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Microbiome Reveals the Effects of Biogas Fertilizer on Soil Microbial Community Structure and Diversity in Perennial Apple Orchards

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Abstract: Fruit trees are perennial crops that grow in one place for their whole lives, which leads to the deterioration of the soil as well as a decline in fruit quality and yield. Microbial diversity and community structure are important soil factors affecting stress resistance and the quality of fruit trees. Additionally, biogas fertilizer also plays an important role in improving fruit quality. Whether biogas fertilizer can improve continuous cropping barriers by affecting microbial diversity and community structure remains to be further investigated. Therefore, 7-year-old Fuji apples were used as material, and biogas fertilizer was applied continuously for three years. The results show that the contents of soil organic matter (SOM), available nitrogen (AN), available phosphorus (AP), and soil porosity (SP) increased by 0.712, 0.217, 1.089, and 0.401 after applying biogas fertilizer, respectively. The concentrations of vitamin C, titratable acid, and soluble solids also significantly increased. We also found that the relative abundance of dominant soil flora significantly increased, such as *Sphingomonas* (*g_Sphingomonas*), *Chlamydomonas* (*g_Chlamydomonas*), and *Stachybotry* (*g_Stachybotry*), while the relative abundance of inferior flora significantly decreased, such as *Cryptococcus* (*g_Cryptococcus*) and *Alternaria* (*g_Alternaria*). In summary, biogas fertilizer can improve the physicochemical properties of the soil as well as the structure and diversity of the microbial communities in rainfed orchards, resulting in higher fruit quality.

Keywords: microbial diversity; microbial community structure; biogas fertilizer; soil physicochemical properties

Citation: Han, C.; Zhang, Z.; Gao, Y.; Wang, W.; Chen, J.; Wang, Y. Microbiome Reveals the Effects of Biogas Fertilizer on Soil Microbial Community Structure and Diversity in Perennial Apple Orchards. *Horticulturae* **2023**, *9*, 1023. https://doi.org/10.3390/horticulturae9091023

Academic Editor: Elena Baldi

Received: 1 August 2023
Revised: 1 September 2023
Accepted: 1 September 2023
Published: 10 September 2023

1. Introduction

Continuous monoculture, known as replanting disease or continuous cropping obstacles, can inhibit plant growth and exacerbate soil-borne diseases [1]. It is reported that crop replanting disorder is a common agricultural problem that can affect the yield and quality of plants [2]. Fruit trees are perennial plants, so monoculture is a huge problem they are facing at present. It was found that the growth and development of apples were affected [3] and the yield significantly decreased during continuous monoculture [4]. Therefore, studying the mechanism of continuous cropping and how to solve the problem of apple monoculture is the focus of current research.

Soil is a complex and dynamic ecosystem, which is estimated to contain billions of microorganisms [5]. As a key driver of soil biochemical processes, soil microorganisms play an important role in maintaining the stability and ecological functions of terrestrial ecosystems [6]. When a crop is monoculture, it produces different microbial communities that tend to have different effects on the host plant. For example, *Dickeya* (*Enterobacteriaceae*) is a well-known pathogen with a broad host range and is widespread in monocultures [7]. A wide range of plant hosts worldwide are affected by members of the genus Dickeya, particularly *Musa nana Lour*, *Chrysanthemum spp*, *Dianthus spp*, *Zea mays L*, *Solanum tuberosum L*, and

Solanum lycopersicum L, which can survive in soil for more than 12 months and adversely affect plants [8]. It has been found that many microorganisms belonging to the genera *Bacillus* (*g_Bacillus*), *Burkholderia* (*g_Burkholderia*), *Pseudomonas* (*g_Pseudomonas*), and *Penicillium* (*g_Penicillium*) have the ability to solubilize phosphorus [9]. These root-associated microorganisms can increase the availability of phosphorus in the soil by releasing phosphate from the soil through the secretion of organic acids or phosphatases [10] The dominant genus *Variovorax* (*g_Variovorax*) can improve replanting barriers by controlling plant hormone levels to balance the influence of microbial communities on root growth [11]. Therefore, it is essential to study microbial community structures for plant growth status.

Biogas is an important part of renewable energy [12], and it is increasingly used as fertilizer produced via anaerobic fermentation [13]. It has been widely reported that biogas fertilizer has positive effects on plant yield and soil properties [14]. The purpose of biogas fertilizer is not only to improve soil fertility and achieve sustainable agriculture [15] but also to provide readily available carbon compounds and nutrients, thereby reducing nutrient losses due to leaching or gas loss [16]. In addition, it can also increase the stability of soil and reduce the content of dispersive clay in soil [17]. A large number of studies have reported strong positive relationships between biogas fertilizer application and plant growth. At the same time, subsurface carbon input from root secretions and root turnover is also increased, thus stimulating rhizosphere fungi and bacteria [18,19]. On the other hand, biogas slurries contain less organic C due to anaerobic fermentation, which may have a negative effect on the soil's organic matter content [20]. There are few studies on the effects of biogas fertilizer application on microbial diversity and structure. Therefore, it is crucial to study the effects of these organic additives on microbial diversity.

We hypothesized that the application of biogas fertilizer might affect soil microbial diversity and community structure by improving soil physicochemical properties, thereby alleviating soil succession barriers, promoting fruit tree growth, and improving fruit quality. Therefore, this paper conducted a study on the effect of biogas fertilizer on the soil microbial community structure of apple orchards in dry rainfed areas.

