

Article

Organic Farming Improves Soil Microbial Abundance and Diversity under Greenhouse Condition: A Case Study in Shanghai (Eastern China)

Jianli Liao, Yun Liang and Danfeng Huang * 

Key Laboratory of Urban Agriculture (South), Ministry of Agriculture, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China; Jerry0427@sjtu.edu.cn (J.L.); yunliangdoreen@gmail.com (Y.L.)

* Correspondence: hdf@sjtu.edu.cn; Tel.: +86-21-3420-6943

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Abstract: Agricultural practices have significant impacts on soil properties and microbial communities; however, little is known about their responses to open field and plastic tunnels under organic and conventional farming. We therefore investigated the responses of soil chemical variables and microbial communities to different agricultural management and cultivation types, including organic management in open field (OF), organic management in plastic tunnels (OP), conventional management in open field (CF) and conventional management in plastic tunnels (CP), by using a pyrosequencing approach of 16S rRNA gene amplicon. Both factors had significant influences on the soil properties and microbial communities. Organic farming increased the nutrient-related soil variables compared to conventional farming regardless of cultivation type, especially for the available N and P, which were increased by 137% and 711%, respectively, in OP compared to CP. Additionally, OP had the highest microbial abundance and diversity among treatments, whereas no difference was found between OF, CF and CP. Furthermore, OP possessed diverse differential bacteria which were mainly related to the organic material turnover (e.g., *Roseiflexus*, *Planctomyces* and *Butyrivibrio*) and plant growth promotion (e.g., *Nostoc*, *Glycomyces* and *Bacillus*). Redundancy analysis (RDA) showed that pH, electrical conductivity (EC), nutrient levels (e.g., available N and available P) and total Zn content were significantly correlated to the structure of the microbial community. Overall, our results showed that the long-term organic farming with high fertilizer input increased soil nutrient levels and microbial abundance and diversity under plastic-tunnel condition compared to other cultivation systems.

Keywords: organic farming; soil microbiota; agricultural practice; greenhouse; horticultural soil; soil properties

1. Introduction

Soil provides important functions in agroecosystems, such as organic material turnover, nutrient cycling and xenobiotics adsorption and degradation [1]. Microbes have been considered as the key drivers in these functions in the agroecosystem. Furthermore, a number of soil microbes could promote crop growth by producing antibiotics and plant hormones and increase nutrient availability (e.g., excrete phosphatase) and, as a consequence, benefit plant quality and productivity [2]. Recently, research on the soil microbial community and its function in the agricultural production system has received increasing attention. These investigations have identified that microbial attributes could reflect ecosystem processes such as crop productivity [3], the regulation of decomposition [4], nutrient cycling [5] and protection against soil-borne pathogens [6]. Several studies have shown that agricultural

practices have significant influences on soil microbial communities and composition, including the tillage regime [7], fertilization [8], monoculture [9], crop residue management [10] and plant protection schemes [11]. Hence, the soil microbial community could be shifted to a positive organization for plant production and ecosystem sustainability by suitable agricultural practices [4].

With the benefits of the green revolution, the development and use of chemical fertilizer, agrochemicals and irrigation systems have significantly increased the efficiency of crop productivity worldwide [12]. In recent years, however, great soil degradation was observed, such as by soil erosion, salinization, acidification, pollution by heavy metals and xenobiotics, and an increased abundance of plant pathogens [4]. These adverse effects led to the reduction of productivity and were mainly due to unsuitable intensive agricultural practices such as repeated tillage and the overuse of synthetic fertilizer and agrochemicals [13]. To minimize these negative impacts, organic farming has been proposed as a sustainable agricultural practice with the main principles of the ecological cycle (e.g., the reuse of composted local organic materials and avoiding the use of agrochemicals) and biodiversity (e.g., rotation and biocontrol) when compared to conventional farming [14]. By the end of 2015, there were approximately 44 million hectares of land that were managed organically over the world. China was the third largest country in terms of its land area under organic management in the world (2.28 million hectares; the first two countries are Australia and Argentina), and the dedicated land area has been steadily growing in recent years [15].

Organic farming has been shown to increase environmental biodiversity in terms of animals, insects and butterflies when compared to conventional farming [14,16]. Several studies also suggested that microbial abundance [4] and diversity [9] were higher in soils under organic management than in soils under conventional practice. The increased microbial diversity could be related to reduced tillage [17], cover cropping [18] and organic fertilizer incorporation [19] by improving soil organic carbon as the energy source of heterotrophic microbiota [20]. Furthermore, high microbial diversity has been linked to high functional diversity in soil [5], which may cause the important ecological processes mentioned above. In addition, most researchers studied the microbial community under different agricultural management practices with field crops such as rice [3,21] and wheat [10], whereas little research has focused on vegetable cultivations [22]. Specifically, we still have a limited understanding of the long-term effects of organic and conventional management on soil microbial communities under open-field and protected conditions.

Protected cultivation methods, such as plastic (e.g., polyethylene) cover and greenhouses which mostly allowed sunlight to penetrate, were built to grow plants with a range of advantages, including early harvest and a high quantity, quality and off-season production [23]. In China, there were approximately 4 million hectares of protected vegetables in 2010, which account for over 80% of all protected vegetable production in the world [23]. There is an increasing concern, however, that this type of cultivation profoundly changes soil properties by regulating water and temperature. Drip and sprinkler irrigation as alternatives due to rainfall limitation under covered cultivation result in increased salinity accumulation on the soil surface by reducing soil leaching. Furthermore, optimal water and temperature control could enhance organic matter degradation in the soil, which leads to lower soil carbon content and results in negative effects on soil microbial communities [22]. In this regard, organic farming has been shown to improve soil carbon content and have a positive effect on soil microbial diversity, which may offset the negative effects of protected cultivation on soil quality and microbiota. However, the impacts of organic farming with protected cultivation on soil properties and the microbial community are not well understood.

