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Soil microbial communities of Japanese apricot (*Prunus mume*) orchard under organic and conventional management

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Abstract

Organic farming has positive effects on soil microbial population, process, and activity. To examine effects of two different management methods (organic farming vs. conventional farming) on the cultivation of Japanese apricot, contents of fatty acid methyl ester (FAME), total glomalin, and soil chemical properties were analyzed and compared. The organic farming practice resulted in significantly higher contents of organic matter, total FAME, total bacteria, Gram-negative bacteria, arbuscular mycorrhizal fungi, and total glomalin than the conventional farming practice. Soil organic matter showed positive correlation with contents of soil microbial biomass, total bacteria, total glomalin, Gram-positive bacteria, Gram-negative bacteria, actinomycetes, and arbuscular mycorrhizal fungi. In 2018, the organic farming practice resulted in lower ratios of cy17:0 and 16:1ω7c than the conventional farming practice, indicating that microbial stress was reduced by the input of organic fertilizer into soil. Based on principal component analyses (PCA) of soil microbial communities, ratios of cy17:0 to 16:1ω7c in orchid soil can be used as microbial indicators to distinguish organically farmed orchard soil from conventionally farmed orchard soil.

Keywords: Organic farming, Soil microbial community, cy17:0/16:1ω7c, Japanese apricot

Introduction

The market for organic agricultural produce is rapidly expanding because more and more people are preferring safe food due to widespread awareness about environmental friendliness, wellness, and food safety. In the global organic produce market, the supply is quickly outpaced by the demand [1]. The number of organic farmers and certified cultivation area have increased steadily from 2000 to 2017 at annual growth rates of 23.6% and 28.1%, respectively. The market size of organic agricultural products has increased by an annual average of 3.6%. It is expected to be reach 574.5 billion won in 2025 [2].

Although soil physio-chemical properties are primarily considered for soil management in the case of organic farming, soil microorganisms are also very important for material cycling as key members of the natural ecosystem [3, 4].

In soil–plant systems, soil microbes play a very important role in the formation of soil structure, decomposition of organic matter, and nutrient cycling [5]. Some soil microbes can maintain and stimulate plant growth. They can also suppresses several diseases caused by soil mediated plant pathogens [5]. The use of soil microbial community structure as an indicator of soil quality has been proposed because it is altered by environmental and anthropogenic factors [6, 7].

As microorganisms are directly or indirectly related to crop growth in nutrient cycling (including carbon, nitrogen, and inorganic matter) and antagonism between microorganisms, it is necessary to analyze microbial distribution characteristics and increase microbial activity to effectively manage organic farms [8–10].

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Soil microbial communities have been evaluated conventionally by culture-dependent techniques [11, 12]. Cavigelli et al. [13] have suggested that soil microorganisms by culture-dependent techniques may represent very small and ecologically insignificant parts of the overall diversity present in various soils. Torsvik et al. [14] have reported the presence of at least 4000 bacterial strains per 30 g in forest soil, with culturable strains representing less than 1% of the total strains present. Although culture-dependent methods allow for the isolation of microbial colonies and spores, there are limitations in understanding microbial community structures within the soil environment. Most soil microorganisms cannot be characterized by classical microbial culture techniques. It is estimated that about 80–90% of soil microorganisms cannot be cultured by classical microbial culture methods [15].

For this reason, the need for new technologies to understand the soil microbial community structure is emphasized. Recently, several useful approaches (culture-independent techniques) have been developed to solve problems with conventional culture-dependent methods, including fatty acid methyl ester (FAME) analysis, 16S rRNA probes, and restriction fragment length polymorphism (RFLP) analysis [9, 13, 16–21].

Most of recent studies comparing organic farming with conventional farming practice have focused on biodiversity [22–24]. Studies that perform a comprehensive comparative analysis on soil microbial properties and fertility are limited.

Therefore, the purpose of this study was to provide useful information about soil management of organic farms by examining changes in soil microbial communities and activities and comparing soil chemical properties between organic farming and conventional farming practiced in Japanese apricot orchards. To determine effects of these two different management practices on soil microbial properties, microbial communities were analyzed by FAME, total glomalin, and chemical properties in soils from organic farming system (OFS) and conventional farming system (CFS) of Japanese apricot orchards.