2. Materials and Methods

2.1. Site Description and Sample Collection

This experiment was conducted (2019–2021) in a 10-year-old 'Huimin Short Branch Fuji' orchard in Zhuhe Village, Zhudian Town, Zhuanglang County, Pingliang City, Gansu Province (35°8′33″ N, 105°58′13 E), where the weather conditions are characterized by an average annual rainfall of 510 mm, an average annual temperature of 8.1 °C, a frost-free period of 159 days, and an average annual sunshine time of 2179 h [21]. The soil texture of the sampled apple orchard was loamy, with a row spacing of 3.5 m × 4.5 m at a planting density of 635 plants/hm^2. The fruit type is a short-branched Fuji apple (rootstock of Malus robustaRehd, tree shape of the delayed happy type). Twelve apple trees with similar growth and no pests or diseases were selected for treatment, and the experiment was set up with two treatments, no biogas application (CK) and biogas application (T), with six replications for each treatment. The application time of biogas was based on the water and fertilizer demand characteristics of different growth stages in the annual growth cycle of fruit trees and the distribution pattern of local rainfall, and it was scheduled to be applied once during the fruiting period of the fruit trees and once after harvesting. The production of the fertilizers was based on pig manure and crop residues, and the amount of fertilizer produced was 60 kg per plant (with a 4-fold dilution of biogas). Starting in 2019, three radial fertilization trenches, 25 cm wide × 100 cm long, were opened outward at the midpoint of the canopy projection radius with the trunk as the center, and the biogas slurry was uniformly injected as a continuous supply for three years. Soil was sampled at 0–0.2 m at six points during both treatments and sent to Shanghai Ouyi Biological for sequencing. Each soil sample was divided into two subsamples: One was brought to the laboratory on dry ice and stored at −80 °C for downstream applications (DNA extraction).

The remainder of the sample was air-dried to determine the soil characteristics (organic matter, pH, available nitrogen, available phosphorus, and available potassium).

2.2. Determination of Soil Physical and Chemical Properties and Fruit Quality

Soil pH was measured using a soil pH meter (FE28, Mettler Toledo, Zurich, Switzerland) with a soil–water ratio of 1:2.5 (*w*/*v*). Soil organic matter (SOM) was determined using the $K_2Cr_2O_7$ redox titration method. Available phosphorus was extracted with a 0.5 mol/L $NaHCO_3$ solution (the Olsen method). Available potassium was extracted using the flame photometric method via digestion with NH_4OAC. Available nitrogen (AN) was determined using the alkali-hydrolyzed diffusion method. Soil bulk density was measured using the ring knife method [22].

Fruit weight was measured with a 1% balance. The longitudinal diameter and transverse diameter of fruits were measured with a digital display vernier caliper, and the fruit shape index was obtained as = longitudinal diameter/transverse diameter. The hardness was measured with a GY-1 hardness tester. Soluble solids were determined with a PAL-1 digital sugar meter. The concentration of vitamin C was determined using molybdenum blue colorimetry [23]. The content of soluble sugar was determined using the anthrone reagent method [24]. The titratable acidity content was determined using standard NaOH solution titration [25].

2.3. DNA Extraction

Soil DNA was extracted using a DNeasy PowerSoil Kit (QIAGEN, Dusseldorf, Germany) following the manufacturer's instructions. The purity and quality of the genomic DNA samples were checked using 0.8% agarose gel electrophoresis, and the concentrations were measured using a NanoDrop 2000 (Thermo Fisher, Waltham, MA, USA).

2.4. PCR Amplification and High-Throughput Sequencing Using Illumina Miseq

The diluted DNA was used as a template for the PCR amplification of bacterial 16S rRNA genes and fungal *ITS1* genes. In the analysis of bacterial diversity, the V3-V4 variable regions of 16S rRNA genes were amplified with the universal primers 343F (343F-5′-TACGGRAGGCAGCAG-3′) and 798R (798R-5′-AGGGTATCTAATCCT-3′). For fungi, the primers ITS1 (5′-CTTGGTCATTTAGGAAGGAAGTAA-3′) and ITS2 (5-GCTGCGTTCTTCATCGATGC-3′) were used. PCR was carried out with a Bio-rad using a 30 µL reaction volume containing 15 µL of 2 × Gflex PCR buffer, 0.6 µL of Tks Gflex DNA Polymerase (1.25 U/µL), 2 µL of the primers (5 pmol/µL), 1 µL of the template DNA, and 11.4 µL of dd H_2O. The cycling parameters were 94 °C for 5 min, followed by 26 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 20 s, with a final extension at 72 °C for 5 min. Three PCR products per sample were pooled to mitigate reaction-level PCR biases. The PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN) and quantified using real-time PCR. Deep sequencing was performed on the Illumina Miseq platform at Ouyi Biomedical Technology Co., Ltd. (Shanghai, China).

2.5. Processing of Sequencing Data

The raw data were first screened, and sequences were removed from consideration if they were shorter than 200 bp, had a low quality score (≤20), contained ambiguous bases, or did not exactly match the primer sequences and barcode tags. The software package UCHIME (version 8.1) was then used to further filter out sequences that were erroneous or chimeric. Finally, the quality sequences obtained from QC were classified by operational taxonomic unit (OTU) at 97% similarity using Vsearch v2.4.2 software [26]. The taxonomy of ITS sequences was analyzed against the UNITE database. The rarefaction curves, alpha diversity index with CHAO1, Good's coverage, and Shannon and Simpson indices were calculated using the software MOTHUR.

To compare the community characteristics in greater detail, heat maps at the genus level were constructed, and Venn diagrams at the OTU level were created with the R

package. Linear discriminant analysis effect size (LEfSe) analysis was performed to identify significantly important microbial taxa. Finally, KEGG function prediction analysis was carried out with the R package. A redundancy analysis (RDA) was performed based on fungal abundance at the OTU level and physicochemical parameters using the R package.

2.6. Statistical Analysis

Microsoft Office Excel 2019 and Origin 8.0 were used for the data processing and mapping, and SPSS 22.0 was used for the analysis of variance and correlation analysis. The LSD of single-factor ANOVA was used for the statistical analysis to compare the significant differences between the treatments ($\alpha = 0.05$).

3. Results

3.1. Physicochemical Properties of Soil and Fruit Quality

The changes in physicochemical properties of the soil and fruit quality under different treatments are shown in Tables 1 and 2. After the application of biogas fertilizer, the soil pH, bulk density, and organic matter, available nitrogen, available phosphorus, and available potassium contents significantly increased (Table 1). The fruit quality, hardness, vitamin C (VC) concentration, titratable acid content, and soluble solids content were significantly increased compared with the unapplied biogas fertilizer treatment (CK). The largest increases were in vitamin C concentration and titratable acid, which increased by 0.939 and 0.444, respectively (Table 2).

