacillus* spp. [33].

For A-P, the content of the EG was higher than that of the CK in May (Figure 2f). This is perhaps a result of the function of the phosphate solubilizing of *Bacillus* spp., some of which are able to solubilize P through acidification or chelation, or enzyme action [33–39]. In Figure 2e,f, the A-P and A-K content followed the same variation trend in both the EG and the CK; the A-P and A-K contents reached their maximum in June, whereas the minimum A-P and A-K contents appeared in July. As discussed earlier, nitrogen is the highest element demanded to expand kiwifruit from April to June. The demands of potassium and phosphorus are not the highest in this stage. Therefore, the A-P and A-K contents improved with the passage of time from April to June in the EG and the CK. On the contrary, July and August are the main period of transformation of OM and formation of the quality of kiwifruit. In this period, the potassium and phosphorus demands increase. This led to the decrease of A-K and A-P in July and August in both the EG and the CK. Moreover, the A-K and A-P contents of the EG were lower than those of the CK in July and August. *B. subtilis* can promote planting upon absorption of potassium and phosphorus [29–31], and *B. amyloliquefaciens* can promote planting upon absorption of phosphorus [38,39]. Thus, the results show that the EMs applied in the study might promote the absorption of potassium and phosphorus for kiwifruit planting.

Our study found that the count of actinomycetes had significant negative correlation with TN content ($p < 0.05$, $r = -0.887$) (Table 1). Therefore, the TN content increased with the decrease in actinomycetes count. This result may be attributed to the TN absorption by actinomycetes. In the future, research should be concentrated on the specific causal mechanism of actinomycetes on TN in soil, as well as concerned about the ratio of actinomycetes in EMs, to retain or improve the content of TN.

5. Conclusions

In this study, the EMs containing *B. subtilis*, *B. stearothermophilus*, *B. amyloliquefaciens*, and *Actinobacteria* sp. were added monthly to kiwifruit planting soil from April to August to investigate the effects of EMs on microbes and nutrients in kiwifruit planting soil. Results show that application of EMs can eventually increase the counts of bacteria, actinomycetes, and fungi in kiwifruit planting soil as well as change the biological diversity of the kiwifruit planting soil ecosystem. Adding EMs to kiwifruit planting soil can reduce soil pH, but the reduction is not obvious, and the pH-values of the EG were still almost neutral. The applied EMs in this study might promote the absorption of major nutrients for kiwifruit tree.

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