Materials and methods

Experimental sites and soil sampling

We selected three leading farms respectively from organically certified farms and conventional farms located in Hadong, Gyeongnam Province. Three replications of 500 g soil samples were taken from each plot at a depth of 20 to 40 cm during harvesting seasons in 2017 and 2018. Collections of soil samples from different farming systems in Japanese apricot orchards are shown in Table 1. Average temperature and precipitation in experimental sites during the experimental period are shown in Fig. 1. Irrigation was dependent on natural rainfall. The variety of Japanese apricot was Namgo in these orchards. Japanese apricot was harvested from June 1st to June 15th annually by hand.

Analysis of soil chemical properties

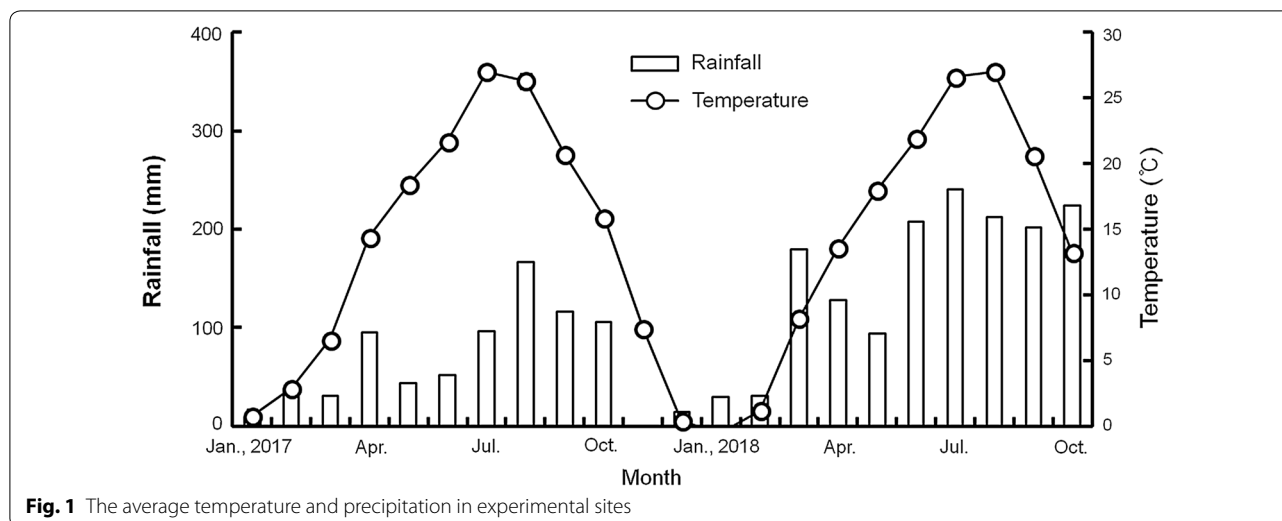
The collected soil samples that were dried in the shade and sieved by a 2 mm sieve were analyzed using the soil chemical analysis method [25]. In terms of the pH and EC of orchard soil, a mixture of soil and distilled water at a ratio of 1:5 was analyzed using the pH meter (Orion 520A pH meter, Orion Research Inc., Boston, USA) and the EC meter (Orion 3STAR EC meter, Orion Research Inc., Boston, USA). Organic matter contents were measured by Tyurin method, and effective phosphate was analyzed using Lancaster method with a colorimeter (Shimadzu Co., Kyoto, Japan). Substituent cations were extracted with 1 M NH₄OAc and analyzed by ICP (Optima 5300 DV, PerkinElmer Co., Shelton, USA).

Analysis of microbial communities and total glomalin in soil samples

Soil microbial contents and communities were analyzed using subsoil with the fatty acid methyl ester (FAME) method to analyze the intrinsic cell wall-bound fatty acids specifically possessed by microorganisms, and the quantification of microorganisms was done using the internal standard 19:0 [26]. The analysis of fatty acids was performed using GC Agilent 6890N (Agilent Technologies, USA) and HP-ULTRA 2 capillary columns

Table 1 Collection of soil samples from different farming system in Japanese apricot orchard