In this context, we investigated the response of soil properties and the microbial community in soils under open-field and protected conditions managed organically and conventionally. We hypothesized that long-term organic farming shifted the soil microbial composition and improved soil organic matter turnover, specifically for soil under the plastic tunnel condition. To test this hypothesis, we used high-throughput sequencing of the 16S rRNA gene markers to analyze microbial communities and measured a range of soil variables in open-field and plastic-tunnel soils under organic

and conventional farming systems. The overall goal of this study was to get a better understanding of how cultivation management (organic vs. conventional) and type (open field vs. plastic tunnel) can affect the composition of the soil microbial community and the cultivation-associated microbiota in the corresponding cultivation system.

2. Materials and Methods

2.1. Sites Description

The study site is located in the urban area of Shanghai (Eastern China), which has a humid subtropical climate with a mean annual temperature of 17.5 °C and rainfall amount of ~1300 mm. Four horticultural soils were selected: (1) organic practice in open field (OF); (2) organic practice in plastic tunnel (OP); (3) conventional practice in open field (CF) and (4) conventional practice in plastic tunnel (CP). The soils were clay loam and were classified as fluvisol according to the World Reference Base for Soil Resources [24]. The organic soils were sampled in the Horticultural Research Station of Shanghai Jiao Tong University, Shanghai, China (27 hectares, Organic Certification No. 134OP1200092), which was established in 2008, approximately 5 km from the East China Sea (30°51' N, 121°5' E). The conventional farm was closed to the research station and has continuously produced vegetables for more than 10 years. The plastic tunnels in both organic and conventional farms have been set up and used for vegetable production for 7 years. The organic soils were fertilized with commercial organic fertilizer, which was produced from mushroom waste and chicken manure, N-P-K (2.3-4.5-2.6) with an organic matter content of 77.3% and total Zn 425 mg kg⁻¹ at an average rate of 90 and 105 ton ha⁻¹ year⁻¹ for open-field and plastic-tunnel cultivation, respectively. A complex fertilizer N-P-K (15:15:15) was used at an average rate of 8 and 10 ton ha⁻¹ year⁻¹ for conventional open-field and protected cultivation, respectively. Additionally, urea was used at an average rate of 0.2 ton ha⁻¹ year⁻¹ for both cultivation types in conventional management. Three and four crops are produced annually in the open fields and plastic tunnel, respectively. The dominant crops cultivated in rotation are onion (*Allium cepa* L.), sweet potato (*Ipomoea batatas* L.), green onion (*Allium fistulosum* L.), Chinese cabbage (*Brassica rapa pekinensis*), and pepper (*Capsicum annuum* L.) for open fields, while these were pakchoi (*Brassica campestris* spp. *chinensis* Makino var. *communis* Tsen et Lee), celery (*Apium graveolens* var. *dulce*), cucumber (*Cucumis sativus* L.), alfalfa (*Medicago sativa* L.), spinach (*Spinacia oleracea* L.), lettuce (*Lactuca sativa* L.) for the plastic tunnel. Pests and diseases were controlled by cultural practices (e.g., rotation) and agricultural chemicals (e.g., mandipropamid, chlorpyrifos and spinosad) in organic and conventional plots, respectively. Weeds were removed manually in organic plots, while herbicides (e.g., glyphosate and linuron) were used in conventional management plots. Furrow irrigation was applied in open fields, while drip and sprinkler irrigations were used in the greenhouse condition to maintain the water content (60–70%) for plant growth.

2.2. Soil Collection

To minimize soil variation by external disturbance, the soils at 10–20 cm depth were collected by 5.5-cm-diameter stainless steel auger after plant harvest in January 2016. We selected an 8 m × 40 m plot from each cultivation system and collected subsamples at regular 2.5 m × 8 m intervals, 15 cm apart from the plant. In total, 15 samples were collected from each plot. After sampling, the soils were stored in polyethylene bags and kept on ice before being transferred to the lab. Three samples were randomly picked from the same cultivation system, mixed homogeneously and then passed through a sterilized 2-mm sieve as one replicate, resulting in five replicates per cultivation system. The sample was divided into three parts: one was stored at 4 °C and another was air-dried and passed through a 2-mm sieve for subsequent chemical analysis, while the other was stored at –80 °C prior to molecular analysis.

2.3. Soil Chemical Analysis

The fresh soil was used for the determination of ammonium (NH_4^+ -N) and nitrate (NO_3^- -N) after extraction with 2 M KCl (1:10, *w/v*) using automated discrete analyzer (Smartchem, Frépillo, France). The air-dried soil was used for the following chemical properties: the soil pH and electrical conductivity (EC) was determined in a 1:5 (*w/v*) soil/water slurry [25]. The cation exchangeable capacity (CEC) was measured by an ammonium acetate method. Soil-available N and available P were measured with the alkaline-hydrolyzable diffusion method [9] and Olsen method. A Vario El III elemental analyzer (Elementar, Langenselbold, Germany) was used to measure total soil C (C_{tot}) and total soil N (N_{tot}). The total amounts of K, Cu and Zn, which were shown as K_{tot}, Cu_{tot} and Zn_{tot}, respectively, were digested by aqua regia and measured using an inductively coupled plasma optical emission spectrometer (ICP-OES, Spectro Arcos, Ametek, Meerbusch, Germany).

