

# 1 **Title:** Seasonal Change of Microbial Diversity and Its Relation with Soil Chemical 2 Properties in Orchard

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14

15    **Abstract**

16    This study aimed to determine the microbial diversity of different soil depths (0-5 and 5-20 cm) in a subtropical  
17    orchard during different seasons (i.e., Spring, Summer and Autumn) for enrich the knowledgements on micorbes  
18    roles in orchard ecosystem balance. In tracking experiments conducted in an orchard (established in 1996), the  
19    phospholipid fatty acid (PLFA) biomarker method was employed to know soil microbial system. Total PLFAs  
20    concentration did not vary significantly between soil depths but changed between seasons. It peaked in the  
21    summer at  $258.97 \pm 23.48 \mu\text{g g}^{-1}$  soil from 0-5 cm and at  $270.99 \pm 58.94 \mu\text{g g}^{-1}$  soil from 5-20 cm. A total of 33  
22    microbial fatty acid biomarkers were observed and identified in the sampled soil. Quantities of PLFAs for 29  
23    microbe groups varied significantly between seasons, except for 15:0 iso 3OH, 15:1 iso G, 16:0 2OH, and 17:0  
24    iso 3OH. The bacterial PLFAs and fungal and actinomycotic PLFAs in the orchard soil collected in Summer were  
25    significantly higher than in the Spring or Autumn ( $P < 0.01$ ). The number of soil microorganism species (Richness)  
26    and the Simpson and Shannon-Wiener indexes were all the highest in summer. The total PLFAs, bacterial PLFAs,  
27    fungal PLFAs, actinomycotic PLFAs, Richness, or the Simpson and Shannon-Wiener indexes were all  
28    significantly negatively correlated with soil pH, total carbon (TOC), total nitrogen (TN) and cation-exchange  
29    capacity (CEC) ( $P < 0.05$ ).

30    **Keywords:** phospholipid fatty acid (PLFA); red soil; soil microorganisms; subtropics

31 **Introduction**

32 The orchard ecosystem is important for fruit production and carbon sequestration, biodiversity decreases [1] and  
33 soil erosion [2], and pollution [3,4]. Hence, maintaining a balanced orchard ecosystem is essential. Soil microbes  
34 are essential for the functioning of terrestrial ecosystems because they play a unique and indispensable role in  
35 ecosystem balance [5].

36 Soil microbial community change by soil quality evolution because of long-term management. The interaction  
37 between microbes and soil quality is very complex. It is reflected in effects on microbial diversity of chemical  
38 properties change [6-11]. Spatial and temporal distribution of microbial community have some value information  
39 to explain the interaction [12-18]. However, few clear information had be found by observation on spatial and  
40 temporal distribution of microbial community in subtropical orchard.

41 Thus, we hypothesized that height seasonal temperature and moisture variation and vertical soil chemical  
42 properties change would lead to obvious differences in spatial and temporal distribution of microbial community in  
43 subtropical orchard. To demonstrate seasonal and vertical changes in microbial diversity and the links between  
44 soil microbial diversity and soil chemical properties in a subtropical orchard, PLFA was employed to monitor  
45 microorganism quantity and diversity in orchard soils under different seasons in the hilly red soil of the subtropical  
46 zone in southern China. The observations of this study regarding ecological parameters of orchard soil  
47 microorganisms can provide a scientific basis for further studies and management strategies.

48 **Materials and methods**

49 **2.1. Experiment Area**

50 The experimental area was located at the Yuchi Village Experimental Station, Xicheng Township, Youxi County,  
51 Fujian Province, southeast China (26° 25' N, 117°57' E). The area has a subtropical humid monsoon climate with  
52 an actual annual sunshine time of 1781.7 h (accounting for 40% of the available annual sunshine hours); an  
53 annual precipitation of 1,284 mm (Fig. 1); an annual average temperature of 19.2°C; an average temperature in  
54 July of 26.6°-28.9°C and in January of 8.0°-12.0°C; and a frost-free period of more than 312 d. As an peach  
55 orchard water and soil conservation monitoring system, the experimental station was established in 1996 with an

56 altitude of 150 m and a slope of 15° facing south-southeast. The soil was a Quaternary age red soil with a clay  
57 soil texture [19]. The experimental field was originally the secondary shrub-barren hill, and the constructive plant  
58 species were *Dicranopteris dichotoma* Bernh, *Misanthus floridulus* War ex Schum, and *M. sinensis* Anderss.  
59 The surrounding vegetation was mainly coniferous and coniferous-deciduous-bamboo mixed stands with  
60 constructive species of *Pinus massoniana* Lamb and *Cunninghamia lanceolata* Hook and a grove of  
61 *Phyllostachys heterocycla* cv. Pubescens. The station was initially established for fixed soil erosion monitoring.

## 62 2.2. Soil sampling

63 Spring, Summer and Autumn as main treatments had been settled for seasonal factors. On April 27, August 22,  
64 and November 4, 2010, at 3 days (d) after raining to keep soil moisture at the same level, soils were sampled  
65 from three random spots in the non-fertilizing zones of the area surrounding the average tree were collected from  
66 the soil depths of 0-5 and 5-20 cm. The soil temperature before sampling was listed in [Table 1](#), with average  
67 temperature in Spring of 21.3°-22.0°C, Summer of 27.0°-28.0°C and Autumn of 18.0°-20.2°C. The air moisture  
68 tested in the same time was 82% for Spring, 86% for Summer, and 82.6% for Autumn. After collection, the soils  
69 from the same soil layer were mixed well in the field, sealed in bags, and immediately brought to the laboratory for  
70 measurement or stored in a -80°C freezer for soil microbial analysis. Other mixed soil samples were brought to  
71 the laboratory for water content test and air-dried for basic chemical properties analysis.

## 72 2.3. Chemical Analysis

73 Soil pH was measured in deionized water (1:5, soil:water). The total organic carbon (TOC) and total nitrogen (TN)  
74 were determined by potassium dichromate [20] and Kjeldahl digestion-distillation [21]. Exchangeable cations (K<sup>+</sup>,  
75 Na<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup>) were extracted by CH<sub>3</sub>COONH<sub>4</sub> solution (pH = 7.0) [22], and analyzed using atomic  
76 absorption spectrophotometry (AA-6800, Shimadzu Corp., Kyoto, Japan). Soil samples was described in  
77 experimental section and conducted in triplicate.

## 78 2.4. Extraction and determination of microbial PLFAs

79 Four steps followed for soil PLFA extraction: 1). Five grams of soil was placed in a centrifuge tube. Then, 15 mL  
80 of a 0.2 M KOH (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and methanol (Fisher Scientific

81 Worldwide (Shanghai) Co., Ltd., Shanghai, China) solution was added before tightening the cap. The centrifuge  
82 tube was shaken for 5 min at 100 rpm. After shaking, the tube was incubated in a CU600 thermostat water bath  
83 for 5 min at 37°C. This procedure was repeated five times to help release the fatty acids from the soil sample. 2).  
84 Then, the tube was opened, and 3 mL of 1.0 M acetic acid (Sinopharm Chemical Reagent Co., Ltd) solution was  
85 added to decrease the pH of the reaction. 3). After adding 10 mL of n-hexane (Merck Co., Darmstadt, German)  
86 and mixing well, the tubes were centrifuged in an N00077 centrifuge for 15 min using the following settings: rotor:  
87 #12,150; speed: 2000 rpm; time: 15 min; and temperature: 4°C. After centrifugation, the supernatant n-hexane  
88 was transferred to a clean flask and air-dried under a fan. 4). The air-dried sample was re-suspended in 0.5 mL of  
89 a mixture of n-hexane:methyl-tert-butyl ether (Tedia Co., Inc., Fairfield, OH) (1:1, v/v) for 3-5 min and transferred  
90 to gas chromatography (GC) vials for PLFA determination.