Table 1. Effect of fertilization on main soil properties.

Treatment	pH	Organic Matter ($g \cdot kg^{-1}$)	Available Nitrogen ($mg \cdot kg^{-1}$)	Available Phosphorus ($mg \cdot kg^{-1}$)	Available Potassium ($mg \cdot kg^{-1}$)	Bulk Density ($g \cdot m^{-3}$)	Soil Porosity (%)
T	7.46 ± 0.05	12.72 ± 0.30 a	72.00 ± 0.77 a	24.71 ± 0.45 a	251.32 ± 1.31	1.69 ± 0.06	64.00 ± 0.07 a
CK	7.14 ± 0.03	7.43 ± 0.22 b	59.16 ± 1.71 b	8.00 ± 0.94 b	236.16 ± 3.25	1.44 ± 0.05	45.67 ± 0.05 b

Mean ± SE of six replicates. Lowercase letters exhibit significant differences ($p < 0.05$) according to LSD test.

Table 2. Effect of fertilization on main fruit quality.

Treatment	Fruit Weight (g)	Hardness ($kg \cdot cm^{-2}$)	Fruit Shape Index	Vc Concentration ($mg \cdot 100\ g^{-1}$)	Soluble Sugar (%)	Titratable Acidity (%)	Soluble Solids Content (%)
T	248.74 ± 5.64	7.26 ± 0.34 a	0.89 ± 0.02	3.49 ± 0.38 a	12.75 ± 0.65	0.13 ± 0.01 a	15.03 ± 0.41 a
CK	236.85 ± 4.52	6.44 ± 0.36 b	0.85 ± 0.03	1.80 ± 0.21 b	11.16 ± 0.72	0.09 ± 0.02 b	13.88 ± 0.27 b

Mean ± SE of six replicates. Lowercase letters exhibit significant differences ($p < 0.05$) according to LSD test.

3.2. Quality of Soil Microorganism Diversity

To investigate the diversity and structure of microbial communities in the apple rhizosphere, we sequenced the 16S rRNA gene and the ITS1 gene. After filtering low-quality reads and trimming adapters and barcodes, 55,551–62,245 and 68,054–75,994 high-quality reads were obtained from bacteria and fungi, respectively. The bacteria and fungi of 3525–4544 and 334–543 OTUs were identified, and the sequence similarity reached 97%.

Microorganism diversity was measured based on OTUs. The bacterial OTU distribution Venn analysis demonstrated that there were a total of 6256 OTUs, with 1460 and 1661 unique OTUs in T and CK, respectively (Figure 1a). The fungal OTU distribution Venn analysis demonstrated that there were a total of 713 OTUs, with 314 and 478 unique OTUs in T and CK, respectively (Figure 1b). The Shannon–Wiener analysis (Figure 1c) and Good's coverage analysis (Figure 1d), which were used in this analysis, also indicated that the sequence amount was enough to represent the true microbes in the sample and that the deeper sequencing identification was successful.

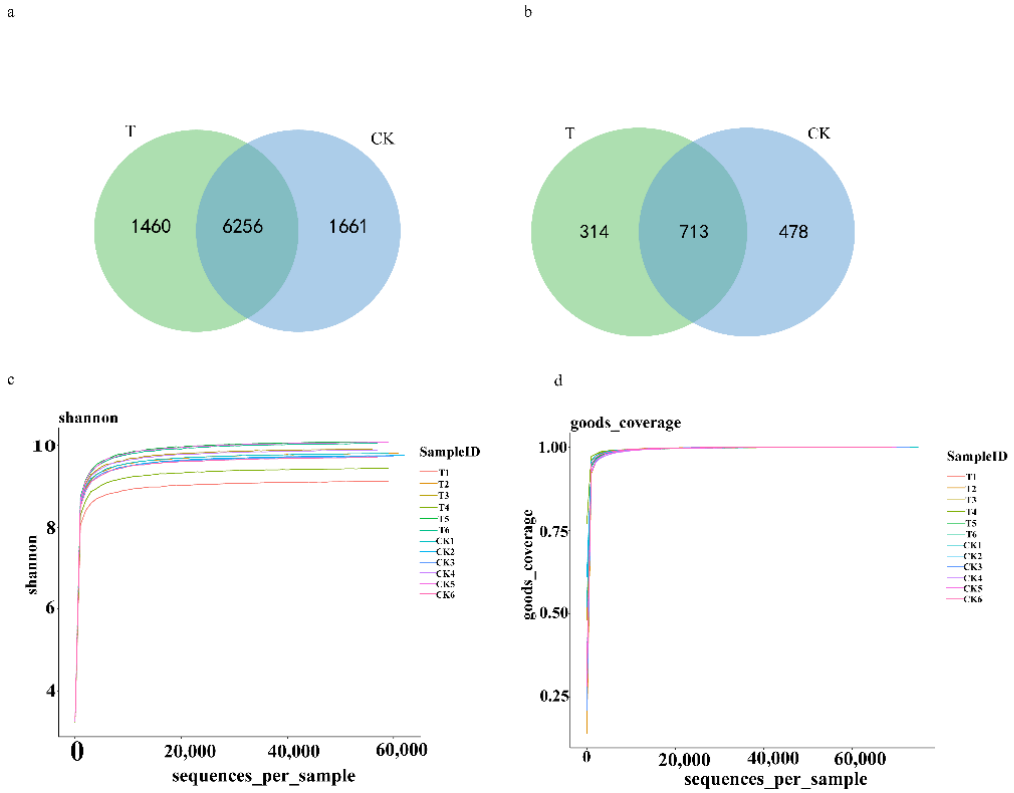

Figure 1. Venn diagram of unique and shared OTUs and diversity indices between bacteria (**a**,**c**) and fungi (**b**,**d**) in different treatments. Note: CK: no biogas fertilizer was applied T: biogas fertilizer was applied. Numbers in the figure represent otus unique and common to different treatments.