2.4. Soil Microbial Analysis

The genomic DNA was extracted from approximately 0.5 g of soil (wet weight) using the E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA); details are provided in the Supplementary Information S1. The quantity and quality of DNA extracts (1 μL) were quantified using a NanoDrop 2000 fluorospectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The 16S microbial primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') were used for amplifying the ~400 bp V4-V5 regions of the 16S rRNA gene. Amplicons from each PCR sample were pooled to equal amounts and sequenced on an Illumina MiSeq sequencer (Illumina Inc., San Diego, CA, USA) with MiSeq Reagent Kit v3 at the Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). Raw sequencing files were deposited in the National Centre for Biotechnology Information Sequence Read Archive (SRA) with the accession number "SRP156388".

Sequences were processed with the quantitative insights into microbial ecology (QIIME) pipeline version 1.8.0 [26]. Briefly, low-quality reads were discarded with an average Phred score lower than 20, lengths of less than 150 bp, ambiguous N bases and mismatched primers. The fast length adjustment of short reads (FLASH) software (v1.2.7) was employed to assemble filtered paired-end reads [27]. The contigs were assigned to operational taxonomic units (OTUs) at 97% similarity using UCLUST [28]. A representative sequence was picked from each out, and BLAST was applied to assign the taxonomic classification against the Greengenes database (Release 13.8) [29]. Only those OTUs accounting for more than 0.001% of all sequence data were used for subsequent analysis.

2.5. Statistical Analysis

We used both multivariate and univariate analyses to assess the overall effects of the management (i.e., organic and conventional) and cultivation type (i.e., open field and plastic tunnel) on soil properties and microbial communities. For the multiple statistical tests, normalized soil variables were used to examine the similarities between cultivation systems by unconstrained principal coordinates analysis (PCoA), relying on a Euclidean distance. This distance matrix was then used to estimate the significance of the management and cultivation type by a permutational multivariate analysis of variance (PERMANOVA). For the univariate analysis, significant differences among cultivation systems were determined using Duncan's new multiple range test ($p < 0.05$).

Alpha-diversity characteristics, including the rarefaction curve, Good's coverage, indices of richness (Chao1 and ACE, i.e., abundance-based coverage estimator) and diversity (Shannon), in each soil sample were calculated by QIIME. Significant differences between these measures were determined by Duncan test ($p < 0.05$). An unconstrained PCoA based on Bray–Curtis index values were used to compare the β -diversity microbial community. PERMANOVA was applied to this distance matrix to examine the significance levels of the management and cultivation types on soil microbial communities. A linear discriminant analysis (LDA) effect size (LEfSe, LDA score > 3.8) was conducted to explore the differential microbial populations between different cultivation systems [30] and then visualized by

using the ggpubr package in R (3.4.1) at the phylum and genus levels. The differential genera generated from LEfSe analysis were further used for the Spearman correlation analysis with soil chemical properties ($p < 0.01$, absolute value of $r > 0.6$). A redundancy analysis (RDA) was applied to examine the relationship between soil environmental factors and the microbial community composition.

An unconstrained PCoA was conducted in the R environment (3.4.1) by a vegan package, while the PERMANOVA of the treatment groups was performed using 999 permutations in PAST3 software [31]. Duncan analysis was carried out in SPSS (IBM, Chicago, IL, USA, version 22.0).

3. Results

3.1. Soil Chemical Properties

The edaphic variables of all four cultivation systems were clustered into groups, with 65.91% and 29.80% of the variance components explained by the first two axes (Figure 1a), which were separated based on cultivation type (i.e., open field and plastic tunnel) and agricultural management (i.e., organic and conventional practices), respectively. PERMANOVA analysis further indicated that cultivation type ($p = 0.001$) was the main driver determining soil parameters while management ($p = 0.025$) was of subordinate importance (Table S1).

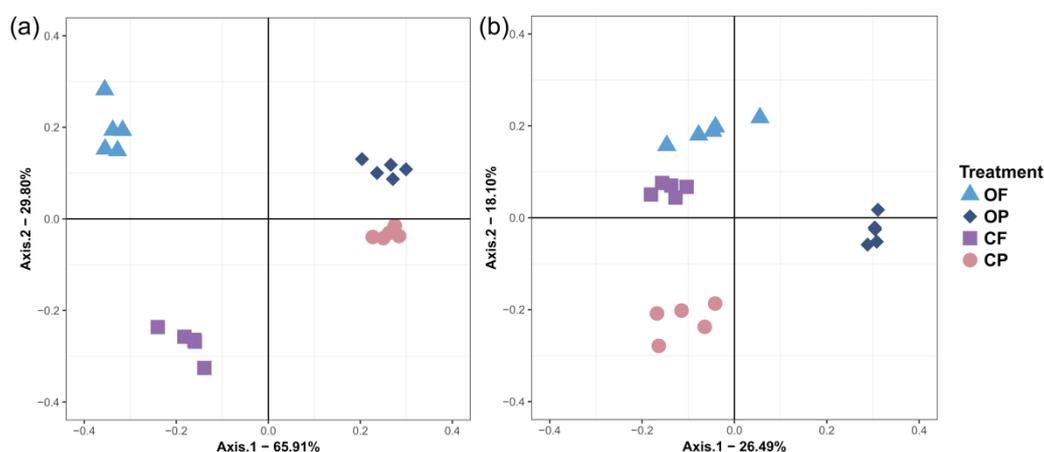


Figure 1. Unconstrained principal coordinates analysis (PCoA) illustrating the effect of fertilizers on (a) the Euclidean distances of soil properties, (b) the Bray–Curtis distance of microbial communities.