91 Microbial PLFAs were determined using the Sherlock Microbial Identification System Sherlock MIS 4.5 (MIDI  
92 Inc., Newark, DE), including a 6890N Gas Chromatograph (GC) system (Agilent Technologies Inc., Palo Alto, CA),  
93 automatic injection devices, quartz capillary column, and flame ionization detector. The standard phospholipid  
94 fatty acid methyl ester (MIDI, Inc.) mixture and extracted samples were analyzed under the following  
95 chromatographic conditions: the temperature increment was controlled by the second-order program, with an  
96 initial temperature of 170°C that increased to 260°C at 5°C min<sup>-1</sup> and then to 310°C at 40°C min<sup>-1</sup> and maintained  
97 at 310°C for 90 sec; vaporization chamber temperature: 250°C; detector temperature: 300°C; carrier gas: H<sub>2</sub> (2  
98 mL min<sup>-1</sup>); blowing gas: N<sub>2</sub> (30 mL min<sup>-1</sup>); pre-column pressure: 68.95 kPa; injection volume: 1 µL; injection split  
99 ratio: 100:1; and ionization mode: electron ionization (EI).

## 100 2.5. Calculation of Microbial Diversity

101 Known concentrations of 19:0 (nonadecanoic methyl ester) were added as internal standards and used to convert  
102 the retention-time peak areas to nanomoles per gram (nmol g<sup>-1</sup>) of soil (absolute abundance) and mole percent  
103 (mol %) (proportional abundance) of liquids. The absolute and proportional abundances of specific microbial  
104 groups were calculated by a summation of diagnostic lipid markers. The sum of 16:1 ω9c (PLFAs configuration  
105 type), 18:1 ω9c and 18:3 ω6c (6, 9, 12) was used to indicate fungi. The sum of 17:0 10 methyl and 18:0 10 methyl  
106 18:1 ω9c and 18:3 ω6c (6, 9, 12) was used to indicate actinomycetes. The sum of 12:0, 14:0, 14:0 anteiso, 14:0  
107 iso, 15:0 2OH, 15:0 3OH, 15:0 anteiso, 15:0 iso, 15:0 iso 3OH, 15:1 iso G, 16:0, 16:0 10 methyl, 16:0 2OH, 16:0

108 anteiso, 16:0 iso, 16:1  $\omega$ 5c, 17:0 anteiso, 17:0 cyclo, 17:0 iso, 17:0 iso 3OH, 17:1  $\omega$ 8c, 18:0, 18:0 iso, 18:1  $\omega$ 7c,  
109 18:1  $\omega$ 7c 11 methyl, 18:3  $\omega$ 6c (6,9,12), 19:0 cyclo  $\omega$ 8c, 19:0 iso, and 20:0 was used to indicate bacteria [23].

110 The number of species (Richness), Simpson diversity, Shannon-Wiener diversity and Alatalo evenness were  
111 used to calculate the ecological parameters of the microbial fatty acid biomarkers. The calculation equations are  
112 expressed as follows:

113 The Simpson diversity index was calculated according to  $D = 1 - \sum Pi^2$  (1);

114 The Shannon-Wiener diversity index was calculated according to  $H = - \sum Pi \bullet \ln(Pi)$  (2)

115 The Alatalo evenness index was calculated according to  $J = \frac{[1/\sum (Pi^2) - 1]}{[\exp(H) - 1]}$  (3)

116 where  $Pi = \frac{Ni}{N}$ ; Ni is the content of the i<sup>th</sup> kind of phospholipid fatty acid (PLFA); and N is the total PLFA  
117 content.

## 118 2.6. Statistical Analysis

119 An one-way ANOVA followed by least significant difference (LSD) multiple comparison test was used to establish  
120 significant differences among the means of soil properties, microbial community indicators (Bacterial PLFAs,  
121 Fungal PLFAs, Actinomycotic PLFAs, and B/F ratio of PLFAs), and microbial diversity indicators (Richness,  
122 Simpson index, Shannon-Wiener index and Alatalo index) in different seasons. A two-way analysis was used to  
123 establish significant differences in the total PLFA and individual PLFA variance between seasons and soil depths.  
124 A Spearman coefficient analysis was used to measure the correlation between soil properties and microbial  
125 indicators. A principle component analysis was used to measure the microbial community change between  
126 different seasons and soil depths. These analyses all used SPSS software version 17.0.

## 127 Results

### 128 3.1. Soil Chemical Properties of Different Seasons in the Trial Orchard

129 The soils could be classified as clay, thermal and Typic Hapludult [24]. Principle chemical properties of the soil  
130 samples collected in the Spring, Summer and Autumn in the 0-5 and 5-20 cm depths are presented in Table 2.

131 Acidic soil pH (0-5 cm: 4.46; 5-20 cm: 4.38), total organic carbon (TOC) (0-5 cm: 12.32 g kg<sup>-1</sup>; 5-20 cm: 10.25 g  
132 kg<sup>-1</sup>), total nitrogen (TN) (0-5 cm: 1.14 g kg<sup>-1</sup>; 5-20 cm: 0.86 g kg<sup>-1</sup>), and cation-exchange capacity (CEC) (0-5 cm:  
133 4.95 cmol(+) kg<sup>-1</sup>; 5-20 cm: 5.34 cmol(+) kg<sup>-1</sup>) were the lowest in the Summer samples, with significant differences  
134 ( $P < 0.05$ ). However, the exchangeable K<sup>+</sup> (0-5 cm: 141.79 g kg<sup>-1</sup>; 5-20 cm: 99.22 g kg<sup>-1</sup>) and Na<sup>+</sup> (0-5 cm:  
135 30.67 g kg<sup>-1</sup>; 5-20 cm: 43.80 g kg<sup>-1</sup>) were significantly higher than in the other seasons. Furthermore, the ratio of  
136 total organic carbon to total nitrogen (C/N ratio) in soil varied significantly between seasons, with the highest  
137 value in the Spring (0-5 cm: 15.81; 5-20 cm: 12.85), the middle value in the Summer (0-5 cm: 10.76; 5-20 cm:  
138 11.88), and the lowest value in the Autumn (0-5 cm: 5.90; 5-20 cm: 9.04) ( $P < 0.05$ ). No significant difference  
139 was detected between seasons in exchangeable Mg<sup>2+</sup> or Ca<sup>2+</sup> ( $P > 0.05$ ).

140 No significant difference of pH, TOC, C/N ratio, CEC, exchangeable Mg<sup>2+</sup> or Ca<sup>2+</sup> was found between soil  
141 depths.

