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Comparative Study on Microbial Community Structure across Orchard Soil, Cropland Soil, and Unused Soil

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Abstract

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We examined the effects of three different soil conditions (orchard soil, cropland soil, unused soil) on the functional diversity of soil microbial communities. The results first showed that orchard and cropland land use significantly changed the distribution and diversity of soil microbes, particularly at surface soil layers. The richness index (S) and Shannon diversity index (H) of orchard soil microbes were significantly higher than the indices of the cropland and unused soil treatments in the 0–10 cm soil layer, while the S and H indices of cropland soil microbes were the highest in 10–20 cm soil layers. Additionally, the Simpson dominance index of cropland soil microbial communities was the highest across all soil layers. Next, we found that carbon source differences in soil layers under the three land use conditions can mainly be attributed to their carbohydrate and polymer composition, indicating that they are the primary cause of the functional differences in microbial communities under different land uses. In conclusion, orchard and cropland soil probably affected microbial distribution and functional diversity due to differences in vegetation cover, cultivation, and management measures.

Keywords: BIOLOG technology; land uses; microbial diversity; soil conditions; soil microorganism population

Land use is the most important human activity to affect soil fertility. Changes in land use patterns directly alter soil ecosystems by influencing organic carbon content, and therefore soil productivity (Zhou & Shi 2006; Tobiašová 2011; Zhang *et al*. 2014). Thus, a better understanding of how different land uses affect various soil properties has strong implications for confronting major agronomic issues. For instance, in recent years, decreased soil fertility has resulted in many orchards that suffer from weakened tree vigor, production decline, and quality deterioration, seriously hampering the healthy economic development of the fruit industry (Li *et al*. 2005). Two major factors contributing to this decreased soil fertility are soil compaction and a reduction in microbial population, which are caused by an overemphasis on increasing fruit yield, lack of orchard investment, and single or non-standard soil management systems (Ramos *et al*. 2011). Generally speaking, long-term farming and management procedures in agricultural land use, such as orchards and croplands, break the ecological balance of natural soil and damage the physical protective layer on soil organic matter, exposing it to microbial decomposition (Campos-Herrera *et al*. 2010). The resultant decrease in soil organic matter causes agricultural soils to differ from natural soils in their organic matter composition (Gosai *et al*. 2010; Lin *et al*. 2010).

Because microbial decomposition majorly influences soil organic matter, soil microorganisms are a very effective biological indicator of soil quality (Liu *et al*. 2006; Raiesi 2007). Soil microbial diversity is

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sensitive to fluctuations in the soil ecosystem, as microbial species both affect and are affected by variation in land uses (Galicia & Garcya-Olivia 2004; Nsabimana *et al*. 2004; Bissetta *et al*. 2011; Jangid *et al*. 2011). Specifically, the diversification of land use results in different types of litter and root exudates, altering soil surface cover, and other soil properties. For example, comparison of two common agricultural land uses, orchards and croplands (rice fields) revealed that due to greater soil surface exposure, orchards are associated with serious water and soil loss, with only a small return of organic matter. However, rice fields use more chemical fertilizers than orchards, and previous research has demonstrated that the long-term, excessive use of chemical fertilizers is likely to have severe negative consequences on soil (Li *et al*. 2007). These different soil characteristics affect microbial number and composition (Hao & Ren 2009; Nautiyal *et al*. 2010; Bai *et al*. 2013), because interspecific variation in carbon resource use results in differential survival of microbial species, depending on the soil habitat. Indeed, variation in long-term land use patterns led to significant differences in soil microbial number and community structure (Bi *et al*. 2010). Finally, the variation in carbonaceous material transformation as a result of changes in microbial community structure leads to changes in soil fertility and quality (Liu *et al*. 2011), which has an obvious influence on land use patterns.

Research on the impact of different land use patterns on soil has mostly focused on physical and chemical properties, such as analyses of microbial biomass carbon and nitrogen (Wang *et al*. 2006; Xu *et al*. 2009; Price *et al*. 2012; Fliessbach *et al*. 2013). Comparatively little research has been performed on how land use variations affect the functional diversity of soil microbes. In particular, there are no reports available on the major fruit-producing region of the northern Bohai Bay area (Liaoning and Hebei Provinces, Beijing, and Tianjin) (28.99–38.35% of Chinese apples). In this study, BIOLOG ECO microplate technology was used to determine the functional diversity of microbial communities in apple orchard soil, cropland soil, and natural soil from the same parent materials. The differences in soil microbial metabolic processes across these three land uses were clarified, in order to better understand the mechanisms underlying fluctuations in the quality of orchard soil from the northern Bohai Bay region.