Univariate analysis showed that organic management had a pH of 7.32 and 7.28 in OF and OP, respectively, which were significantly lower than those in conventional management (7.59 and 7.83 in CF and CP, respectively, Table 1). The soil salinity in terms of EC was approximately twofold higher in OP (0.34 dS m^{-1}) than the other treatments and decreased in the order $\text{CP} > \text{CF} > \text{OF}$, while a similar trend was observed in available N. In addition, available P, Ctot, Ntot and Ktot ($\text{OP} > \text{OF} > \text{CF} = \text{CP}$) were significantly increased in soils under organic management compared to conventional management. The C/N ratio was significantly higher in CP than OP, whereas opposite trends were observed in CEC, NO_3^- -N, Cutot and Zntot. Soil NH_4^+ -N showed the highest content in OF (7.86 mg kg^{-1}), followed by OP (7.42 mg kg^{-1}) and CP (6.40 mg kg^{-1}), while CF had the lowest content (5.11 mg kg^{-1}). In general, organic management, especially for the plastic tunnel, had higher nutrient content (NH_4^+ -N, NO_3^- -N, available N, available P and Ntot) and Zntot but lower values of soil pH and C/N ratio than conventional management.

Table 1. The soil chemical properties under different cultivation systems.

Parameter	OF		OP		CF		CP	
pH	7.32 ± 0.03	c	7.28 ± 0.10	c	7.59 ± 0.26	b	7.83 ± 0.13	a
EC (dS m ⁻¹)	0.14 ± 0.01	d	0.34 ± 0.01	a	0.17 ± 0.01	c	0.19 ± 0.02	b
Available N (mg kg ⁻¹)	114.6 ± 14.3	c	2099 ± 410	a	106.1 ± 8.92	c	883.8 ± 31.2	b
Available P (mg kg ⁻¹)	181.7 ± 58.1	b	247.6 ± 32.0	a	9.40 ± 4.63	c	30.5 ± 4.63	c
Ctot (%)	1.93 ± 0.40	b	2.83 ± 0.45	a	1.33 ± 0.16	c	1.24 ± 0.04	c
Ntot (%)	0.19 ± 0.05	b	0.29 ± 0.04	a	0.13 ± 0.01	c	0.12 ± 0.01	c
Ktot (%)	2.06 ± 0.30	ab	2.28 ± 0.16	a	1.89 ± 0.16	b	1.21 ± 0.12	c
C/N	10.0 ± 0.50	b	9.74 ± 0.24	b	10.1 ± 0.57	ab	10.5 ± 0.47	a
CEC (cmol ₍₊₎ kg ⁻¹)	13.2 ± 0.91	b	17.9 ± 1.41	a	12.2 ± 0.99	b	9.58 ± 0.50	c
NO ₃ ⁻ -N (mg kg ⁻¹)	2.67 ± 2.07	c	46.4 ± 4.42	a	18.2 ± 13.1	b	3.36 ± 2.20	c
NH ₄ ⁺ -N (mg kg ⁻¹)	7.86 ± 0.97	a	7.42 ± 1.08	ab	5.11 ± 0.47	c	6.40 ± 1.08	b
Cutot (mg kg ⁻¹)	17.5 ± 1.74	ab	23.2 ± 1.31	a	20.0 ± 7.94	ab	16.1 ± 1.15	b
Zntot (mg kg ⁻¹)	129.5 ± 13.3	ab	156.7 ± 3.08	a	120.1 ± 31.1	b	123.0 ± 22.1	b

Different letters indicate statistically significant differences ($p < 0.05$) according to Duncan's test ($n = 5$). OF: organic management in open fields; OP: organic management in plastic tunnels; CF: conventional management in open fields; CP: conventional management in plastic tunnels; EC: electrical conductivity; CEC: cation exchangeable capacity.

3.2. Microbial α - and β -Diversity

A total of 812,526 ($40,276 \pm 10,768$ per sample) 16S sequences were obtained from the 20 samples covering five replicates for each of the four cultivation systems, which clustered into 7091 OTUs with the number of OTUs ranging from 5074 to 5657 per sample (Table 2). Rarefaction curves showed that the sampling effort was close to the plateau for all treatments, with Good's coverage values ranging between 0.89 and 0.93 (Figure S1 and Table 2), indicating that this processed dataset was sufficient for subsequent analysis.

Table 2. Richness estimators and diversity index for different cultivation systems at a 97% identify threshold.

Cultivation System	Reads	OTUs	Coverage	ACE	Chao 1	Shannon
OF	41,616	5380	0.93	2227 ± 604.3 ab	1439 ± 204.0 ab	9.10 ± 0.19 b
OP	48,983	5657	0.90	2738 ± 563.4 a	1640 ± 169.9 a	9.43 ± 0.13 a
CF	36,064	5161	0.89	1866 ± 92.50 b	1343 ± 85.83 b	9.09 ± 0.08 b
CP	35,842	5047	0.89	1810 ± 196.9 b	1332 ± 144.0 b	9.18 ± 0.17 b

Different letters indicate statistically significant differences ($p < 0.05$) according to Duncan's test ($n = 5$). OTUs: operational taxonomic units; ACE: abundance-based coverage estimator.

Unconstrained PCoA indicated that the composition of microbial communities was clustered clearly under different cultivation systems, while a strong effect of OP was found on the first axis (Figure 1b). PERMANOVA further suggested that management ($p = 0.001$) and cultivation types ($p = 0.002$) significantly influenced microbial β -diversity (Table S1). Moreover, the microbial abundances estimated by ACE and Chao 1 indices were higher in OP than those in conventional management (Table 2). However, no clear difference of microbial abundance was observed between the open field and plastic tunnel under the same agricultural management. In addition, the microbial diversity represented by the Shannon index was significantly higher in OP than in the other cultivation systems.