### 142 3.2. PLFAs of Total Microbial, Bacteria, Fungi and Actinomycotic of Different Seasons

143 The total microbial PLFAs peaked in the Summer in both the 0-5 and 5-20 cm soil depths (0-5 cm: 258.97 µg g<sup>-1</sup>  
144 soil,  $P < 0.01$ ; 5-20 cm: 270.99 µg g<sup>-1</sup> soil,  $P < 0.01$ ). The results of the two-way analysis demonstrated that  
145 the total microbial PLFAs were significantly different between seasons ( $P < 0.001$ ). The quantities of bacterial,  
146 fungal and actinomycotic PLFAs in the orchard soil increased significantly in the Summer compared to those in  
147 the Spring and Autumn in the 0-5 and 5-20 cm soil depths ( $P < 0.01$ ). In the 0-5 cm soil depth, the peak values  
148 of bacterial PLFAs, fungal PLFAs and actinomycotic PLFAs were 216.05, 33.94 and 8.96 µg g<sup>-1</sup> soil, respectively.  
149 For the 5-20 cm soil depth, bacterial PLFAs, fungal PLFAs and actinomycotic PLFAs summit to 230.00, 31.15 and  
150 9.83 µg g<sup>-1</sup> soil, respectively (Table 3). Microbial PLFAs contents (µg g<sup>-1</sup> soil) of different seasons sampled at 0-5  
151 and 5-20 cm soil depths in orchard is shown in Table 4. Two-way analysis of variance for the effects of seasons  
152 (Spring, Summer, Autumn) and soil depths (0-5 cm, 5-20 cm) on microbial PLFAs contents are demonstrated on  
153 Table 5. Quantities of PLFAs for 29 microbe groups varied significantly between seasons, except for 15:0 iso  
154 3OH, 15:1 iso G, 16:0 2OH, and 17:0 iso 3OH.

### 155 3.3. Microbial Diversity Change between Seasons in the Trial Orchard

156 The microbial diversity analysis of the orchard soil showed that the Richness (number of microbial species) was in  
157 the range of 18 to 31, and the Simpson index, Shannon-Wiener index and Alatalo index were 0.76 to 0.93, 3.16 to  
158 3.97 and 0.59 to 0.76, respectively (Fig. 2). The microbial diversities of the orchard soil in different seasons varied.  
159 In the 0-5 cm soil depth, the Richness and the Simpson, Shannon-Wiener and Alatalo indexes peaked in the  
160 Summer, with values of 30.0, 0.92, 3.91 and 0.65, respectively. The Simpson and Shannon-Wiener indexes in the  
161 Summer were significantly different from those in the Spring and Autumn ( $P < 0.05$ ). A significantly difference in  
162 Richness was been found between the Summer and Spring. No significant difference in the Alatalo index  
163 between seasons was found ( $P > 0.05$ ). In the 5-20 cm soil layer, the highest values of Richness and the  
164 Simpson and Shannon-Wiener index were still been found in the Summer, with values of 29.2, 0.92 and 3.81,  
165 respectively. The Richness and the Simpson index of the soil microbes in the Summer were significantly higher  
166 than those in the Spring ( $P < 0.05$ ). There was no significant difference in the Shannon-Wiener index. However,  
167 the Alatalo index of the soil microbes in the Autumn (0.76) was significantly higher than that in the Spring (0.62) ( $P$   
168  $< 0.05$ ). Overall, the microbial diversity of the orchard soil in the Summer was higher than in the Spring and  
169 Autumn.

#### 170 3.4. Correlation between Microbial Communities and Soil Properties

171 The correlation analysis was carried out between the soil properties (pH, TOC, TN, C/N ratio, CEC, and  
172 exchangeable  $K^+$ ,  $Na^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$ ), microbial quantities (total PLFAs, bacterial PLFAs, fungal PLFAs,  
173 actinomycotic PLFAs, B/F ratio) and microbial diversities (Richness, the Simpson, Shannon-Wiener and Alatalo  
174 indexes). The results showed that the microbial quantities (total PLFAs, bacterial PLFAs, fungal PLFAs, and  
175 actinomycotic PLFAs) were significantly negatively correlated with the pH, TOC, TN and CEC, with Spearman  
176 correlation coefficients of -0.530 to -0.618 ( $P < 0.01$ ), -0.572 to -0.642 ( $P < 0.01$ ), -0.401 to -0.422 ( $P < 0.05$ ) and  
177 -0.791 to -0.831 ( $P < 0.01$ ), respectively (Table 5). The microbial diversity (Richness, and the Simpson and  
178 Shannon-Wiener indexes) was significantly negatively correlated with the pH, TOC, TN and CEC, with Spearman  
179 correlation coefficients of -0.460 to -0.753 ( $P < 0.05$ ), -0.419 to -0.707 ( $P < 0.05$ ), -0.450 to -0.526 ( $P < 0.05$ ), and  
180 -0.446 to -0.722 ( $P < 0.05$ ), respectively (Table 6).

181 **Discussion**

182 **4.1. Microbial Population Variation of Soil Ecosystem Among Seasons**

183 Usually, microbial population varies by moisture and temperature change among seasons. However, whether this  
184 change could be found easily depend on how long the suitable season last and how hard the extreme water and  
185 temperature condition affect on soil microorganisms. In subtropical mountain area, the soil was utilized as  
186 planting peach, obvious microbial change was confirmed in this article. And, principal component 1 explained  
187 46.8 % of the variation in the soil microbial community (Fig. 3). The total, bacterial, fungal, and actinomycotic  
188 PLFAs, the B/F ratio, and the Richness were the main drivers of principal component 1 (Table S1). The  
189 remarkable change in microbial community was related to the peak soil microorganism growth in the Summer  
190 because of comfortable temperature and rainfall.

191 Principal component 2 explained 26.5 % of the variation in the soil microbial community (Fig. 3). The Simpson  
192 index and Shannon-Wiener index were its major drivers (Table S1). This could be explained by the  
193 microorganism propagating well and maintaining a good balance in the Summer in the tested orchard soil system.

194 The results of our study are consistent with the results of Zhu et al. [14] in an evergreen broadleaf forest and  
195 Qi et al. [15] in a bamboo grove in the subtropical climate zone. An investigation of an orchard [13], grassland [12]  
196 and forest [17] in the temperate monsoon climate zone was reported similar trends. However, unlike our results,  
197 Shi et al. [16] observed seasonal variations characterized by low values for most of the microbial biomass (C, N,  
198 and P), enzyme activities and PLFAs in the Summer in southwestern Quebec, Canada. This difference is related  
199 to the particularly dry climate (drier than the long-term average of the season) with a significantly lower moisture  
200 content than in the Spring and Autumn. It is well documented that temperature and moisture are the main factors  
201 related to microbial abundance and distribution.

202 **4.2. Relationship between Soil Physicochemical Properties and Microbial Communities**

203 The reported relationship between soil physicochemical properties and microbial communities varies among  
204 studies. In this study, in the view of distribution among seasons and soil depths, the total PLFAs, bacterial PLFAs,  
205 fungal PLFAs, actinomycotic PLFAs, number of species (Richness), Simpson and Shannon-Wiener diversity  
206 indexes were significantly negatively correlated with the soil TOC, TN and CEC. In the Summer, of most soil  
207 microbes experienced rapid growth, and a significant decrease in the soil pH, TOC, TN, and CEC was found

208 (Table 1). Liu et al. [18] also found the microbial dominance index and Shannon-Wiener index to be negatively  
209 related to soil  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N in an apple system by observation on different growing periods. However, in the  
210 view of change by utilization, Yao et al. [6] illustrated microbial biomass C, basal respiration and total PLFA to be  
211 highly correlated with organic C and TN on red soil orchard ecosystem. In some ecosystem soil microbes grew  
212 rapidly with energy and nutrition consumption [25, 26]. It is clearly suggested that the propagation and growth of  
213 soil microorganisms from Spring to Summer require energy from TOC and nutrition from N in an subtropical  
214 orchard system. Microbes mainly act as “consumers” could be well documented.