Farming system	Latitude	Longitude	Field size (m ²)	Soil texture	Disease and pest control
Organic farm	35° 05' 22.73"	127° 43' 31.14"	42,975	Loam	Bordeaux mixture, cinnamon and garlic extract, sulfur 80%
	35° 11' 12.17"	127° 42' 02.82"	9917	Loam	Shrubby sophora extract, pyrethrum flower, sulfur
	35° 06' 44.39"	127° 44' 17.27"	14,214	Loam	Shrubby sophora extract, castor oil, plant extract
Conventional farm	35° 01' 33.24"	127° 55' 11.46"	6611	Loam	Buprofezin, imidacloprid, chromafenozide, trifloxystrobin
	35° 05' 27.29"	127° 46' 00.80"	3966	Loam	Acetamidrid, trifloxystrobin
	35° 05' 19.31"	127° 46' 08.31"	13,223	Loam	Sulfoxaflor, acetamidrid, trifloxystrobin



(25 m × 0.2 mm × 0.33 μm film thickness, Agilent Technologies, USA). The column temperature was increased from 170 to 270 °C by 5 °C min⁻¹, and then was maintained at 27 °C for 2 min. In case of analysis on microbial cell wall-bound fatty acids, the microbial communities of each fatty acid were analyzed using a MIDI software program package (MIDI, Inc., Newark, DE) [27]. Total bacteria were analyzed by adding up the contents of i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7, i17:0, a17:0, 17:0, cy17:0, 18:1ω7c, and cy19:0 [18, 26]. Gram-negative bacteria were analyzed by adding up the contents of fatty acids 16:1ω7c, 18:1ω7c, cy17:0, and cy19:0, whereas Gram-positive bacteria were analyzed by adding up the contents of fatty acids i15:0, a15:0, i16:0, i17:0, and a17:0 [28]. Actinomycetes were represented by the fatty acids 10Me18:0 [29], and fungi were represented by the fatty acids 18:1ω9c and 18:2ω6c. The fatty acid 16:1ω5c was used as a biomarker of arbuscular mycorrhizal fungi [30–32]. And the cy17:0 to 16:1ω7c ratios were used as indicators of microbial stress in soil [33].

Total glomalin content was determined using the 100 mM (pH 9.0) sodium pyrophosphate extraction method [34]. 2.0 g of subsoil was placed in a tube and then was added by 8 mL of 100 mM sodium pyrophosphate (pH 9.0). After closing it with a cap, the tube was shaken for about 10 s, and was pressurized at 121 °C for 1 h for extraction. After separating the supernatant by centrifugation at 5000 × g for 10 min, glomalin content was analyzed using the Bradford dye-binding assay method with the colorimeter (UV-1650PC, Shimadzu Co., Kyoto, Japan).

Statistical analysis

All data were statistically analyzed using the SAS software version 9.2 for Windows (SAS Institute, Cary, NC).

Comparisons of chemical properties, total glomalin, microbial biomass and communities in soil samples were performed using two-way ANOVA. The *F*-test was used to detect and separate the mean treatment differences at 0.1% ($p < 0.001$), 1.0% ($p < 0.01$), and 5.0% ($p < 0.05$) levels of significance when significant effects of interactions between independent variables were detected. In addition, significant effects of cy17:0/16:1ω7c ratios in soils were detected, and the LSD was used to detect and separate the mean treatment differences at 5.0% ($p < 0.05$) levels of significance. The microbial communities in soil were analyzed by principal component analysis (PCA) to determine the overall effects of OFS and CFS.

Results and discussion

Soil chemical properties and yield of Japanese apricot

Analysis results of soil chemical compositions of organic Japanese apricot orchards and their conventional counterparts in 2017 and 2018 are summarized in Table 2. Soil organic matter contents in organic farm soil samples were measured to be 58 g kg⁻¹ in 2017 and 46 g kg⁻¹ in 2018, whereas those in conventional farming soil samples were measured to be 34 g kg⁻¹ in 2017 and 32 g kg⁻¹ in 2018, with organic farms showing significantly ($p < 0.05$) higher levels than conventional farms, consistent with results of Li [35] which reported that organic pear orchards had higher organic matter content than their conventional counterparts.