To get a better understanding of the microbial community composition under these four cultivation systems, we first conducted microbial analysis at the phylum level. The result of pyrosequencing obtained 40 bacterial phyla and three archaea phyla across all soil samples. The three most dominant phyla under different soils were Proteobacteria (32.6% in average), Chloroflexi (12%) and Planctomycetes (9.3%), which account for more than 50% of the total (Figure 2).

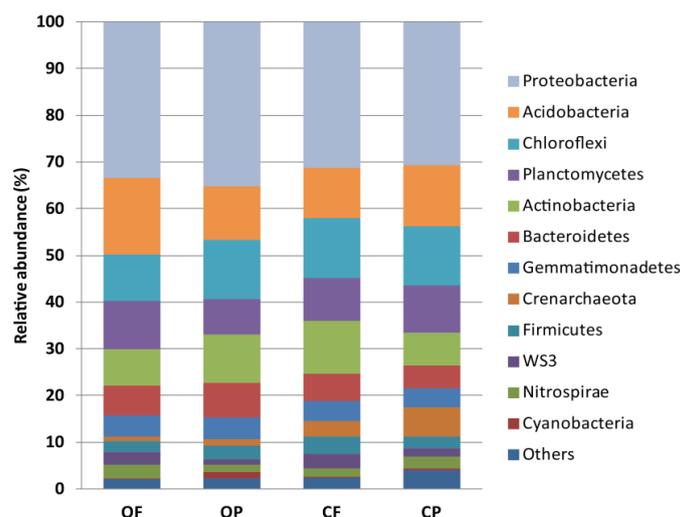


Figure 2. Relative abundance of dominant microbial phyla under different cultivation systems.

3.3. Cultivation System-Associated Microbial Taxa

We further applied LEfSe analysis to explore the potential distinct microbial communities under respective cultivation systems (Figure 3). The results indicated that OP enriched the most potential differential bacterial lineages in these environments. The relative abundances of the OTUs from phyla Planctomycetes and Cyanobacteria were significantly increased in OP. At the genus level, *Roseiflexus* (Chloroflexi) was the most differential taxon in OP (LDA score = 4.52), followed by *Planctomyces* (Planctomycetes), *Butyrivibrio* (Firmicutes), *Leptolyngbya* (Cyanobacteria), *Nostoc* (Cyanobacteria), *Glycomyces* (Actinobacteria), *Bacillus* (Firmicutes), *Microbulbifer* (Proteobacteria), *Pseudoxanthomonas* (Proteobacteria) and *Methylocaldum* (Proteobacteria). In addition, the relative abundances of the genera *Rhodoplanes* (Proteobacteria) and *Skermanella* (Proteobacteria) were significantly enriched in OF and CF, respectively. Notably, the preferential taxa in CP were the Archaeal phylum Crenarchaeota and the genus *Candidatus Nitrososphaera* (Crenarchaeota).

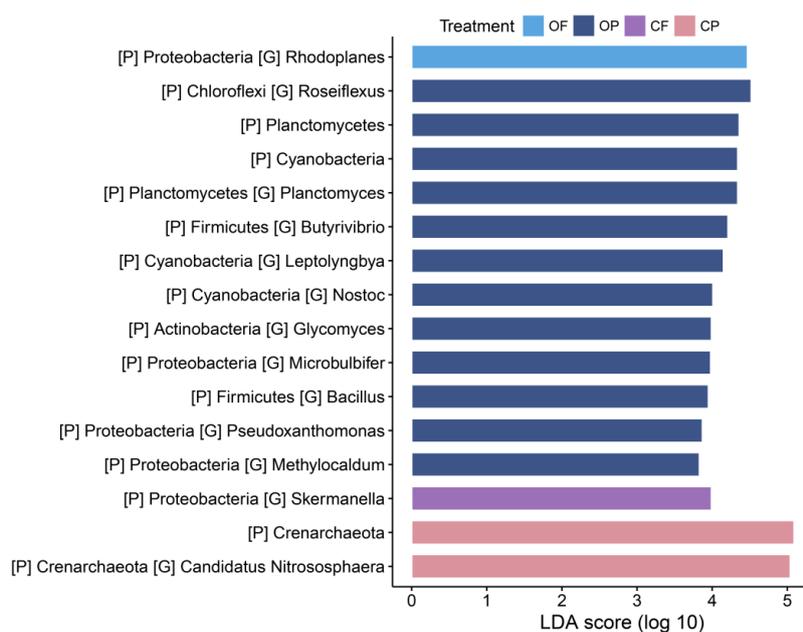


Figure 3. Comparison of microbial variations using the linear discriminate analysis (LDA) of effect size (LEfSe) online tool, with a histogram of the LDA scores for differential phyla and genera among groups. The threshold on the logarithmic LDA score for discriminative features was set to 3.8.

3.4. Relationships between the Microbial Community and Edaphic Properties

RDA and Spearman correlation analyses were used to reveal how microbial communities are related to edaphic properties or may participate in the fundamental nutrient processes. The results of the RDA analysis showed that soil EC, CEC, available N, available P, Ctot and Ntot were the main factors which were highly associated to the soil microbial community in these studied sites ($p = 0.001$, Figure 4, Table S2). In addition, significant correlations were also observed for soil pH, NO_3^- -N and Zntot content ($p = 0.005$, $p = 0.008$ and $p = 0.043$, respectively). Under the genus level, the correlations between differential genera and soil properties are shown in Table 3 (C/N ratio, NH_4^+ -N and Zntot had no significant correlations with any genus). Soil pH was positively correlated to OTUs belonging to *Candidatus Nitrososphaera* and negatively correlated to OTUs belonging to *Rhodoplanes*. The genera associated with conventional management (i.e., *Skermanella* and *Candidatus Nitrososphaera* for open field and plastic tunnel, respectively) were negatively correlated to most of the nutrient-related properties. In contrast, all taxa that positively correlated with the nutrient-related variables and Ctot were highly associated with organic management. These genera were preferentially found in plastic tunnel soils, except for the genus *Rhodoplanes*.