3.3. Overall Structural Changes in Microorganism Communities after Applying Biogas Fertilizer

Alpha diversity indices were evaluated based on OTUs. As can be seen in Table 3, after applying biogas fertilizer, the Shannon index of bacteria increased compared with CK, the choa1 index was lower than the CK index, and the Simpson index remained unchanged. In addition, the diversity index of fungi was lower than that of the CK group. However, there was little difference in the microbial diversity index between T and CK (Table 3).

Table 3. Diversity indices of bacteria and fungi in different treatments.

Treatment		Choa1 Index	Shannon Index	Simpson Index
Bacteria	T	4982.218	9.81	1
	CK	5005.635	9.754	1
Fungi	T	396.709	2.34	0.51
	CK	427.371	4.4423	0.87

The OUT-based PCA successfully represented the sample data. The results show that the bacterial samples were grouped based on the different treatments. The first principal component axis (PC1), which contributed 0.397 of the total variation, and the second component axis (PC2), which contributed 0.258 of the variation, explained 0.655 of the variation (Figure 2a). The PCA analysis of the fungal community displayed that the contribution of PC1 was 0.448 and that of PC2 was 0.197 (Figure 2b). The results show that the repetitions of T and CK were separated and that the repetition of T was more similar than that of CK. In summary, the application of biogas fertilizer in continuous monoculture soil can change the structure of the soil microbial community.

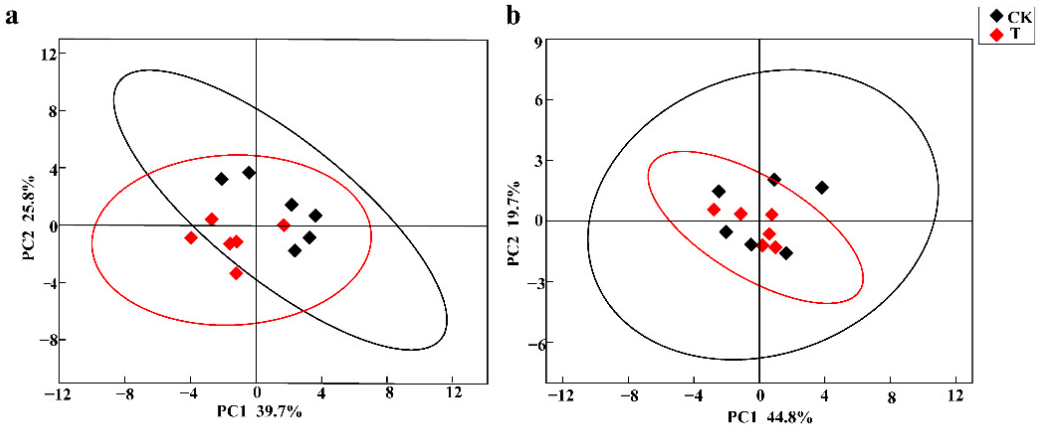

Figure 2. PCA analysis of bacterial (**a**) and fungal (**b**) community compositions in different treatments.

3.4. Taxonomic Distributions of Microbes Enriched in the Soil with Application of Biogas Fertilizer

From the data, it can be seen that among bacteria, the most abundant phyla were *p_Proteobacteria* (0.364–0.372), *p_Bacteroidetes* (0.169–0.219), *p_Gemmatimonadetes* (0.133–0.154), *p_Actinobacteria* (0.095–0.166), *p_Acidobacteria* (0.037–0.051), *p_Nitrospirae* (0.028–0.034), and *p_Verrucomicrobia* (0.019–0.035), which accounted for more than 90% of sequences among all groups (Figure 3a). *Proteobacteria* dominated the apple rhizosphere, making up 0.364–0.372 of all sequences.

At the phylum level, there were seven species with significant differences. Among them, *Actinobacteria* were significantly abundant ($p < 0.05$) in soil samples from the groups. In contrast, a significant decrease ($p < 0.05$) was observed in the relative abundances of *Verrucomicrobia*, *Elusimicrobia* (*p_Elusimicrobia*), *Dependentiae* (*p_Dependentiae*), *Deferribacteres* (*p_Deferribacteres*), *Omnitrophicaeota* (*p_Omnitrophicaeota*), and *Margulisbacteria* (*p_Margulisbacteria*) in T soils.

Among the sequences identified at the genus level, the relative abundances of 22 genera were significantly higher ($p < 0.05$) in the CK group than in the T group (Figure 4a). Conversely, the relative abundances of 26 genera in the rhizosphere soils of the continuous replanting orchard were significantly higher ($p < 0.05$) than those in the CK group, and the relative abundances of *Dongia*, *Gaiella* (*g_Gaiella*), *Steroidobacter* (*g_Steroidobacter*), and *Nordella* (*g_Nordella*) in the T group were significantly higher than those in the CK group among the top 30 genera. In contrast, the relative abundances of *Is-44* (*g_Is-44*) and *Polyclovorans* (*g_Polyclovorans*) in the CK group were significantly higher than those in the T group.

For the fungal community, the rhizosphere of the T group contained 7 bacterial phyla, 28 classes, 73 orders, 136 families, and 254 genera. The phylum *Ascomycota* (*p_Ascomycota*) was the most abundant across all samples, followed by *Basidiomycota* (*p_Basidiomycota*), *Mucoromycota* (*p_Mucoromycota*), *Glomeromycota* (*p_Glomeromycota*), *Chytridiomycota* (*p_Chytridiomycota*), *Cercozoa* (*p_Cercozoa*), and *Rozellomycot* (*p_Rozellomycot*) (Figure 3b). The relative abundance of *Basidiomycota* in the rhizosphere of the T group was higher than that in the CK group. In contrast, the other fungal communities were lower than those in the CK group. Among the sequences identified at the genus level, the relative abundances of *Diversisporales* and *Chaetomium* in the T group were significantly lower than those in the CK group (Figure 4b).