This result might be because organic farming inputs a large quantity of organic matter manure every year to maintain soil organic contents and supply nutrients [35, 36]. However, the study by Kim et al. [37] reported that the organic pear orchard showed a higher level of effective phosphorus at 754 mg kg⁻¹ than the conventional pear farm, although the difference was not statistically

Table 2 Soil chemical properties of the OFS and CFS in soils cultivated with Japanese apricot

System	Year	pH (1:5)	EC (dS m ⁻¹)	OM (g kg ⁻¹)	Av. P ₂ O ₅ (mg kg ⁻¹)	K (Ex. Cat. cmol _c kg ⁻¹)	Ca (Ex. Cat. cmol _c kg ⁻¹)	Mg (Ex. Cat. cmol _c kg ⁻¹)	Na (Ex. Cat. cmol _c kg ⁻¹)
OFS	2017	6.0	0.48	58	370	1.54	6.2	1.6	0.12
	2018	6.0	0.48	46	391	1.28	5.6	1.3	0.05
CFS	2017	5.8	0.61	34	238	0.82	6.2	1.2	0.05
	2018	4.9	0.53	32	268	0.57	2.6	1.1	0.05
Sig. ^a	System	NS	NS	*	NS	NS	NS	NS	NS
	Year	NS	NS	NS	NS	NS	NS	NS	NS
	System x year	NS	NS	NS	NS	NS	NS	NS	NS

OFS organic farming system, CFS conventional farming system, OM soil organic matter

^a Significant effects were obtained from two-way analysis of variance: NS not significant; * $p < 0.05$

significant. There was no significant year-over-year difference in soil organic matter content for organic farms or conventional farms in the present study. Compositions other than organic matter did not show any significant difference depending on farming type or by year either in this study.

The study by Li [35] reported that organic farming showed a higher level of substitutional potassium content than conventional farming, although the difference was not statistically significant. These results might be due to different input amounts of organic matter such as livestock manure which is used by organic farms instead of chemical fertilizer commonly used by conventional farms (Table 3) [38, 39].

Comparative analysis results on application rates of livestock manure, organic fertilizer, nitrogen, phosphoric acid, and potassium fertilizer, as well as damages caused by the outbreak of *Eurytoma maslovskii* between organic farms and conventional farms are summarized in Table 3. In 2018, the application amount of livestock manure in organic farms was measured to be 1167 kg 10a⁻¹, which was higher than that in conventional farms at 800 kg

10a⁻¹. In case of organic matter fertilizers, organic farms used an amount of 488 kg 10a⁻¹ in 2017, which was not significantly higher than that in conventional farms. However, organic farms' Japanese apricot fruit damage rate caused by *Eurytoma maslovskii* was measured to be 36.7% in 2017 and 23.3% in 2018, both of which were significantly higher than conventional farms' fruit damage rate. Choi et al. [40] reported that the overall Japanese apricot fruit damage rate caused by *Eurytoma maslovskii* was 67.3% in 2013 and 33.3% in 2014 in Jeonnam Province. It soared to 90% in one of the most serious cases in the past [41]. This might be because the conventional farm can easily control *Eurytoma maslovskii* with an insecticide. In the case of organic farming, although several studies have reported that various plant extracts can be used by organic farms [42], it is difficult to verify their effects at a field site. Japanese apricot yield in organic farms was decreased to 302 kg 10a⁻¹ in 2017 and 400 kg 10a⁻¹ in 2018 due to fruit damages caused by the outbreak of *Eurytoma maslovskii*. In contrast, Japanese apricot yield in conventional farms was 650 kg 10a⁻¹ in 2017 and 727 kg 10a⁻¹ in 2018, showing significant ($p < 0.01$)

Table 3 Amount of fertilizer, damaged fruit rate due to *Eurytoma maslovskii* and yield by OFS and CFS in Japanese apricot orchard

System	Year	Compost (kg 10 ⁻¹)	Organic fertilizer (kg 10 ⁻¹)	Total application of fertilizer (kg 10 ⁻¹)			Damaged fruit rate (%)	Yield (kg 10 ⁻¹)
				N	P	K		
OFS	2017	568	488	24.5	14.8	13.5	36.7	302
	2018	1167	153	23.5	25.6	18.4	23.3	400
CFS	2017	590	149	9.0	5.9	7.7	0.0	650
	2018	800	184	21.3	26.7	16.3	0.0	727
Sig. ^a	System	NS	NS	NS	NS	NS	*	**
	Year	NS	NS	NS	NS	NS	NS	NS
	System x year	NS	NS	NS	NS	NS	NS	NS

OFS organic farming system, CFS conventional farming system

^a Significant effects were obtained from two-way analysis of variance: NS not significant; * $p < 0.05$

differences in the yield between these two different farming practices. Fruit yield decrease and the outbreak of *Eurytoma maslovskii* showed a negative correlation as shown in Fig. 2 ($y = -6.76x + 621$, $p < 0.05$). These results suggest that although soil nutrient management is important for organic farms to secure a stable yield, how to prevent the outbreak of *Eurytoma maslovskii* in an organic way is also very important.