## 215 **Conclusions**

216 In subtropical orchards, the temperature and humidity in the Summer are conducive to the growth of soil  
217 microorganisms. PLFA analysis showed that the quantity of soil microbes in the soil samples collected in the  
218 Summer was significantly higher than in the Spring and Autumn. The Simpson and Shannon-Wiener indices also  
219 both peaked in the Summer sample collections. The total PLFAs, bacterial PLFAs, fungal PLFAs, actinomycotic  
220 PLFAs, Richness, and the Simpson and Shannon-Wiener indexes were significantly negatively correlated with  
221 seasonal changes in the soil pH, TOC, TN and CEC.

222 The function of microbial community in the translation and accumulation of soil nutrition from Summer to  
223 Autumn should be studied further. The changes in functional flora (including Archaea) in the soil microorganisms  
224 resulting from different orchard management strategies merit further investigations, and the relationships between  
225 the flora change and the orchard litter, soil organic matter (SOM), soil C/N ratio, ammonia N, nitrate N, pH, soil  
226 respiration, and other parameters should be merited further studied.

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233 **Author Contributions**

234 **Funding acquisition:** BW XL.  
235 **Project administration:** BW.  
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237 **Chemical Analysis:** XL.  
238 **Determination of microbial PLFAs:** GH.  
239 **Statistical Analysis:** XL.  
240 **Writing-original draft:** XL.  
241 **Writing-review & editing:** MW BW.

242 **References**

- 243 1. Simon S, Bouvier JC, Debras JF, Sauphanor B. Biodiversity and pest management in orchard systems. A  
244 review. *Agron. Sustainable Dev.* 2010; **30**: 139-152.
- 245 2. Labrière N, Locatelli B, Laumonier Y, Freycon V, Bernoux M. Soil erosion in the humid tropics: A systematic  
246 quantitative review. *Agric. Ecosyst. Environ.* 2015; **203**: 127-139.
- 247 3. Rowlings DW, Grace PR, Scheer C, Kiese R. Influence of nitrogen fertiliser application and timing on  
248 greenhouse gas emissions from a lychee (*Litchi chinensis*) orchard in humid subtropical Australia. *Agric.*  
249 *Ecosyst. Environ.* 2013; **179**: 168-178.
- 250 4. Cai MF, McBride MB, Li KM. Bioaccessibility of Ba, Cu, Pb, and Zn in urban garden and orchard soils.  
251 *Environ. Pollut.* 2016; **208**: 145-152.
- 252 5. Young IM, Crawford JW. Interactions and self-organization in the soil-microbe complex. *Science (New*  
253 *series)*, 2004; **304**: 1634-1637.
- 254 6. Yao H, He Z, Wilson MJ, Campbell CD. Microbial biomass and community structure in a sequence of soils  
255 with increasing fertility and changing land use. *Microb. Ecol.* 2000; **40**: 223-237.

256 7. Li R, Khafipour E, Krause DO, Entz MH, de Kievit TR. Pyrosequencing reveals the influence of organic and  
257 conventional farming systems on bacterial communities. *PLoS One*; 2012; **7**, e51897.

258 8. Mercier A, Dector MC, Harris-Hellal J, Breeze D, Mouvet C. Distinct bacterial community structure of 3  
259 tropical volcanic soils from banana plantations contaminated with chlordcone in Guadeloupe (French West  
260 Indies). *Chemosphere*. 2013; **92**: 787-794.

261 9. Joa JH, Weon YH, Hyun HN, Jeun YC, Koh SW. Effect of long-term different fertilization on bacterial  
262 community structures and diversity in citrus orchard soil of volcanic ash. *J. Microbiol.* 2014; **52**: 995-1001.

263 10. Yang DW, Zhang MK. Effects of land-use conversion from paddy field to orchard farm on soil microbial  
264 genetic diversity and community structure. *Eur. J. Soil Biol.* 2014; **64**: 30-39.

265 11. Gilbert JA, Field D, Swift P, Thomas S, Cummings D, Temperton B, Weynberg K, Huse S, Hughes M, Joint I,  
266 Somerfield PJ, Mühlung M, Rodriguez-Valera F. The taxonomic and functional diversity of microbes at a  
267 temperate voastal site: a 'Multi-Omic' study of seasonal and diel temporal variation. *PloS One*. 2010; **5**: 1-17.

268 12. Bardgett RD, Lovell RD, Hobbs PJ, Jarvis SC. Seasonal changes in soil microbial communities along a  
269 fertility gradient of temperate grasslands. *Soil Biol. Biochem.* 1999; **31**: 1021-1030.

270 13. Shishido M, Sakamoto K, Yokoyama H, Momma N, Miyashita S. Changes in microbial communities in an  
271 apple orchard and its adjacent bush soil in response to season, land-use, and violet root rot infestation. *Soil  
272 Biol. Biochem.* 2008; **40**: 1460-1473.

273 14. Zhu WZ, Cai XH, Liu XL, Wang JX, Cheng JX, Cheng S, Zhang XY, Li DY, Li MH. Soil microbial population  
274 dynamics along a chronosequence of moist evergreen broad-leaved forest succession in southwestern  
275 China. *J. Mount. Sci.* 2010; **7**: 327-338.

276 15. Qi LH, Ai WS, Fan SH, Du MY, Meng Y, Mao C. Soil microbial biomass carbon dynamics of *Phyllostachys*  
277 *edulis* forests under different managing patterns in the hilly region of central Hunan, southern China. *J.*  
278 *Nanjing Forest Univ.* 2013; **37**: 45-48 (in Chinese with English abstract).

279 16. Shi Y, Lalande R, Hamel C, Ziadi N, Gagnon B, Hu Z. Seasonal variation of microbial biomass, activity, and  
280 community structure in soil under different tillage and phosphorus management practices. *Biol. Fert. Soils.*  
281 2013; **49**: 803-818.

282 17. Kim CS, Nam JW, Jo JW, Kim SY, Han JG, Hyun MW, Sung GH, Han SK. Studies on seasonal dynamics of  
283 soil-higher fungal communities in Mongolian oak-dominant Gwangneung forest in Korea. *J. Microbiol.* 2016;  
284 **54**: 14-22.

285 18. Liu LZ, Qin SJ, Lu DG, Wang BY, Yang ZY. Variation of potential nitrification and ammonia-oxidizing  
286 bacterial community with plant-growing period in apple orchard soil. *J. Integr. Agric.* 2014; **13**: 415-425.

287 19. Zhuan WM. Map of Fujian Province. Fuzhou: Fujian provincial map and atlas publishing house; 2008.

288 20. Nelson DW, Sommers LE. Total carbon, organic carbon and organic matter. In Page AL, Miller RH. and  
289 Keeney DR. editors. *Methods of Soil Analysis*. Madison WI: Soil Sci. Soc. Am. 1982; 539-577.

290 21. Bremner JM, Mulvaney CS. Total nitrogen. In: Page AL, Miller RH, Keeney DR, editors. *Methods of Soil*  
291 *Analysis*. Madison WI: Soil Sci. Soc. Am.; 1982.

292 22. Jackson ML. *Soil Chemical Analysis*. Published by Author, 2nd ed. Madison WI; 1979.

293 23. Zhu YJ, Hu GP, Liu B, Xie HA, Zheng XF, Zhang JF. Using phospholipid fatty acid technique to analysis the  
294 rhizosphere specific microbial community of seven hybrid rice cultivars. *J. Integr. Agric.* 2012; **11**:  
295 1817-1827.

296 24. Soil Survey Staff. *Keys to Soil Taxonomy*. 11th ed. Washington, DC: USDA-Natural Resources Conservation  
297 Service; 2010.

298 25. Huang PM, Wang MK, Chiu CY. Soil mineral-organic matter-microbe interactions: impacts on  
299 biogeochemical processes and biodiversity in soils. *Pedobiogia*. 2005; **49**: 609-635.