In order to further clarify the possible interactions between bacterial dependencies in the rhizosphere soil, the biomarkers of different species were quantitatively analyzed using linear discriminant analysis (LDA) and effect quantity analysis (Figure 5a). A total of five biomarkers were identified from all rhizosphere soil samples, as shown in the branch diagram. In the soil bacterial community of the experimental group, there was only one different phylum at the phylum classification level, which was *Actinobacteria* (*p_Actinobacteria*). There was a difference at the class level. At the order level, there was a

different order, *Gaillales* (*o_Gaillales*). In the soil bacterial community of the CK group, there was a different order, *Chitinophagales* (*o_Chitinophagales*), at the order level and a different family, *Chitinophagaceae* (*f_Chitinophagaceae*), at the family level.

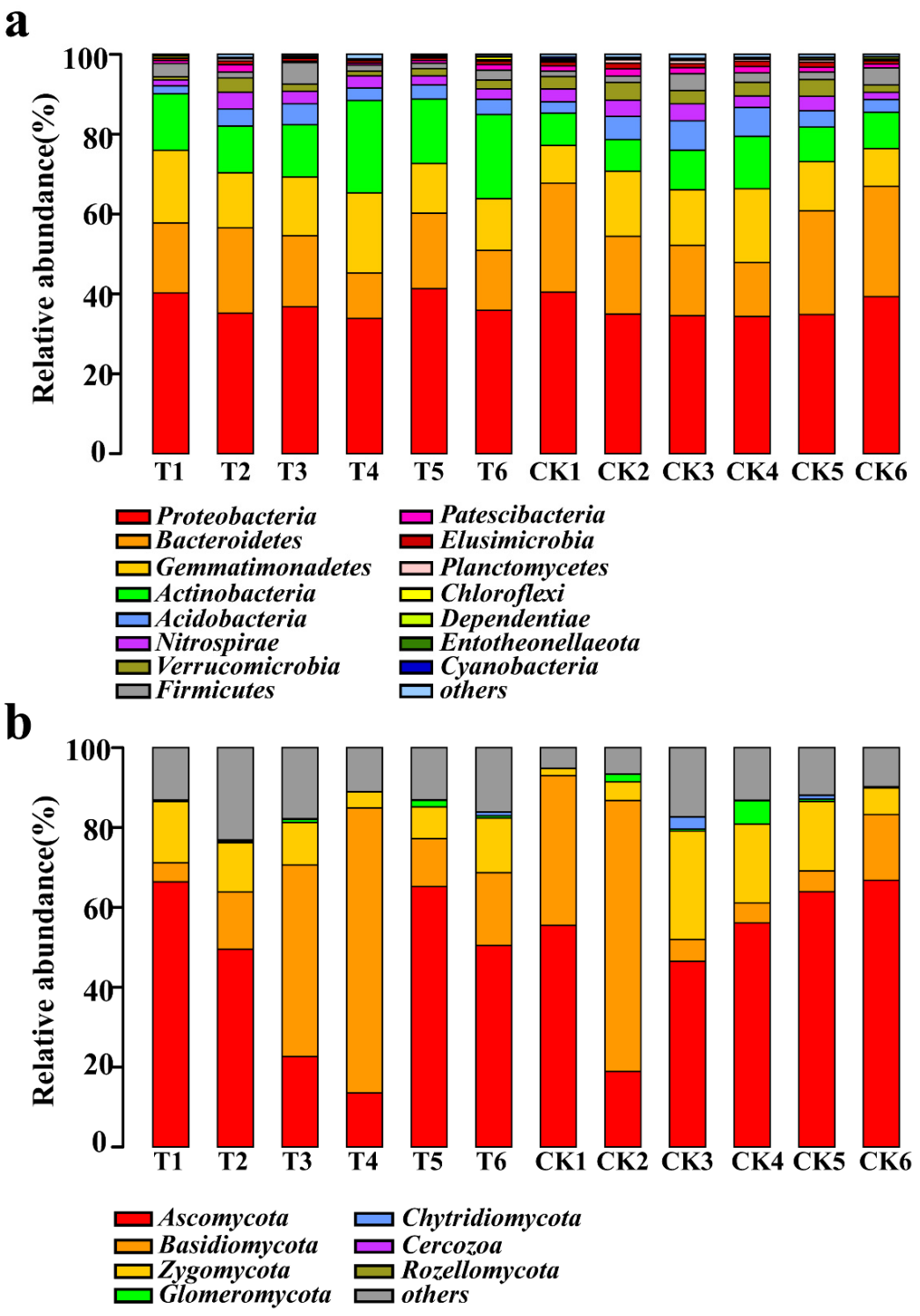

Figure 3. Relative abundances in bacterial (**a**) and fungal (**b**) community compositions at phylum level. Note: Columns indicate samples, and different colors indicate different annotation information.