Soil microbial biomass

Comparative analysis results of soil microbial content between organic Japanese apricot orchards and conventional orchards are shown in Table 4. In 2017, organic farms showed the following mean contents: FAME at 363 nmol g⁻¹, total bacterial at 114 nmol g⁻¹, Gram-negative bacteria at 52 nmol g⁻¹, arbuscular mycorrhizal fungi at 13.5 nmol g⁻¹, and glomalin at 3.22 mg g⁻¹. In 2018, organic farms showed the following mean contents: FAME at 227 nmol g⁻¹, total bacteria at 70 nmol g⁻¹, Gram-negative bacteria at 31 nmol g⁻¹, arbuscular mycorrhizal fungi at 6.5 nmol g⁻¹, and glomalin at

2.26 mg g⁻¹. In contrast, in 2017, conventional farms showed the following mean contents: FAME at 155 nmol g⁻¹, total bacteria at 49 nmol g⁻¹, Gram-negative bacteria at 20 nmol g⁻¹, arbuscular mycorrhizal fungi at 5.0 nmol g⁻¹, and glomalin at 1.32 mg g⁻¹. In 2018, conventional farms had the following mean contents: FAME at 221 nmol g⁻¹ of, total bacteria at 68 nmol g⁻¹, Gram-negative bacteria at 29 nmol g⁻¹, arbuscular mycorrhizal fungi at 4.7 nmol g⁻¹ of, and glomalin at 1.12 mg g⁻¹. Organic orchards showed significantly higher contents of FAME, total bacteria, Gram-negative bacteria, arbuscular mycorrhizal fungi, and glomalin than conventional orchards (all $p < 0.05$). However, there was no significant difference in the content of Gram-positive bacteria, actinomycetes, or fungi between the two. On the other hand, there was no significant difference in microbial content depending on the year. These results were consistent with findings of a previous study which reported that the organic farming practice showed higher levels of microbial contents and bacterial contents than the conventional farming practice [10, 43, 44]. These results were also consistent with findings of a study by Lee and Yun [20], which reported that organic farming showed a significantly higher content of glomalin than conventional farming. More studies on soil microbial diversity at genus level between organic and conventional farming practices need to be conducted in the future.

Ratio of cy17:0 to 16:1ω7c as an indicator of microorganisms' activities are shown in Fig. 3. In 2018, organic farming soil showed a cy17:0 to 16:1ω7c ratio at 0.52, which was significantly lower than conventional farming soil at 0.90 ($p < 0.05$). Generally, if the ratio of cy17:0 to 16:1ω7c ratio is higher, the activity of microorganism is lower [45]. This is due to the accumulation of cyclopropyl fatty acids caused by various factors such as acid soil, nutrient source, and lack of soil moisture, leading to a decrease in microbial activity but an increase

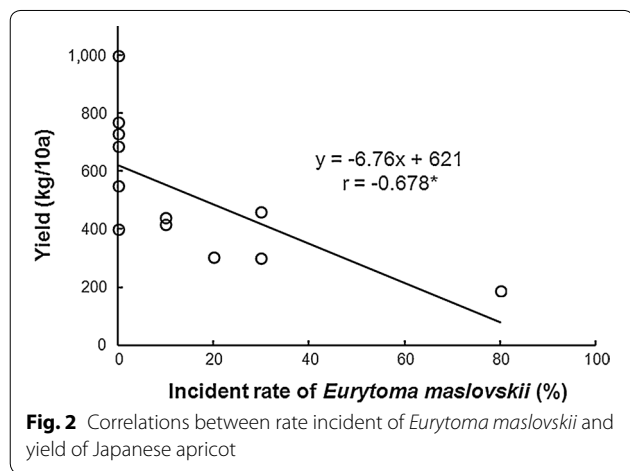


Fig. 2 Correlations between rate incident of *Eurytoma maslovskii* and yield of Japanese apricot

Table 4 Soil microbial properties of the OFS and CFS in soils cultivated with Japanese apricot