MATERIAL AND METHODS

Study site and soil characteristics. The study was conducted from April to November 2012 at the Lishan Base of the Fruit Tree Research Institute, Chinese Academy of Agricultural Sciences. All three treatments were located in the same hilly zone of the Songling mountain range, with the parent material mainly consisting of weathered granitic gneiss. Orchard soil (OS) was planted with 7-year Hanfu apple trees under an inter-row natural grass-growing management system. Cropland soil (CS) was planted with a corn-peanut rotation for 10 years, while only peanuts were planted in 2012. Unused soil (US) was from an uncultivated area mainly populated with grass weeds for twelve years.

Soil sample collection. In 2012, samples were collected from soil profiles at pre-selected test points during the following apple phenophases: germination (A: April 30, 2012), rapid spring shoot growth (B: June 6, 2012), suspended spring shoot growth (C: July 13, 2012), rapid fruit expansion (D: September 9, 2012), and defoliation (E: October 24, 2012).

At each test site, soil samples were collected at five points in a Z-shaped pattern. The ground cover and litter were first removed. Then, samples were collected from the soil profiles at 0–10, 10–20, 20–40, and 40–60 cm depths. After the removal of large stones and plant roots, samples of the same soil layer were thoroughly mixed. About 2 kg of each sample was obtained by quartering the mixed soil. Samples were brought to the laboratory and immediately sieved (1 mm, 20 mesh), and then maintained at 4°C before microbial functional diversity analysis.

Measurement methods. The functional diversity of soil microbes was determined using BIOLOG test plates (EcoPlates^{TM}, Matrix Technologies Corporation, Hudson, USA). A conical flask containing 10 g of dry soil sample was added to flasks containing 90 ml of sterile 0.85% NaCl. The flasks were sealed and agitated for 30 min on a shaker at 200 rpm. The solution was diluted to 10–3 via a 10-fold serial dilution with sterile 0.85% NaCl solution. The resulting soil dilution was used for inoculation. Each well of the BIOLOG Eco Plate was injected with 150 µl of inoculation suspension and then incubated at 25°C for 192 h. The absorbance of the culture at 590 nm was measured every 24 h using a BIOLOG reader (Garland 1996).

Calculation of microbial functional diversity indices. The average absorbance among three rep-

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licates of the 31 substrates in each plate was calculated. The substrates were also divided into six functional guilds of carbon sources (amines, amino acids, carbohydrates, carboxylic acids, polymers, and aromatics), and the average absorbance for all wells within each guild was calculated. The overall colour change in BIOLOG Eco Plates was expressed by the average well colour development (AWCD), which is an indicator of overall microbial activity and can be used to determine the carbon useability by soil microbial communities (Zabinski & Gannon 1997).

$$AWCD = \Sigma (Ci\text{-}R)/31$$

where:

Ci – absorbance value of the sample well at 590 nm

R – absorbance of the control well

In cases where $Ci - R < 0$, AWCD was considered to be zero.

The calculation of functional diversity indices was carried out using the optical density (OD) of the microplates, measured with the BIOLOG reader after a 96-h incubation period (Garland 1997).

The following microbial community functional diversity indices were calculated:

Richness index (S): S refers to the number of substrates utilized by microbial communities. OD ≥ 0.25 was considered positive and included in S (i.e. the sum of microwells with positive OD) (Ratcliff *et al.* 2006).

Shannon diversity index (H): $H = \Sigma Pi \times \ln Pi$, where $Pi = (Ci - R)/\Sigma (Ci - R)$, representing the ratio of OD difference between reaction and control wells over the overall OD differences in the entire plate (Rogers & Tate 2001).

Evenness index (E): $E = H/\ln S$ (Pielou 1966)

Simpson dominance index (Ds): $Ds = 1 - \Sigma Pi2$, a simple mathematical measure that characterizes species diversity in a community (Simpson 1949).