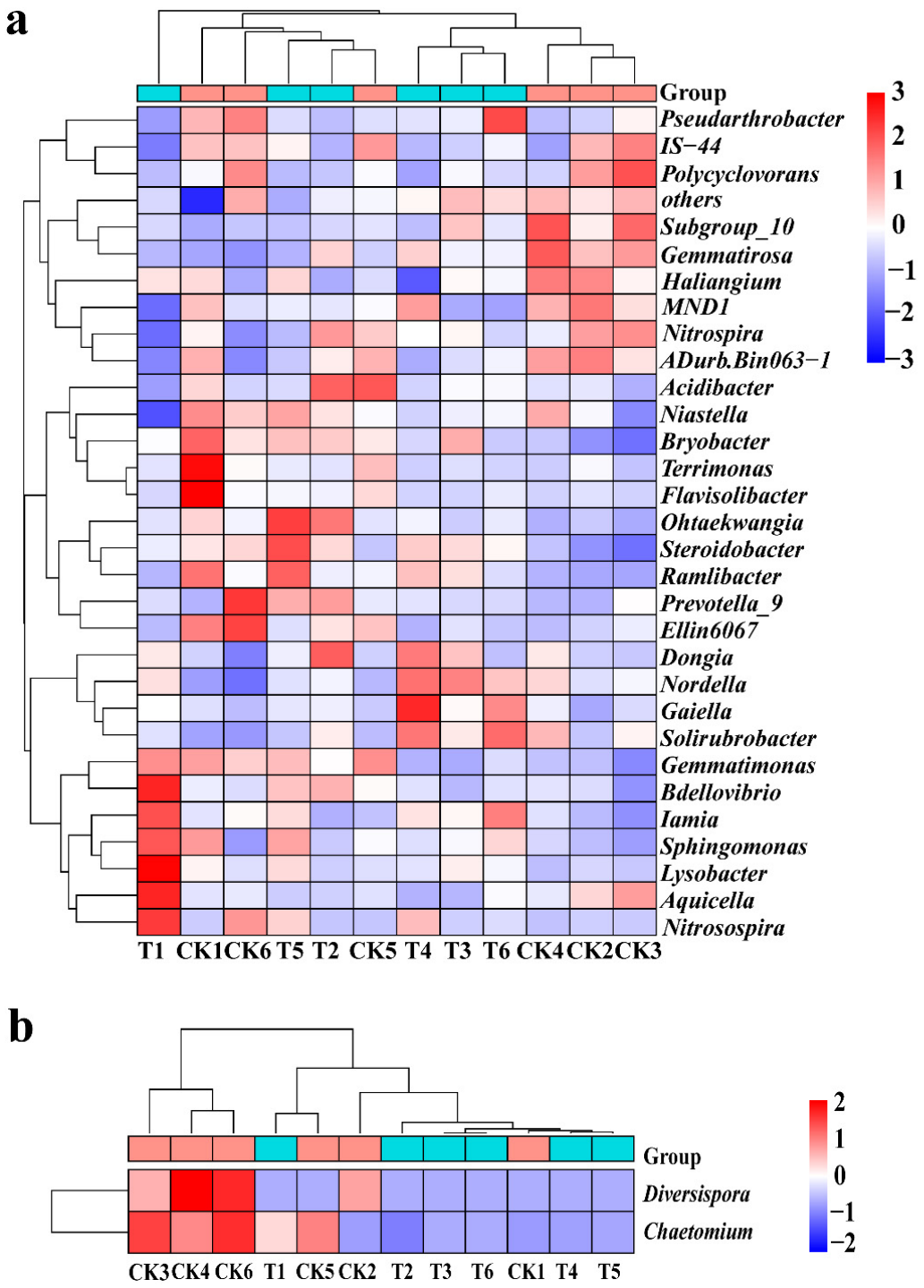

Figure 4. Cluster analysis of bacterial (**a**) and fungal (**b**) community compositions at the genus level. Note: Group represents different subgroups, and the left clustering tree represents clustering of species. The top clustering branch Group represents samples from different subgroups. Orange color indicates higher relative abundance of species, and blue color indicates lower relative abundance of species.

Cladograms obtained from the LEfSe analysis provided a deep insight into the changes in identified fungi accumulated in the rhizosphere soil under different treatments (Figure 5b). Firstly, there were many unidentified fungi in the CK group. *Guehomyces*, which are a part of the phylum *Basidiomycota* (*p_Basidiomycota*), preferentially colonized the T group. The genus *Botrytis* (*g_Botrytis*) also preferentially colonized it. The order

Diversisporales (*o_Diversisporales*) and its families *Diversisporaceae* (*f_Diversisporaceae*) and *Diversispora* were previously planted in the CK group. In the dominant family *Togniniaceae* (*f_Togniniaceae*) of the order *Diaporthales*, the most abundant genus was *Phaeoacremonium* (*g_Phaeoacremonium*), which preferentially colonized the CK group. The family *Bionectriaceae* (*f_Bionectriaceae*) was dominant in the CK group.

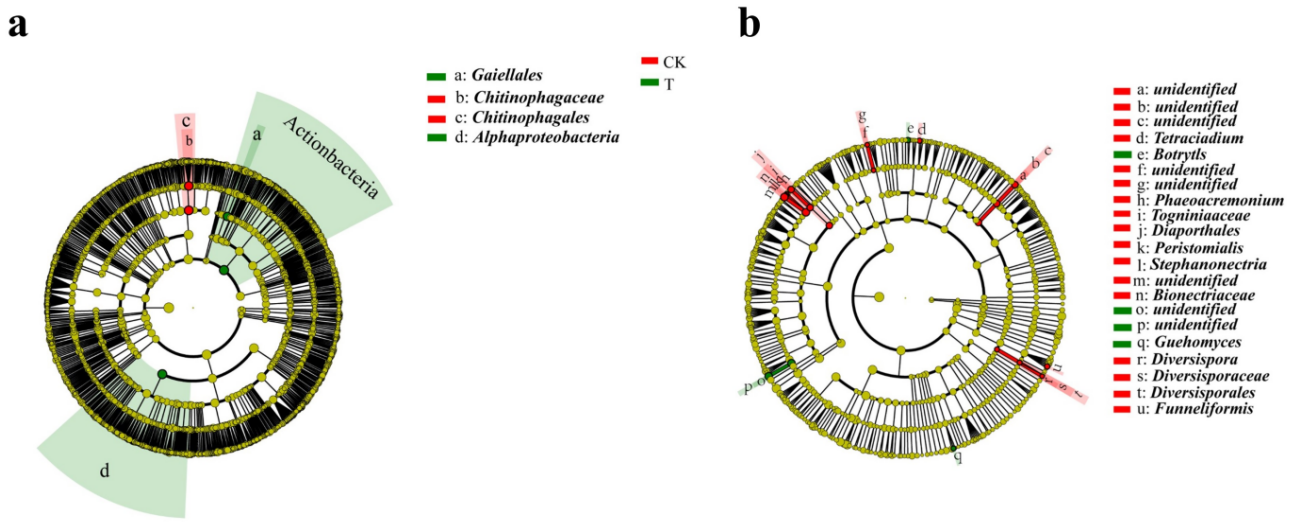

Figure 5. Annotated species analysis of bacteria (**a**) and fungi (**b**). Note: The size of the node diameter is proportional to the size of the relative abundance, and the nodes in each layer indicate the phylum/class/order/family/genera from the inside to the outside, and the annotations of the species markers in each layer indicate the phylum/class/order/family/genera from the outside to the inside, respectively.