System	Year	FAMEs (nmol g ⁻¹) ^b	TB (nmol g ⁻¹)	G(-) (nmol g ⁻¹)	G(+) (nmol g ⁻¹)	A (nmol g ⁻¹)	F (nmol g ⁻¹)	AMF (nmol g ⁻¹)	TG (mg g ⁻¹)
OFS	2017	363	114	52	56	6.4	60	13.5	3.22
	2018	227	70	31	35	4.4	29	6.5	2.26
CFS	2017	155	49	20	26	2.8	29	5.0	1.32
	2018	221	68	29	36	6.1	37	4.7	1.12
Sig. ^a	System	*	*	*	NS	NS	NS	*	*
	Year	NS	NS	NS	NS	NS	NS	NS	NS
	System × year	*	*	*	NS	NS	NS	NS	NS

OFS organic farming system, CFS conventional farming system, FAME total ester-linked fatty acid methyl ester, TB total bacteria, G(-) Gram negative bacteria, G(+) Gram positive bacteria, A actinomycetes, F fungi, AMF arbuscular mycorrhizal fungi, TG total glomalin

^a Significant effects were obtained from two-way analysis of variance: NS not significant; * $p < 0.05$

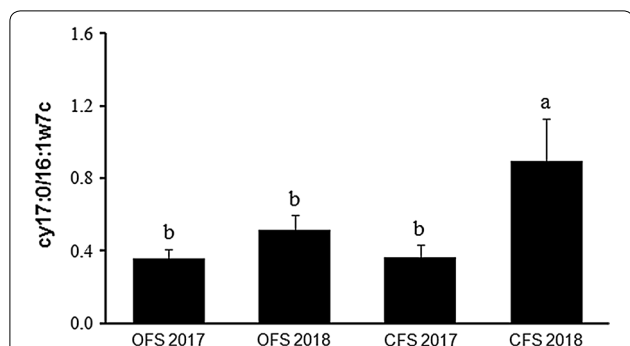


Fig. 3 Ratio of cy17:0 to 16:1w7c in soils subject to the different soil managements. *OFS* organic farming system, *CFS* conventional farming system. The bars represent one standard deviation of the mean. The LSD was used to detect and separate the mean treatment differences at 5.0% levels of significance. Means with the same letter are not significantly different

in stress [46, 47]. This study found that the organically farmed soil had higher pH and organic matter content than the conventionally farmed soil, thus leading to an increase in microbial activity but a decrease in microbial stress.

Relationship among organic matter and microbial biomass
 Analysis results on the correlation between soil organic matter and microbial content are shown in Table 5. Soil organic matter content showed positive correlations with soil FAME ($p < 0.01$), total bacteria ($p < 0.01$),

Gram-negative bacteria ($p < 0.01$), Gram-positive bacteria ($p < 0.05$), arbuscular mycorrhizal fungi ($p < 0.05$), and glomalin content ($p < 0.001$). These results were consistent with findings of Lee and Lee [48] and Lee et al. [49], both of which reported that microbial content was reduced because of an insufficient amount of feed when the soil organic matter content was low. Dinesh et al. [50] have also reported that the addition of organic matter to the soil can increase microbial biomass. Results of the correlation between organic matter content and glomalin content in Japanese apricot orchard soil in the present study were consistent with findings of Kim et al. [51] and Lee et al. [52], both of which reported that the organic matter content had a positive correlation with glomalin content in greenhouse cultivation farms and paddy soil.

Soil microbial community

Results of analyzing microbial communities by dividing soil microbial contents by FAME contents are shown in Table 6. In 2017, the organic farm soil consisted of 31.6% of total bacteria, 14.3% of Gram-negative bacteria, 15.6% of Gram-positive bacteria, 1.7% of actinomycetes, 16.3% of fungi, and 3.6% of arbuscular mycorrhizal fungi. In 2018, the organic farm soil consisted of 31.1% of total bacteria, 13.9% of Gram-negative bacteria, 15.4% of Gram-positive bacteria, 1.9% of actinomycetes, 12.9% of fungi, and 3.0% of arbuscular mycorrhizal fungi. In 2017, the conventional farm soil consisted of 31.2% of total bacteria, 13.2% of Gram-negative bacteria, 16.8% of Gram-positive bacteria, 1.7% of actinomycetes, 18.3% of

Table 5 Relationship among organic matter and microbial concentrations in soils

	FAME	TB	G(-)	G(+)	A	F	AMF	TG
Organic matter	0.804**	0.787**	0.779**	0.767*	0.544	0.473	0.681*	0.934***