Data analysis. One-way analysis of variance (ANOVA) was performed to analyze data in the same soil layers among the three different land use conditions. A Duncan's multiple range test was used for multiple comparisons of treatment means. A principle component analysis (PCA) was used to display the diversity of BIOLOG metabolic types produced by different microbial communities. Replicates of the same model community clustered when analyzed with PCA, and model communities with different compositions were clearly separated on the PCA axis. We then performed a cluster analysis on the covariance matrix of different carbon sources in the microwells, allowing similar soil microbial communities to be classified. All statistical tests were performed in SPSS 12.0. Graphs were created in MS Excel 2003. The significance level was defined as $\alpha = 0.05$.

RESULTS

Basic parameters of the test soils. Table 1 shows that there were significant differences in soil organic carbon (SOC) contents under the three land use conditions, with CS > OS > US. The contents of total N and available N in US were far higher than those in

Table 1. Basic parameters of the test soils

Soil layer (cm)	Land use pattern	SOC	Total			Available			Ca (g/kg)	Mg	Fe	Mn
			N	P	K	N	P	K				
		(g/kg)				(mg/kg)				(mg/kg)		
0~10	OS	13.19	1.21	0.320	32.58	128.93	13.89	206.83	1.11	60.66	38.19	68.83
	CS	14.17	1.05	0.336	29.21	101.56	17.94	156.19	1.37	61.16	21.30	51.18
	US	9.00	1.73	0.231	28.80	180.08	4.73	128.23	1.17	47.88	20.50	51.29
10~20	OS	9.55	1.16	0.281	31.36	115.79	7.96	142.51	0.96	49.78	38.64	85.31
	CS	12.58	0.92	0.280	23.84	99.15	11.94	150.33	1.61	69.47	28.31	68.66
	US	7.74	1.46	0.249	31.32	145.82	2.48	63.32	1.43	48.90	23.60	30.20
20~40	OS	5.05	0.59	0.204	31.74	67.53	2.33	85.86	1.31	56.08	36.94	53.78
	CS	8.84	0.56	0.205	30.23	56.15	4.58	115.87	1.73	75.62	21.96	39.57
	US	3.96	1.08	0.169	34.59	118.18	1.65	48.87	1.57	54.66	11.44	10.69
40~60	OS	4.19	0.56	0.147	27.58	68.53	1.80	62.12	1.29	54.68	13.94	55.08
	CS	7.98	0.39	0.145	30.18	33.71	4.20	100.23	1.94	75.93	12.36	44.45
	US	2.37	0.86	0.134	30.95	88.54	2.18	49.80	1.23	73.69	7.40	33.62

OS – orchard soil; CS – cropland soil; US – unused soil; SOC – soil organic carbon

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OS and CS. The total P contents in CS and OS were similar, and both were far higher than those in US. The available P contents in CS were higher than those in OS and US. For the 0–20 cm soil layer, the total K contents in OS were higher than those in CS and US. For the 20–60 cm soil layer, the total K contents in US were higher than those in OS and CS. For the 0–10 cm soil layer, the availble K contents in OS were higher than those in CS and US. For the 10–60 cm soil layer, the availble K contents in CS were higher than those in OS and US. The Ca and Mg contents in CS were higher than those in OS and US. The Fe and Mn contents in OS were higher than those in CS and US.

Impact of different land uses on AWCD growth curve of soil microbial communities. Figure 1 shows that within the first 24 h of incubation, AWCD values were small for microbial communities across all of the soils under the three land use conditions. After 24 h, AWCD values gradually increased with increasing incubation time. After 144 h, the rate of AWCD increase gradually plateaued.

AWCD decreased with depth (Table 2). The magnitude of AWCD changes in OS and CS was significantly greater than that in US (Table 2). There were significant differences in AWCD for surface soils (0–10 cm) under the three land use conditions, with OS > CS > US. For the 10–20 cm soil layer, the OS and US AWCD curves were similar and significantly lower than the CS AWCD curve. For the 20–40 cm soil layer, the CS and US AWCD curves were similar, and both were far higher than the OS AWCD value. For the 40–60 cm soil layer, the AWCD profiles of OS and CS were similar and far below the US AWCD profile.