3.5. Prediction of Bacterial Functions

PICRUST was applied to predict the abundances of different metabolic pathways based on the 16S rRNA sequencing data. Overall, six pathways with significant enrichment of differentially expressed genes were found in the predicted pathway (Figure 6). The results show that basic metabolic pathways dominated, including human diseases, environmental information, processing organismal systems, metabolism, and genetic information processing. Among these pathways, environmental information processing, organism systems, and metabolism in the T group were significantly higher than those in the CK group.

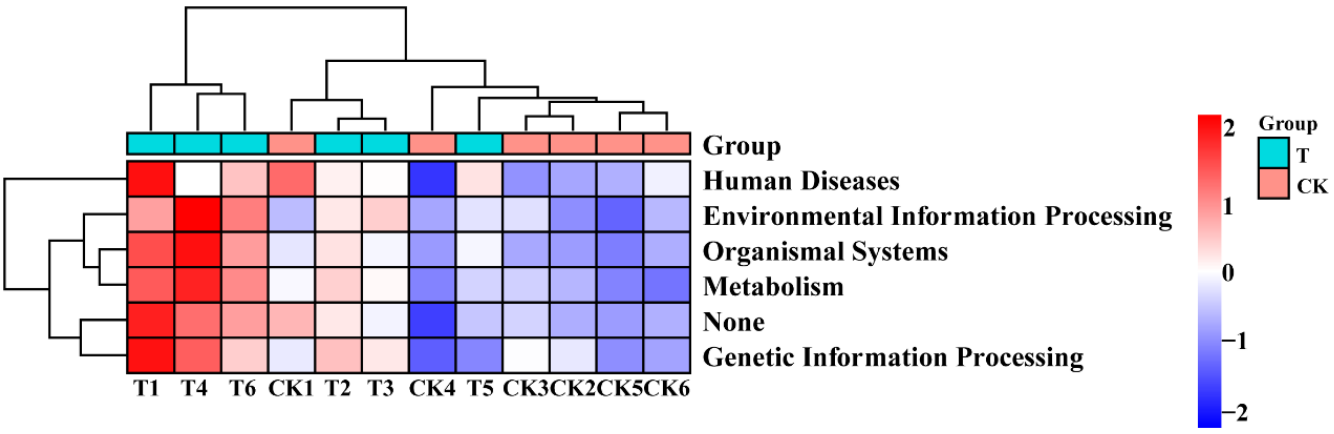

Figure 6. Clustering heat map of bacterial KEGG difference results.

3.6. Links between Microbial Community and Soil Physicochemical Properties

Soil physicochemical properties significantly changed with the application of biogas fertilizer (Table 1). The RDA model was applied to investigate the relationships between these soil parameters and the microbial community compositions (Figure 7). Associations between the bacterial and fungal groups and soil physicochemical properties were evaluated using the soil parameter data as the explanatory matrix. For bacteria, RDA1 and RDA2 explained 0.929 of the total variance. Except for *Chloroflexi* bacteria, other bacteria were closely related to the soil's physicochemical properties, and Bacteroidetes was inversely proportional to all soil physical and chemical properties. For fungi, RDA1 and RDA2 explained 0.995 of the total variance. Regarding phyla, *Ascomycota*, *Basidiomycota*, and *Zygomycota* were closely related to these soil parameters. On the contrary, *Glomeromycota*, *Chytridiomycota*, *Cercozoa*, and *Rozellomycota* were not closely related to soil physicochemical properties.

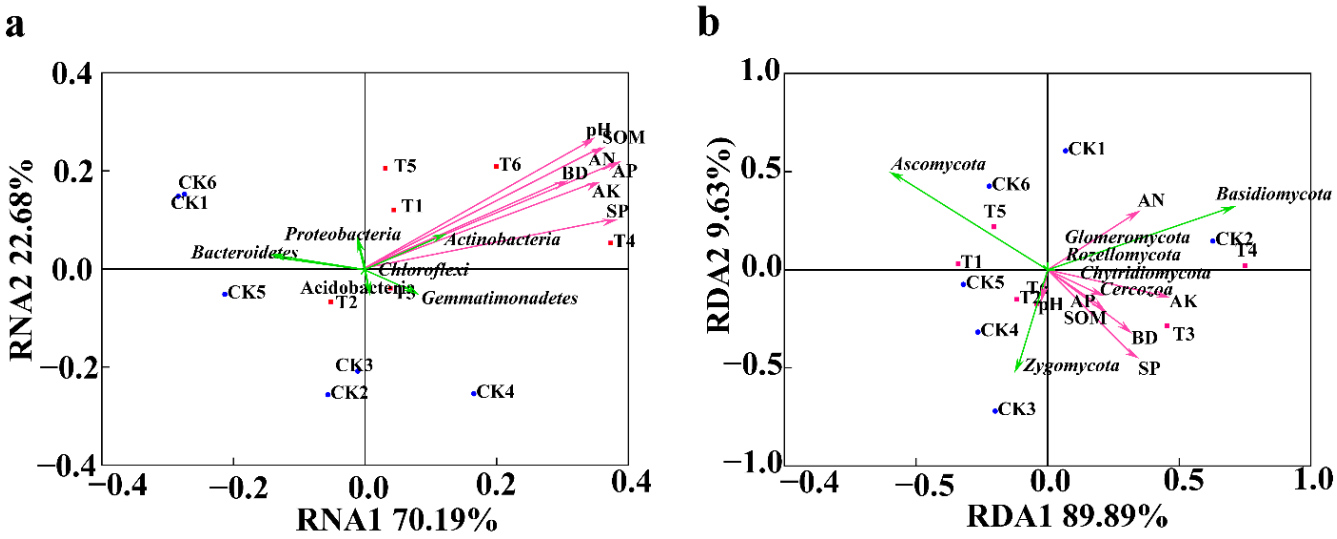

Figure 7. Redundancy analysis of dominant bacteria (**a**) and fungi (**b**) and soil physicochemical properties. Note: SOM, AN, AP, AK, BD, and SP represent soil organic matter, available nitrogen, available phosphorus, available potassium, bulk density, and soil porosity, respectively.