300 26. Huang PM, Wang SL, Tzou YM, Huang YB , Weng BQ, Zhuang SY, Wang MK. Physicochemical and  
301 biological interfacial interactions: impacts on soil ecosystem and biodiversity. *Environ. Earth. Sci.* 2013; **28**:  
302 2199-2209.

303

304 **Table 1**  
305 Soil temperature and air moisture at different sampling stages in orchard

Item	Spring	Summer	Autumn
Soil temperature (°C)			
0 cm	21.3	27.5	18.0
5 cm	22.0	27.7	19.0
10 cm	21.5	28.0	19.5
15 cm	21.7	27.3	20.2
20 cm	21.4	27.0	19.7
Air relative moisture (%)	82.0	86.0	82.6

306 Soil temperature and air moisture was the average of two weeks data before sampling that was on April 27 for  
307 Spring, on August 22 for Summer and on November 4 for Autumn.  
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**Table 2**

Chemical characteristics of soil sampling in different seasons at 0-5 and 5-20 cm soil depths in orchard

Item	Spring	Summer	Autumn
0-5 cm			
pH	4.87±0.06 a (5)	4.46±0.08 b (5)	4.95±0.04 a (5)
TOC (g kg <sup>-1</sup> )	20.90±1.07 a	12.32±1.53 b	20.92±1.17 a
TN (g kg <sup>-1</sup> )	1.46±0.24 bc	1.14±0.08 c	3.95±0.75 a
C/N ratio	15.81±3.30 a	10.76±0.93 b	5.90±1.18 c
CEC (cmol(+) kg <sup>-1</sup> )	9.42±0.26 a	4.95±0.29 c	7.09±0.25 b
Exchangeable K <sup>+</sup> (mg kg <sup>-1</sup> )	124.00±15.64 a	141.79±30.11 a	99.67±13.16 b
Exchangeable Na <sup>+</sup> (mg kg <sup>-1</sup> )	17.75±0.62 b	30.67±3.61 a	14.09±1.13 b
Exchangeable Mg <sup>2+</sup> (mg kg <sup>-1</sup> )	30.14±3.83 a	28.41±7.56 a	24.80±2.79 a
Exchangeable Ca <sup>2+</sup> (mg kg <sup>-1</sup> )	163.98±20.25 a	122.72±20.74 a	192.83±33.25 a
5-20 cm			
pH	4.81±0.06 a (5)	4.38±0.07 b (5)	4.83±0.08 a (5)
TOC (g kg <sup>-1</sup> )	19.15±0.92 a	10.25±0.89 b	18.78±0.67 a
TN (g kg <sup>-1</sup> )	1.61±0.14 ab	0.86±0.06 b	2.20±0.25 a
C/N ratio	12.85±0.81 ab	11.88±0.55 b	9.04±1.10 bc
CEC (cmol(+) kg <sup>-1</sup> )	9.29±0.56 a	5.34±0.39 c	6.87±0.18 b
Exchangeable K <sup>+</sup> (mg kg <sup>-1</sup> )	62.85±7.54 ab	99.22±12.24 a	55.95±7.85 ab
Exchangeable Na <sup>+</sup> (mg kg <sup>-1</sup> )	15.22±1.26 b	43.80±5.91 a	10.21±0.85 b
Exchangeable Mg <sup>2+</sup> (mg kg <sup>-1</sup> )	22.71±6.17 a	34.47±8.80 a	20.05±4.82 a
Exchangeable Ca <sup>2+</sup> (mg kg <sup>-1</sup> )	121.38±22.12 a	117.97±51.79 a	164.97±18.31 a

311 TOC = total organic carbon, TN = total nitrogen, CEC = cation exchange capacity, C/N ratio = ratio of total organic  
312 carbon to total nitrogen. Values followed by the same letter (s) in a low are not significantly different at  $P < 0.05$   
313 using LSD post hoc tests. Case number is shown in parentheses  
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**Table 3**

Contents of total PLFAs, bacterial PLFAs, fungal PLFA, actinomycetal PLFAs, and the ratio of bacterial to fungal of soils sampling on different seasons at 0-5 and 5-20 cm depths in orchard

Item	Spring	Summer	Autumn
0-5 cm			
Total PLFAs ( $\mu\text{g g}^{-1}$ soil)	14.07 $\pm$ 8.23 c B (4)	258.97 $\pm$ 23.48 a A (5)	99.93 $\pm$ 18.62 b B (4)
Bacterial PLFAs ( $\mu\text{g g}^{-1}$ soil)	11.46 $\pm$ 6.75 b B	216.05 $\pm$ 20.44 a A	79.79 $\pm$ 14.56 b B
Fungal PLFAs ( $\mu\text{g g}^{-1}$ soil)	2.27 $\pm$ 1.23 c B	33.94 $\pm$ 3.96 a A	16.09 $\pm$ 3.64 b B
Actinomycetal PLFAs ( $\mu\text{g g}^{-1}$ soil)	0.34 $\pm$ 0.26 b B	8.96 $\pm$ 0.81 a A	4.05 $\pm$ 1.00 b B
B/F ratio	4.79 $\pm$ 0.29 a A	6.37 $\pm$ 0.61 a A	5.15 $\pm$ 0.60 a A
5-20 cm			
Total PLFAs ( $\mu\text{g g}^{-1}$ soil)	10.63 $\pm$ 1.58 b B (5)	270.99 $\pm$ 58.94 a A (5)	51.28 $\pm$ 7.17 b B (5)
Bacterial PLFA ( $\mu\text{g g}^{-1}$ soil)	8.66 $\pm$ 1.29 b B	230.00 $\pm$ 50.36 a A	44.60 $\pm$ 6.05 b B
Fungal PLFA ( $\mu\text{g g}^{-1}$ soil)	1.71 $\pm$ 0.32 c B	31.15 $\pm$ 6.26 a A	5.37 $\pm$ 0.83 bc B
Actinomycetal PLFA ( $\mu\text{g g}^{-1}$ soil)	0.24 $\pm$ 0.05 b B	9.83 $\pm$ 2.45 a A	1.30 $\pm$ 0.60 b B
B/F ratio	5.33 $\pm$ 0.59 b B	7.38 $\pm$ 0.24 a AB	8.70 $\pm$ 1.07 a A

318 Mean  $\pm$  standard error. the values followed by the same lowercase and capital letter (s) in a column are not  
319 significantly different at  $P < 0.05$  and  $P < 0.01$  using LSD post hoc tests, respectively. Case number is shown  
320 in parentheses, 1 case was missed for Spring and Autumn at 0-5 cm depth respectively.  
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**Table 4**

Microbial PLFAs contents ( $\mu\text{g g}^{-1}$  soil) of different seasons sampled at 0-5 and 5-20 cm soil depths in orchard