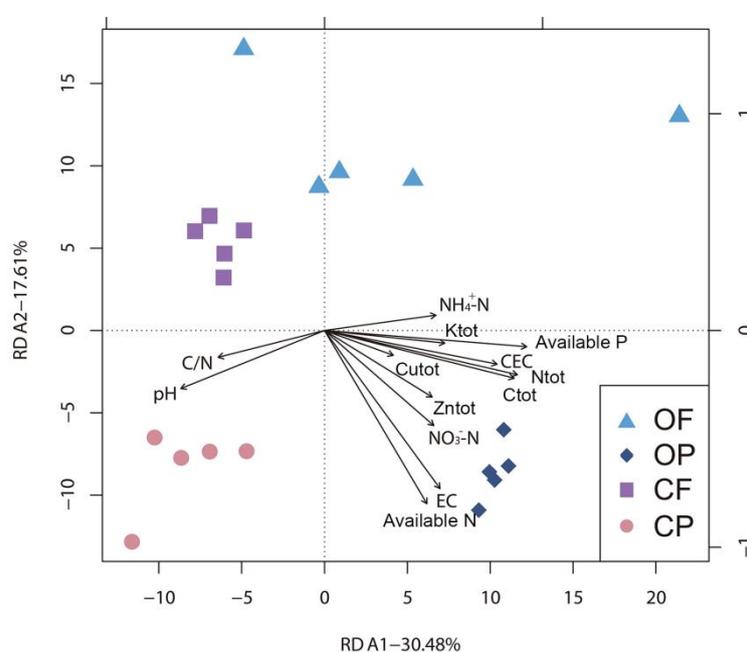


Figure 4. Redundancy analysis (RDA) of soil characteristics and microbial communities for individual samples.

Table 3. Spearman correlation analysis between edaphic properties and differential genera of four cultivation systems.

Treatment	Genus	pH	EC	Available N	Available P	Ctot	Ntot	Ktot	CEC	NO ₃ ⁻ -N	Cutot
CF	<i>Skermanella</i>	0.12	-0.68 **	-0.90 **	-0.69 **	-0.43	-0.41	-0.27	-0.38	-0.34	-0.31
CP	<i>Candidatus</i> <i>Nitrososphaera</i>	0.66 **	0.02	-0.28	-0.74 **	-0.81 **	-0.86 **	-0.84 **	-0.85 **	-0.33	-0.60
OF	<i>Rhodoplanes</i>	-0.64 **	-0.34	-0.10	0.57	0.71 **	0.72 **	0.62 **	0.66 **	0.04	0.30
	<i>Roseiflexus</i>	-0.49	0.76 **	0.75 **	0.65 **	0.69 **	0.70 **	0.48	0.73 **	0.75 **	0.64 **
	<i>Pseudoxanthomonas</i>	-0.06	0.62 **	0.71 **	0.54	0.57	0.49	0.54	0.46	0.45	0.45
	<i>Butyrivibrio</i>	-0.57	0.59	0.60	0.67 **	0.72 **	0.74 **	0.48	0.74 **	0.61 **	0.62 **
	<i>Leptolyngbya</i>	-0.44	0.67 **	0.71 **	0.64 **	0.47	0.53	0.37	0.55	0.36	0.46
OP	<i>Nostoc</i>	-0.23	0.74 **	0.64 **	0.43	0.51	0.50	0.69 **	0.58	0.82 **	0.54
	<i>Glycomyces</i>	-0.35	0.56	0.54	0.43	0.59	0.56	0.33	0.58	0.53	0.61 **
	<i>Microbulbifer</i>	-0.12	0.69 **	0.88 **	0.60 **	0.50	0.42	0.51	0.46	0.47	0.49
	<i>Bacillus</i>	-0.16	0.58	0.53	0.44	0.62 **	0.53	0.32	0.49	0.58	0.49
	<i>Planctomyces</i>	-0.05	0.67 **	0.86 **	0.57	0.45	0.36	0.41	0.30	0.35	0.33
	<i>Methylocaldum</i>	-0.58	0.49	0.54	0.68 **	0.64 **	0.70 **	0.64 **	0.72 **	0.45	0.64 **

Significant correlation was showed in bold. Significance levels: **: $p < 0.01$.

4. Discussion

In this study, we assessed the soil chemical properties and microbial communities in horticultural soils under different management (organic and conventional farming) and cultivation types (open field and plastic tunnel conditions). Overall, our results showed that edaphic variables and the microbial community structure were significantly influenced by management and cultivation type (Figure 1 and Table S1). We further explored the differential microbial taxa that may possess important ecological functions in corresponding cultivation systems (Figures 3 and 4). Moreover, the shifted soil properties under different cultivation systems had strong correlations with the microbial communities in these horticultural soils (Figure 4).