FAME fatty acid methyl ester, TB total bacteria, G(-) Gram negative bacteria, G(+) Gram positive bacteria, A actinomycetes, F fungi, AMF arbuscular mycorrhizal fungi, TG total glomalin

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 6 Soil microbial communities of the OFS and CFS in soils cultivated with Japanese apricot

System	Year	TB (%)	G(-) (%)	G(+) (%)	A (%)	F (%)	AMF (%)
OFS	2017	31.6	14.3	15.6	1.7	16.3	3.6
	2018	31.1	13.9	15.4	1.9	12.9	3.0
CFS	2017	31.2	13.2	16.8	1.7	18.3	3.5
	2018	30.7	13.4	15.9	2.8	16.8	2.2
Sig. ^a	System	NS	NS	NS	NS	NS	NS
	Year	NS	NS	NS	NS	NS	NS
	System × year	NS	NS	NS	NS	NS	NS

OFS organic farming system, CFS conventional farming system, TB total bacteria, G(-) Gram negative bacteria, G(+) Gram positive bacteria, A actinomycetes, F fungi, AMF arbuscular mycorrhizal fungi

^a Significant effects were obtained from two-way analysis of variance: NS not significant

fungi, and 3.5% of arbuscular mycorrhizal fungi. In 2018, the conventional farm soil consisted of 30.7% of total bacteria, 13.4% of Gram-negative bacteria, 15.9% of Gram-positive bacteria, 2.8% of actinomycetes, 16.8% of fungi, and 2.2% of arbuscular mycorrhizal fungi. In general, the organically farmed soil showed higher distributions of total bacteria, Gram-negative bacteria, and arbuscular mycorrhizal fungi than the conventionally farmed soil, although differences between these two soil samples were not statistically significant.

Comparative analysis results of microbial community between the organic orchard and the conventional orchard are shown in Fig. 4. After extracting several components from soil microbial communities, main components were analyzed, explained, and used for prediction [20, 49]. The primary component was 35.2% and the secondary component was 26.7%, both of which accounted for 63.9% of all components. In terms of primary components, Gram-positive bacteria and actinomycetes in soil made positive contributions, while arbuscular mycorrhizal fungi and Gram-negative bacteria made negative contributions. According to analysis results of secondary components, the ratio of cy17:0 to 16:1ω7c made the largest contribution. Thus, it could be used as a microbial biomarker to distinguish organically farmed soil from conventionally farmed soil.

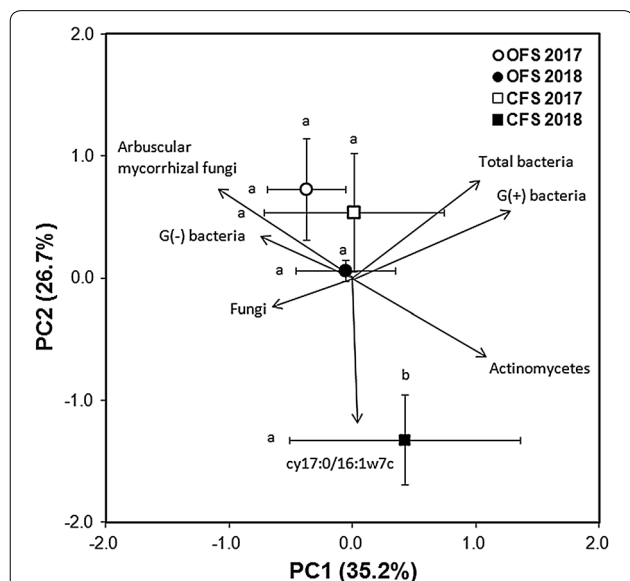


Fig. 4 Principal component analyses of soil microbial communities from organic farming system and conventional farming system. The variance explained by each principal component (PC) axis is shown in parentheses. PC analysis shows loading values for the individual microbial biomarkers. The bars represent one standard error of the mean. The LSD was used to detect and separate the mean treatment differences at 5.0% levels of significance. Means with the same letter are not significantly different

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Authors' contributions

HJC, YHL, JYH and CSD designed and conducted the experiment as well as wrote the manuscript. SLC and SRM inspired the overall work and revised the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data is available in the main text.

Competing interests

The authors declare that they have no competing interests.

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