Fatty acid configuration	0-5 cm			5-20 cm		
	Spring (4)	Summer (5)	Autumn (4)	Spring (5)	Summer (5)	Autumn (5)
12:0	0.05 $\pm$ 0.04	2.67 $\pm$ 0.44	0.65 $\pm$ 0.17	0.00 $\pm$ 0.00	1.68 $\pm$ 1.03	0.23 $\pm$ 0.14
14:0	0.19 $\pm$ 0.10	4.89 $\pm$ 0.54	1.43 $\pm$ 0.26	0.16 $\pm$ 0.03	5.15 $\pm$ 1.06	0.76 $\pm$ 0.06
14:0 anteiso	0.04 $\pm$ 0.04	0.00 $\pm$ 0.00	0.15 $\pm$ 0.15	0.03 $\pm$ 0.03	0.00 $\pm$ 0.00	0.68 $\pm$ 0.23
14:0 iso	0.03 $\pm$ 0.03	1.72 $\pm$ 0.20	0.38 $\pm$ 0.09	0.01 $\pm$ 0.01	1.70 $\pm$ 0.41	0.05 $\pm$ 0.05
15:0 2OH	0.06 $\pm$ 0.06	0.37 $\pm$ 0.18	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	0.46 $\pm$ 0.19	0.00 $\pm$ 0.00
15:0 3OH	0.19 $\pm$ 0.14	3.99 $\pm$ 0.88	1.14 $\pm$ 0.37	0.18 $\pm$ 0.04	4.33 $\pm$ 0.86	0.61 $\pm$ 0.30
15:0 anteiso	0.40 $\pm$ 0.15	9.07 $\pm$ 1.17	3.22 $\pm$ 0.68	0.43 $\pm$ 0.08	9.25 $\pm$ 1.85	2.13 $\pm$ 0.23
15:0 iso	0.58 $\pm$ 0.38	15.08 $\pm$ 0.91	5.44 $\pm$ 1.15	0.22 $\pm$ 0.07	13.97 $\pm$ 5.74	2.74 $\pm$ 0.86
15:0 iso 3OH	0.02 $\pm$ 0.02	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.07 $\pm$ 0.04	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
15:1 iso G	0.04 $\pm$ 0.03	0.00 $\pm$ 0.00	0.15 $\pm$ 0.11	0.94 $\pm$ 0.84	13.96 $\pm$ 13.96	2.29 $\pm$ 2.25
16:0	3.85 $\pm$ 2.34	56.42 $\pm$ 4.23	23.36 $\pm$ 3.44	2.14 $\pm$ 0.45	48.29 $\pm$ 14.45	9.61 $\pm$ 2.13
16:0 10 methyl	0.68 $\pm$ 0.36	16.04 $\pm$ 1.90	5.92 $\pm$ 1.22	0.47 $\pm$ 0.07	17.58 $\pm$ 3.72	3.54 $\pm$ 0.82
16:0 2OH	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
16:0 anteiso	0.24 $\pm$ 0.04	4.01 $\pm$ 1.50	1.30 $\pm$ 0.24	0.37 $\pm$ 0.07	2.49 $\pm$ 0.36	0.92 $\pm$ 0.17
16:0 iso	0.64 $\pm$ 0.40	17.89 $\pm$ 2.61	6.09 $\pm$ 1.33	0.38 $\pm$ 0.06	18.19 $\pm$ 3.91	3.70 $\pm$ 0.64
16:1 $\omega$ 9c	0.00 $\pm$ 0.00	1.86 $\pm$ 0.57	2.76 $\pm$ 0.61	0.00 $\pm$ 0.00	1.14 $\pm$ 0.41	0.00 $\pm$ 0.00
16:1 $\omega$ 5c	1.13 $\pm$ 0.88	7.33 $\pm$ 0.77	0.00 $\pm$ 0.00	0.52 $\pm$ 0.17	7.86 $\pm$ 2.04	1.22 $\pm$ 0.19
17:0 10 methyl	0.14 $\pm$ 0.11	3.22 $\pm$ 0.26	1.33 $\pm$ 0.28	0.08 $\pm$ 0.02	3.89 $\pm$ 0.76	0.59 $\pm$ 0.20
17:0 anteiso	0.46 $\pm$ 0.14	8.94 $\pm$ 1.36	3.06 $\pm$ 0.64	0.52 $\pm$ 0.11	8.70 $\pm$ 1.52	1.78 $\pm$ 0.17
17:0 cyclo	0.09 $\pm$ 0.07	4.38 $\pm$ 0.77	1.04 $\pm$ 0.29	0.08 $\pm$ 0.01	4.31 $\pm$ 0.77	0.52 $\pm$ 0.15
17:0 iso	0.48 $\pm$ 0.25	17.13 $\pm$ 2.33	5.35 $\pm$ 1.21	0.33 $\pm$ 0.05	18.53 $\pm$ 3.27	3.42 $\pm$ 0.28
17:0 iso 3OH	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.12 $\pm$ 0.07	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
17:1 $\omega$ 8c	0.08 $\pm$ 0.08	2.23 $\pm$ 0.49	0.40 $\pm$ 0.18	0.02 $\pm$ 0.02	1.73 $\pm$ 0.50	0.00 $\pm$ 0.00
18:0	0.70 $\pm$ 0.35	12.99 $\pm$ 1.20	5.89 $\pm$ 1.07	0.66 $\pm$ 0.13	13.39 $\pm$ 2.69	3.19 $\pm$ 0.37
18:0 iso	0.03 $\pm$ 0.03	2.24 $\pm$ 0.24	0.47 $\pm$ 0.27	0.03 $\pm$ 0.02	2.73 $\pm$ 0.52	0.20 $\pm$ 0.13
18:0 10 methyl	0.20 $\pm$ 0.15	5.75 $\pm$ 0.58	2.72 $\pm$ 0.76	0.16 $\pm$ 0.04	5.79 $\pm$ 1.72	0.70 $\pm$ 0.43
18:1 $\omega$ 9c	1.93 $\pm$ 1.06	28.87 $\pm$ 3.45	14.79 $\pm$ 3.42	1.44 $\pm$ 0.30	27.09 $\pm$ 5.88	4.57 $\pm$ 0.74
18:1 $\omega$ 7c	0.53 $\pm$ 0.34	7.86 $\pm$ 0.86	3.28 $\pm$ 0.56	0.38 $\pm$ 0.15	8.90 $\pm$ 2.45	1.55 $\pm$ 0.28
18:1 $\omega$ 7c 11 methyl	0.08 $\pm$ 0.08	1.66 $\pm$ 0.14	0.58 $\pm$ 0.42	0.00 $\pm$ 0.00	1.56 $\pm$ 0.48	0.00 $\pm$ 0.00
18:3 $\omega$ 6c (6,9,12)	0.33 $\pm$ 0.17	3.21 $\pm$ 0.80	1.30 $\pm$ 0.23	0.28 $\pm$ 0.03	3.06 $\pm$ 0.40	0.79 $\pm$ 0.24
19:0 cyclo $\omega$ 8c	0.65 $\pm$ 0.38	15.95 $\pm$ 0.72	6.39 $\pm$ 1.43	0.43 $\pm$ 0.06	22.14 $\pm$ 5.22	4.83 $\pm$ 0.84
19:0 iso	0.01 $\pm$ 0.01	0.53 $\pm$ 0.32	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	0.44 $\pm$ 0.14	0.00 $\pm$ 0.00
20:0	0.20 $\pm$ 0.10	2.67 $\pm$ 0.10	1.34 $\pm$ 0.24	0.16 $\pm$ 0.04	2.66 $\pm$ 0.62	0.63 $\pm$ 0.12

324 Case number is shown in parentheses. Mean $\pm$ standard error.  
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**Table 5**

Two-way analysis of variance for the effects of seasons (Spring, Summer, Autumn) and soil depths (0-5 cm, 5-20 cm) on microbial PLFAs contents