4.1. Agricultural Management Affects Soil Properties

Most of the studies to date have suggested that organic farming systems increase soil pH when compared to conventional farming in acidic soils by buffering from bicarbonates and organic acids in manure [32], while chemical fertilizer addition decreased soil acidity by acidification and nitrification [21]. However, opposite trends were observed here in a moderate alkaline soil in both open-field and plastic-tunnel cultivations. This observation was consistent with our previous study in the same county, which reported that the difference of pH was related to soil microbial activity under different management types [33]. In addition, the available N, available P, N_{tot}, K_{tot}, CEC and NO₃⁻-N were significantly higher in soils managed organically than conventional practices, suggesting that organic farming could provide higher soil nutrient contents and nutrient capacity, potentially through long-term practice. Furthermore, the soil nutrient-related variables also reflected the over-use of fertilizers in both organic and conventional farming systems, especially for those soils under plastic tunnel conditions. For example, the available N in OP and CP was 2099 mg kg⁻¹ and 883.8 mg kg⁻¹, respectively, which was belonged to the extremely high level of soil available N (>150 mg kg⁻¹) according to the national soil survey in China [34]. Similarly, the available P in OP and CP belonged to the extremely high level (>40 mg kg⁻¹) and high level (20–40 mg kg⁻¹), respectively. Notably, the Zn content in the studied soils was between the background level (100 mg Zn kg⁻¹) and regulatory standard (300 mg Zn kg⁻¹) in China [35]. The highest Zn content in soil was observed in OP, which was introduced by organic fertilizer with high Zn content (425 mg kg⁻¹). Although organic farming could improve soil quality, it should be noted that inappropriate application of organic fertilizer might cause potential risks for food safety (e.g., Zn accumulation in plants) and environmental quality (e.g., soil salinity and eutrophication) under long-term use. In addition, the over-use of chemical fertilizer also has negative effects on the environmental quality. To avoid these issues, soil testing has been promoted by the Ministry of Agriculture to determine the application rate of fertilizers and the introduced “action plan for zero increase of fertilizer use in 2020” in 2015 aims to protect the soil quality and increase the effectiveness of fertilizer utilization in China [36].

Plastic-tunnel production provides a physical protection to prevent pests and rainfall from plants and results in a higher rate of evaporation caused by the greenhouse effect than open fields. This phenomenon could be found in our study in terms of the higher salinity (i.e., EC) and nutrient levels in plastic tunnels under both organic and conventional managements. Meanwhile, the optimal temperature in covering cultivation could enhance organic matter degradation in plastic-tunnel production [22], as could be observed from the fact that C_{tot} in CP was 6.8% lower than in the CF plot. However, organic farming increased C_{tot} in both open-field and plastic-tunnel conditions, which could lead to lower bulk density and well aggregated soil in these soils and thus provide better soil conditions for plant growth.

4.2. Soil Microbial Community Shifted by Cultivation Systems

The cultivation histories were different in these cultivation systems, implying that the difference of crops could be a factor to alert soil microbial communities by plant root exudates and/or residues.

However, this possibility was minimized by sampling bulk soil (10–20 cm), and the sampling site was 15 cm away from the plant. In addition, a previous study has suggested that soil properties play predominant roles in shaping the soil bacterial community rather than the influence of crops [37]. In this regard, the effects of agricultural practices (e.g., fertilization) on edaphic properties are stronger than the effects of crops. Thus, we suppose that the shifted soil properties caused by agricultural practices could have a greater influence on the microbial community in soil than the effect of different cultivation histories in these agricultural practices. Additionally, the microbial abundance and diversity in OP were significantly higher than conventional management (i.e., CF and CP). This indicated that plastic-tunnel cultivation could potentially enlarge the effect of organic farming practice on the microbial community, which might be related to the sufficient C supply and the optimal temperature and water conditions [38] when compared to the open-field condition. Thus, organic farming increased the microbial richness and diversity in soils, leading to a more stable composition of the bacterial community under the organic plastic-tunnel condition. However, it should be noted that the studied sites were over-fertilized and the nutrient levels were extremely high in OP. In this regard, this studied soil might not be sustainable. Therefore, the effects of an appropriate lower input of organic farming system on the soil bacterial community should be investigated to get a better understanding of how the microbial community would change under sustainable agricultural practices. Meanwhile, no difference was found between these two cultivation types under conventional farming, which might be related to the use of agricultural chemicals (e.g., pesticides and fungicide) that result in a harmful environment for microbial propagation.

4.3. Differential Taxa for Cultivation Systems

LEfSe analysis showed that the phyla Planctomycetes and Cyanobacteria were the preferential bacterial communities in OP soil. Members belonging to Planctomycetes have been reported as major groups to decompose heteropolysaccharide [39] and chitin [40] and are involved in the C cycle in soils, which might be related to the highest C_{tot} content being in OP soil. OTUs from Cyanobacteria are photoautotrophs and play key roles in biogeochemical processes in nature and improve the turnover of C and N in the soil [41]. In addition, we also observed that the archaeal phylum Crenarchaeota was significantly increased in CP. OTUs belonging to this phylum have been shown to play important role in the N cycle as ammonia oxidizers [21]. However, it is difficult to define their functions at the phylum level, and we thus further considered them at the lower classification [10].