Comparison of soil microbial community diversity indices under different land use conditions. Table 1 shows that after 96 h of incubation in BIOLOG microplates, OS AWCD in the 0–10 cm soil layer was by 29.88% and 56.12% higher than the corresponding values of CS and US, respectively ($P < 0.05$). For the 10–20 cm soil layer, the CS AWCD was by 35.82% and 22.76% higher than the AWCD values of US and OS ($P < 0.05$), while the latter two soil treatments did not significantly differ. In the 20–40 cm layer, OS AWCD was by 85.71% and 86.53% lower than CS and US AWCD values, respectively. Finally, in the

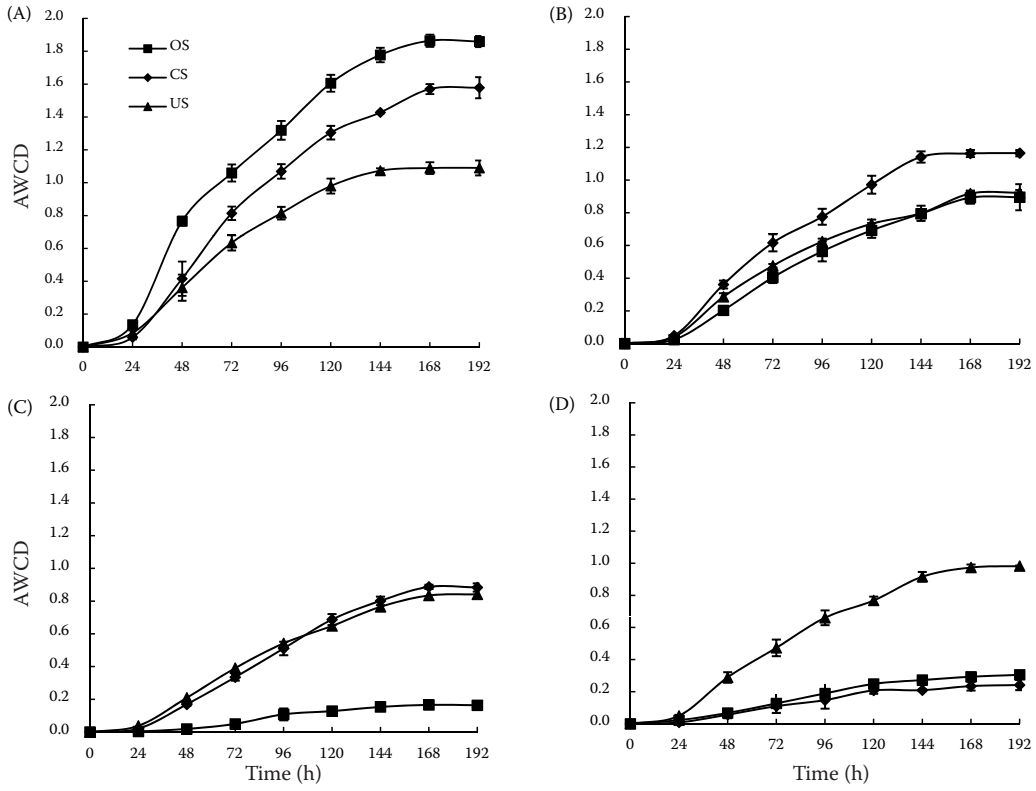

Figure 1. Average well colour development (AWCD) of soil microbial process under three land uses and soil depths: (A) 0–10 cm, (B) 10–20 cm, (C) 20–40 cm, and (D) 40–60 cm
OS – orchard soil; CS – cropland soil; US – unused soil

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40–60 cm soil layer, the AWCD values of OS and CS were by 97.15% and 88.98% lower than the US AWCD value ($P < 0.05$), respectively.

Analyses of the diversity indices revealed that in the 0–10 cm layer, OS S and H values were significantly higher than those of CS and US (Table 1). For the 10–20 cm soil layer, OS S and H values were significantly lower than those of CS, but not significantly different from those of NS. For the 20–40 cm soil layer, OS R and H values were significantly lower than those of CS and US. In the 40–60 cm soil layer, OS S values were significantly lower than those of US but not significantly different from those of CS. Finally, E values were not significantly different across any of the soil layers and land use treatments.