4. Discussion

Biogas projects are considered to be effective methods for the anaerobic digestion of animal manure or crop residues and have been widely used. Biogas fertilizer is rich in organic matter, and increasing the application of biogas fertilizer is an important means to increase the content of soil organic matter and can also increase the number of soil microorganisms and regulate the balance between microbial populations [27]. With the application of biogas fertilizer in crop production, the activity of root microorganisms can be effectively increased, the root epidermis is more capable of absorbing nutrients from the soil, and the activity of soil enzymes is improved [28]. A study by Xu et al. (2020) found that after years of biogas fertilizer application, soil nutrient content, pH, total nitrogen, and other physicochemical properties significantly increased, and the soil microbial community structure and diversity significantly changed [6]. In terms of improving the quality of crops, the application of biogas fertilizer not only has a significant enhancing effect on the vitamin C, protein, amino acid, and sugar contents in crops but also reduces the content of nitrates and nitrites in crops. For example, some studies have shown that the contents of amino acids, proteins, soluble sugars, and vitamin C in the fruits of tomatoes treated with biogas fertilizers are significantly increased [29], which is consistent with our findings. In our study, we found that the application of biogas fertilizer significantly increased the soil porosity and organic matter, available N, and available P contents in the continuous cropping soil of apple orchards, and the VC concentration in the fruit increased by 0.938.

This may be due to the fact that the application of biogas fertilizer provides a variety of nutrients to plants, improves soil conditions, and creates a suitable soil environment for the growth of plant roots, which in turn promotes the growth and development of plants to improve the quality of fruits.

To further understand whether biogas fertilizer affected the structure and diversity of soil microbial communities, we performed microbial transcriptomic sequencing. We found that in bacteria, the dominant phyla (average RA > 1%) were *Proteobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Actinobacteria*, *Acidobacteria*, *Nitrospirae*, *Verrucomicrobia*, and *Firmicuteshe*. These phyla were widespread in the soil [30]. Many *Betaproteobacteria*, *Bacteroidetes*, and *Actinobacteria* are copiotrophic soil bacteria, which become abundant if labile substrates are available [31]. This successfully explains why *Proteobacteria* and *Actinobacteria* were the most dominant phyla. Among these dominant phyla, *Proteobacteria* was the most dominant phylum. *Proteobacteria* have commonly been reported as the first dominant phylum in soils [32]. *Sphingomonas*, which belongs to *Proteobacteria*, is a plant growth promoter. Some microorganisms in *Sphingomonas* can secrete indole-3-acetic acid and other substances, promote plant growth, and degrade organic matter [33] and have activity in the biological control of pathogenic bacteria [34]. In this study, we found that the relative abundance of *Actinobacteria* increased after applying biogas fertilizer. Similar results have also been reported, where the relative abundance of *Actinomycetes* with long-term chemical fertilizer application was lower than that with organic fertilizer application [35]. As the main phylum in bacterial groups, *Actinomycetes* have the ability to resist stress and pathogen infection, so they may have important potential in fruit trees to adapt to environmental stress [36]. In this study, we also found that after applying biogas fertilizer, the relative abundance of *Chloroflexi* increased by 1.360. Most microorganisms in the phylum *Chloroflexi* are strictly anaerobic bacteria that can ferment sugars and polysaccharides into organic acids and hydrogen to accelerate the decomposition of soil organic matter [37]. The dominant phylum *Chloroflexi* has been largely reported as a group of bacteria that frequently live in a nutritious environment, and numerous nutrients are beneficial to their growth and reproduction [38].

For fungi, the most dominant phyla *Ascomycota*, *Basidiomycota*, and *Mucoromycota*, which frequently occur in nature, were also detected in this study. Among these phyla, *Ascomycota* has been found to be associated with a wide range of crop monoculture systems [39]. The class *Sordariomycetes* of *Ascomycota* is the dominant fungus, which is consistent with many studies that found *Sordariomycetes* to be the most common fungal class in different agricultural systems, with members being ubiquitous as pathogens and plant endophytes in almost all ecosystems [40]. The phylum *Basidiomycota* includes a large and complicated group of fungi with abundant saprophytic (wood decomposers and litter decomposers), ectomycorrhizal, and parasitic fungi [41]. Within this phylum, the predominant group is the agaric bacteria that accumulate during treatment and are reported to be a key decomposer, containing “soft”, “brown”, and “white” decay fungi that produce hydrogen peroxide and enzymes to degrade complex plant compounds, including cellulose and lignin [42]. Therefore, this class may have caused the increase in apple soil organic matter from the constant biogas fertilizer input under long-term continuous culture. The genera *Cryptococcus* are oligotrophic organisms and single-celled microorganisms (yeast) with a wide range of enzymatic activities [43]. This shows that the application of biogas fertilizer reduces the relative abundance of oligotrophic organisms. Thus, this genus can be regarded as a fungal indicator for soil nutrient degradation in apple field cropping.

Therefore, we believe that the application of biogas fertilizer can improve the physicochemical properties of orchard soil and the structure of the soil microbial community and thus improve the quality of fruit.

We expect to lay the foundation for orchard production through microbial isolation and characterization by systematically isolating and identifying dominant microorganisms in pure cultures, such as *Sphingomonas* and *Chlamydomonas*.

5. Conclusions

The continuous application of biogas fertilizer in dry and rain-fed areas significantly increased soil physicochemical properties such as soil organic matter, available nitrogen, available phosphorus, and soil porosity. Additionally, the fruit quality was also significantly improved, for instance, the contents of vitamin C, titratable acid, and soluble solids in fruits also significantly increased. Finally, microbiomics also revealed that the application of biogas fertilizer can increase the relative abundance of dominant floras, such as *Sphingomonas*, *Chlamydomonas*, and *Stachybotry*, which significantly increased and provided favorable support for orchard production.

Author Contributions: C.H. and Y.W. designed this research. C.H., Y.G. and Z.Z. performed the experiments. J.C. and W.W. performed the data analysis and interpretation. Y.G. and Y.W. prepared the figures and tables. C.H. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (project no. 31960581).

Data Availability Statement: All data generated or analyzed in this study are included in this published article.

Conflicts of Interest: The authors declare no conflict of interest associated with this manuscript.

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