Fatty acid configuration	Microbial group	Season	Soil depth	$r^2$
12:0	Gram-negative bacteria	**a	n.s.	0.522
14:0	Gram-negative bacteria	**	n.s.	0.815
14:0 anteiso	Gram-positive bacteria	**	n.s.	0.554
14:0 iso	Gram-positive bacteria	***	n.s.	0.795
15:0 2OH	Gram-negative bacteria	**	n.s.	0.425
15:0 3OH	Gram-negative bacteria	***	n.s.	0.723
15:0 anteiso	Gram-positive bacteria	***	n.s.	0.796
15:0 iso	Gram-positive bacteria	***	n.s.	0.599
15:0 iso 3OH	Gram-negative bacteria	n.s.	n.s.	0.297
15:1 iso G	Bacteria	n.s.	n.s.	0.155
16:0	Bacteria	***	n.s.	0.733
16:0 10 methyl	Sulfate-reducing bacteria	***	n.s.	0.785
16:0 2OH	<i>Ralstonia</i> spp.	n.s.	n.s.	0.170
16:0 anteiso	Gram-positive bacteria	**	n.s.	0.508
16:0 iso	Gram-positive bacteria	***	n.s.	0.774
16:1 $\omega$ 9c	Fungi	***	n.s.	0.704
16:1 $\omega$ 5c	Methane-oxidizing bacteria	***	n.s.	0.613
17:0 10 methyl	Actinomycetes	***	n.s.	0.814
17:0 anteiso	Gram-positive bacteria	***	n.s.	0.806
17:0 cyclo	Gram-negative bacteria	***	n.s.	0.801
17:0 iso	Gram-positive bacteria	***	n.s.	0.806
17:0 iso 3OH	Gram-negative bacteria	n.s.	n.s.	0.352
17:1 $\omega$ 8c	Gram-negative bacteria	***	n.s.	0.693
18:0	<i>Hydrogenobacter</i>	***	n.s.	0.808
18:0 iso	Gram-positive bacteria	***	n.s.	0.821
18:0 10 methyl	Actinomycetes	***	n.s.	0.691
18:1 $\omega$ 9c	Fungi	***	n.s.	0.775
18:1 $\omega$ 7c	<i>Pseudomonas</i> spp.	***	n.s.	0.705
18:1 $\omega$ 7c 11 methyl	<i>Cellulomonas</i> spp.	***	n.s.	0.670
18:3 $\omega$ 6c (6,9,12)	Fungi	***	n.s.	0.700
19:0 cyclo $\omega$ 8c	<i>Burkholderia</i>	***	n.s.	0.758
19:0 iso	Bacteria in general	**	n.s.	0.375
20:0	Bacteria in general	***	n.s.	0.782

329 \* , \*\*and \*\*\* indicate significant differences in that row at  $P < 0.05, 0.01, 0.001$ , respectively. Not significant  
330 results are labelled n.s. ( $P > 0.05$ ).  
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**Table 6**

Spearman correlation coefficients matrix for microbial community variations and chemical properties in orchard soils

Item	pH	TOC	TN	C/N ratio	CEC	Exchangeable			
						K <sup>+</sup>	Na <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>
Total PLFAs	-0.614**	-0.617**	-0.401*	-0.302	-0.817**	0.284	0.493**	0.119	-0.177
Bacterial PLFAs	-0.618**	-0.642**	-0.422*	-0.317	-0.831**	0.296	0.485**	0.141	-0.160
Fungal PLFAs	-0.530**	-0.572**	-0.401*	-0.313	-0.791**	0.390*	0.482**	0.229	-0.074
Actinomycetal PLFAs	-0.593**	-0.555**	-0.434*	-0.248	-0.744**	0.320	0.525**	0.150	-0.165
B/F ratio	-0.517**	-0.565**	-0.354	-0.119	-0.595**	-0.259	0.131	-0.189	-0.263
Richness	-0.460*	-0.419*	-0.526**	0.051	-0.446*	0.403*	0.622**	0.332	-0.085
Simpson diversity	-0.753**	-0.707**	-0.498**	-0.159	-0.722**	0.132	0.517**	-0.012	-0.328
Shanon-Wiener diversity	-0.602**	-0.446*	-0.450*	0.007	-0.556**	0.330	0.504**	0.090	-0.380*
Alatalo evenness	-0.054	0.096	0.215	-0.062	0.028	-0.176	-0.252	-0.203	-0.092

335 FLFA = phospholipid fatty acid, B/F ratio = ratio of bacterial to fungal PLFA, TOC = total organic carbon, TN =  
336 total nitrogen, CEC = cation exchange capacity, C/N ratio = ratio of total organic carbon to total nitrogen.  
337 \* , \*\* indicate spearman coefficient significant differences at  $P < 0.05, 0.01$ , respectively ( $n = 28$ ).  
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## Figure Captions

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342 Fig. 1. Monthly dynamics of precipitation (A), average air temperature (B), and extremes air temperature (C) at  
343 the trail location from 1997 to 2010. Annul precipitation is shown in parentheses (A).

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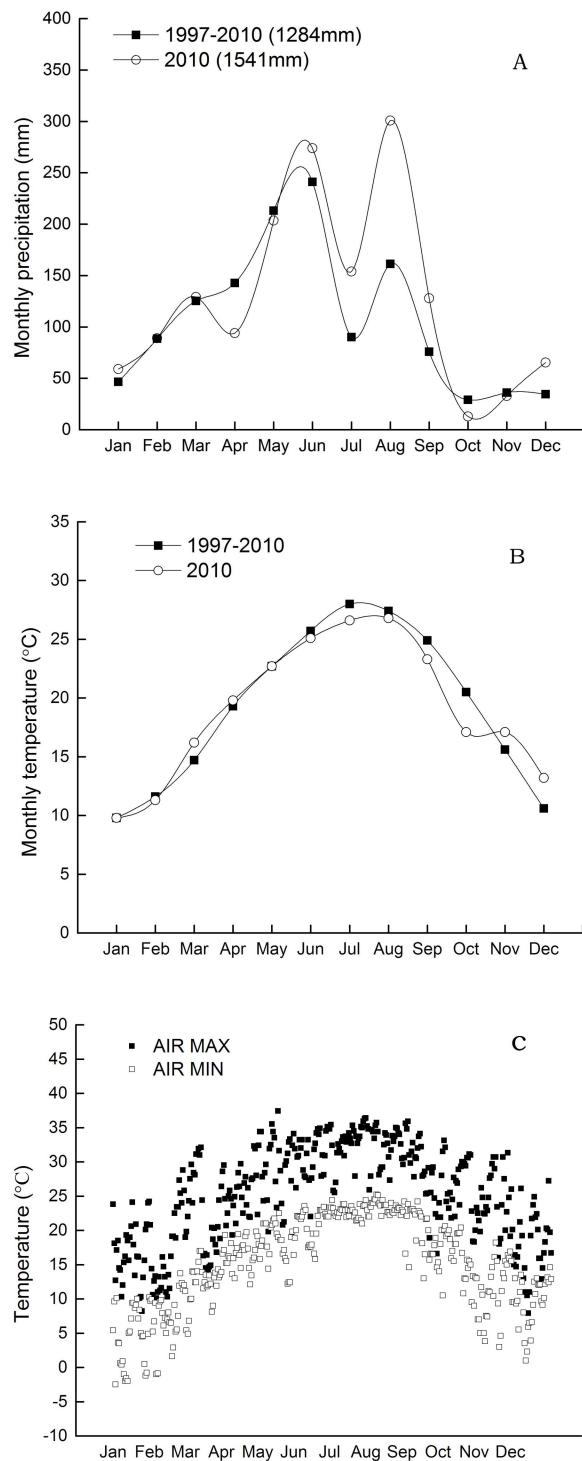
346 Fig. 2. Microbial diversity of Richness (A) Simpson index (B), Shannon-Wiener index (C), and Alatalo index (D) in  
347 orchard soil of 0-5 and 5-20 cm s at different seasons. Bars with the same letter (s) are not significantly  
348 differences between seasons for each depth at  $P < 0.05$  using LSD post hoc tests.