Among the genus levels, a total of 13 treatment-associated genera were found. Among the 10 genera that were enriched in OP, most of them have different signatures related to plant growth promotion and organic material turnover. For example, OTUs of the genera *Nostoc* (Cyanobacteria), *Pseudoxanthomonas* (Proteobacteria), *Glycomyces* (Actinobacteria), *Leptolyngbya* (Cyanobacteria) and *Bacillus* (Firmicutes) have been reported as beneficial bacteria. Specifically, *Nostoc* spp. and *Pseudoxanthomonas* spp. have been shown as N fixers [41,42]. OTUs within the genera *Glycomyces* and *Leptolyngbya* could inhibit other pathogens by producing antibiotics (e.g., streptomycin) [43,44]. *Bacillus* spp. are known as versatile plant growth-promoting bacteria (PGPB) by producing plant hormones (e.g., auxin), antibiotics (e.g., surfactin) and/or phosphatase [2]. This was in line with our previous study, which indicated that organic fertilizer addition could increase the abundance of PGPB and improve the nutrient processes in the soil [45]. In addition, the genera *Roseiflexus* (Chloroflexi), *Planctomyces* (Planctomycetes), *Butyrivibrio* (Firmicutes), *Microbulbifer* (Proteobacteria) and *Methylocaldum* (Proteobacteria) have been shown to be involved in organic substance degradation in soil. *Roseiflexus* spp., *Planctomyces* spp. and *Butyrivibrio* spp. are able to degrade organic substances such as cellulose, hemicelluloses or chitin [46–48]. *Microbulbifer* spp. belong to the aerobic sulfate-reducing bacteria, which have been shown to be dominant carbohydrate degraders in sandy sediments [49]. OTUs assigned to *Methylocaldum* have been reported to possess the *pmoA* gene involved in methane oxidation [50]. We also found positive correlations between these organic substance degraders and nutrient-related soil variables (Table 3), indicating that the increased abundance of

these taxa could potentially improve nutrient availability in the organic protected soil environment. Altogether organic plastic-tunnel soil possessed a versatile differential bacterial species, which may change the soil functions to improve plant fitness and nutrient availability.

The OF soil was significantly enriched the genus of *Rhodoplanes* (Proteobacteria). Most members of *Rhodoplanes* initiate the process of denitrification, which could reduce NO_3^- -N to NO or N_2O and result in N loss [51]. In addition, the relative abundance of *Candidatus Nitrososphaera* (Crenarchaeota belonged to Archaea) and *Skermanella* (Proteobacteria) were significantly increased in CP and CF, respectively. *Candidatus Nitrososphaera* spp. was the only differential genus belonging to the ammonia-oxidizer Archaea (AOA) in this study and able to oxidize NH_4^+ -N to NO_3^- -N, which plays an important role in the N cycle in the environment [52]. This was in line with previous studies, which showed that the abundance of AOA was higher in chemical fertilizer-amended soil than that of organic fertilizer [53]. Members belonging to *Skermanella* are aerobic bacteria and have been isolated from the oligotrophic environment (e.g., desert and coal mine soil) [54,55], which were negatively correlated to nutrient-related soil properties (i.e., EC, available N and available P, Table 3). It has been shown that the relative abundance of *Skermanella* was higher in conventional farming than organic practice under open-field conditions in Canada [56]. Consistently, another study in Mexico indicated that *Skermanella* could be regarded as the bioindicator for conventional open-field soil [10]. This observation implied that a distinct microbial community response to different cultivation systems could be used as a potential bioindicator for evaluating soil condition as soil microbes were the first responders to the changed soil environment.

4.4. Relationships between Soil Properties and Microbial Communities

Given that microbiota have a strong relationship with the soil environment, the shifted environmental factors could reshape the composition of the soil microbial community [9]. Here, RDA analysis showed that different cultivation practices alerted microbial communities in response to the changed soil chemical measurements. Consistent with the previous study, we found that pH had a significant influence on the soil microbial community structure, which was the well-known predictor of the microbial community in soils [11]. Interestingly, nutrient-related properties, except for inorganic N content and K_{tot} , had a higher significant level than pH (Table S2). This phenomenon might be due to the lower pH variation in such small-scale assessment. Therefore, we speculated that the shifted microbial community was mainly caused by the changed nutrient content, especially for the accelerated accumulation of salinity and nutrients under the plastic-tunnel condition when compared to open field (Figure 1b). Notably, despite the Zn content in the soil being lower than the regulatory standard in China (300 mg kg^{-1} when soil pH > 7.5), we observed a significant relationship with the composition of the microbial community, which agreed with previous studies in rice fields [57]. Thus, the potential risk of increased Zn content in soil should be noted in terms of the soil quality and microbial community structure. Further study is needed to focus on the responses of the soil microbial community to the heavy metal content introduced by such organic fertilizer.

5. Conclusions

In this study, we highlight the impacts of agricultural management and cultivation type with high fertilizer input on soil chemical properties and microbial communities. The results showed that organic management significantly improved soil nutrient levels and increased microbial abundance and diversity, especially in the plastic tunnel, when compared to conventional farming. Differential microbial taxa analysis suggested that organic plastic tunnel cultivation-enriched diverse bacterial linkages related to plant growth promotion and organic material turnover were positively correlated to a range of soil nutrient parameters. Furthermore, the microbial community composition was significantly correlated to the soil environment, such as pH, EC, Zn_{tot} and nutrient-related properties (i.e., available N, available P, C_{tot} , N_{tot} , CEC, NO_3^- -N). Our findings indicated that organic farming improved the soil carbon and nutrient content, microbial abundance and diversity, and the abundance

of beneficial microorganisms. However, it should be concerned that the inappropriate application of organic fertilizer containing heavy metals (e.g., Zn) should have more attention paid to it. The excess nutrients and Zn content may negatively affect the soil environmental health and the microbial community in terms of their sustainable agricultural development.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2071-1050/10/10/3825/s1>, Figure S1: Rarefaction curves of microbial communities based on observed species at a 97% identify threshold, Table S1: Effects of agricultural management and cultivation type on soil variables and microbial community, Table S2: Significant level of soil variables in explaining the microbial community based on RDA analysis.

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