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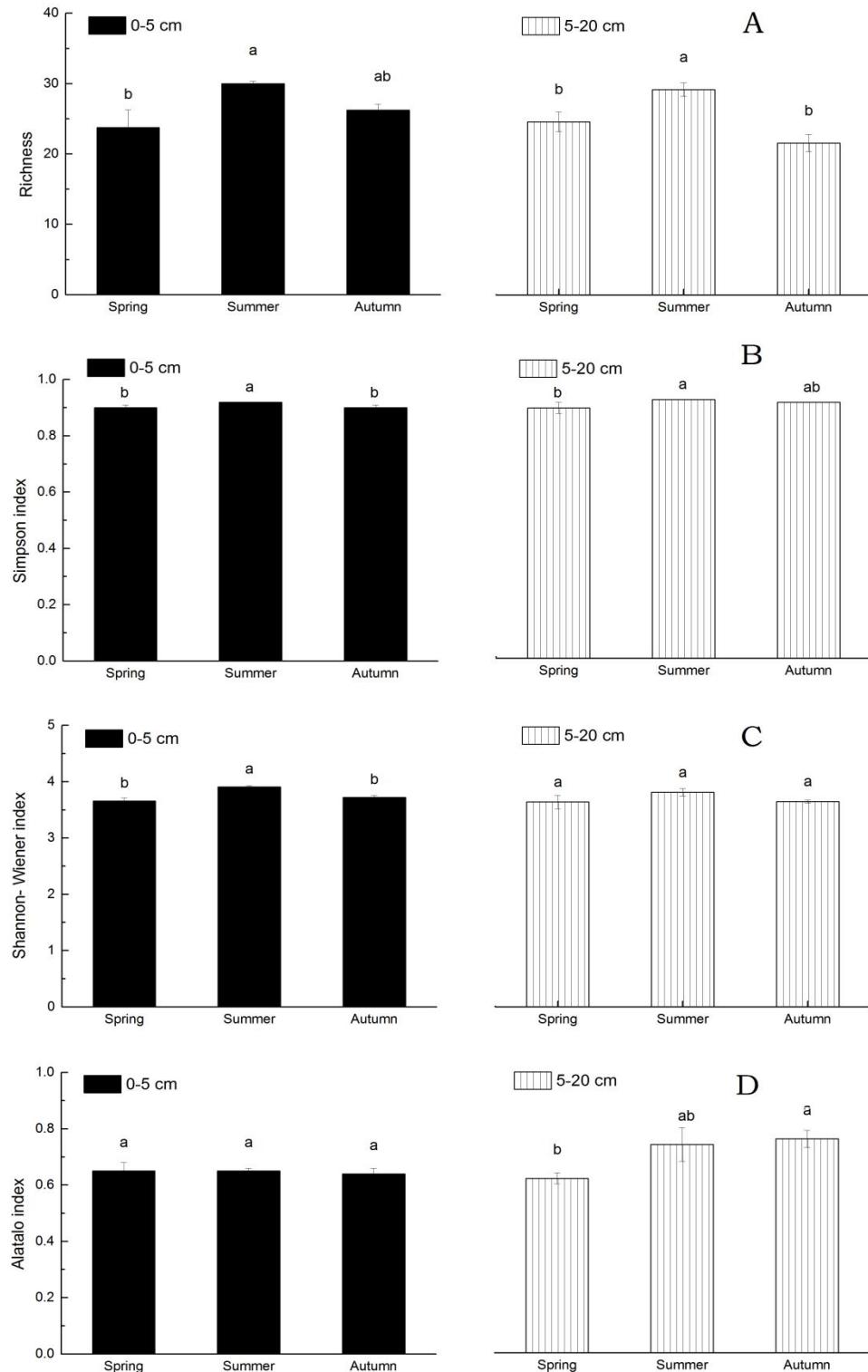
350 Fig. 3. Principal components analysis (PCA) of microbial community from orchard soil sampled at different  
351 seasons and depths. Percent variance explained by each component (PC) is shown in parentheses. Error  
352 bars represent standard error ( $n = 28$ ).

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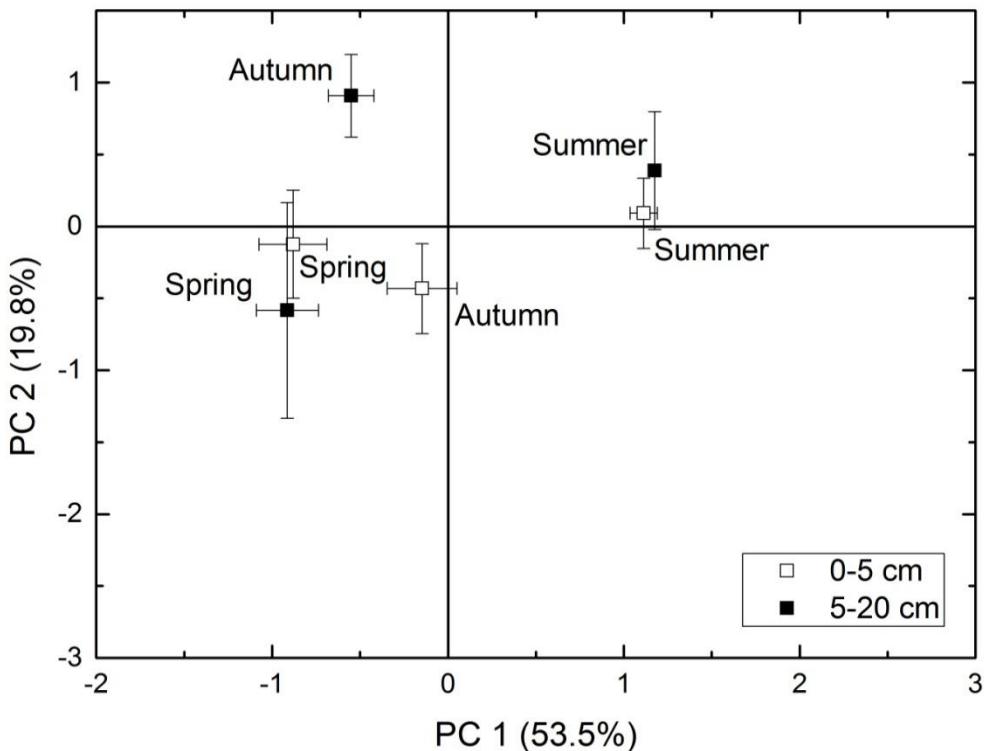


355  
356 Fig. 1



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Fig. 2



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Fig. 3

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## 364 Supplemental Information

### 365 Table S1

366 Principle component (PC) factors value and total eigenvector coefficients between PC factors and microbial  
367 community indexes after varimax rotation

PC	Value	Contribution rate		Factor									
		Pr Var	Cum Var	Total	Bacterial	Fungial	Actinomy	B/F ratio	Richness	Simpson	Shannon-	Alatalo	Wiener
		...%...		.....PLFAs.....					.....Indexes.....				
1	5.35	53.50	53.50	0.96	0.97	0.95	0.96	0.30	0.81	0.62	0.56	0.35	
2	1.98	19.80	73.30	-0.09	-0.07	-0.14	-0.14	0.40	-0.36	0.71	0.54	0.89	

368 PC= principle component, Pr Var= Principle variance, Cum Var= cumulative variance, PLFA=phospholipid fatty  
369 acid, B/ F ratio =ratio of bacterial to fungal PLFA.

370 Factor loadings | x | >5% of total eigenvector coefficients after varimax rotation.