Analysis of the Ds values revealed that microbial communities in CS were significantly more dominant than those in US and OS, across all soil layers except for the 10–20 cm depth. The Ds values of OS in the 0–10 cm and 10–20 cm soil layers were significantly higher than those of US (39.54% and 1.91%, respectively). There was no significant difference in Ds between US and OS for the 20–40 cm layer. The Ds of OS was significantly lower than that of US (26.11%) for the 40–60 cm soil layer.

In sum, the OS land use treatment significantly altered soil microbe diversity across different soil layers, whereas CS significantly increases the dominance of soil microbes.

Utilization of six carbon sources by soil microbial communities under different land use conditions. The analysis of carbon use by soil microbial communities revealed significant effects of both land use and soil layer, indicating differing microbial metabolic function across those conditions (Figure 2). Under the same land use conditions, soil layers significantly affected the microbial use of soil carbons. At the same soil layer, differing land use conditions also significantly affected microbial use of soil carbons.

Specifically, in the 0–10 cm soil layer, OS, CS, and US microbial communities significantly differed in their usage of carbon from carbohydrates, amino acids, polymers, and multi-amine carbons, all following the order of OS > CS > US. OS and CS microbes used a similar level of carbons from carboxylic acids and aromatics, and both were significantly higher than the usage levels of US microbes (Figure 2A). In the 10–20 cm soil layer, OS, CS, and US microbes significantly differed in their use of carbons from carbohydrates, amino acids, polymers, multi-amines, and aromatics; however, no clear order among them emerged. At this layer, OS and US microbes used a similar level of carbons from carboxylic acids, and both were significantly lower than the usage levels of CS microbes (Figure 2B). In the 20–40 cm soil layer, OS, CS, and US microbes significantly differed in their use of carbons from carbohydrates, amino acids, polymers, and multi-amines, with OS being the lowest (Figure 2C). Finally, in the deepest (40–60 cm) soil layer, CS microbes had the lowest carbon use levels across the six categories of compounds (Figure. 2C), while US microbes had the highest use levels (Figure 2D).

Table 2. Analysis of soil microbial community diversity index under different land uses and soil layers

Soil layer (cm)	Land use pattern	AWCD	Richness index (S)	Shannon diversity index (H)	Evenness index (E)	Simpson dominance index (Ds)
0–10	OS	1.319 ± 0.057^a	31 ± 1^a	3.256 ± 0.058^a	0.951 ± 0.018^a	1.341 ± 0.102^b
	CS	1.071 ± 0.044^b	25 ± 1^b	3.108 ± 0.010^b	0.966 ± 0.012^a	1.909 ± 0.197^a
	US	0.819 ± 0.039^c	22 ± 2^b	3.019 ± 0.079^b	0.973 ± 0.008^a	0.961 ± 0.052^c
10–20	OS	0.564 ± 0.061^b	18 ± 1^b	2.884 ± 0.067^b	0.951 ± 0.018^a	0.960 ± 0.003^a
	CS	0.776 ± 0.049^a	22 ± 1^a	3.042 ± 0.029^a	0.966 ± 0.012^a	0.949 ± 0.001^b
	US	0.624 ± 0.018^b	19 ± 1^b	2.893 ± 0.032^b	0.973 ± 0.008^a	0.942 ± 0.005^b
20–40	OS	0.073 ± 0.008^b	3 ± 0^b	1.458 ± 0.108^b	0.998 ± 0.005^a	0.930 ± 0.009^b
	CS	0.511 ± 0.041^a	18 ± 2^a	2.978 ± 0.045^a	0.984 ± 0.013^a	0.942 ± 0.001^a
	US	0.542 ± 0.008^a	19 ± 2^a	2.974 ± 0.078^a	0.983 ± 0.016^a	0.928 ± 0.004^b
40–60	OS	0.176 ± 0.014^b	7 ± 2^b	2.569 ± 0.149^a	1.327 ± 0.098^a	0.693 ± 0.038^b
	CS	0.068 ± 0.007^c	3 ± 0^c	2.097 ± 0.216^b	1.032 ± 0.020^a	0.939 ± 0.005^a
	US	0.617 ± 0.008^a	19 ± 2^a	2.840 ± 0.083^a	1.011 ± 0.006^a	0.938 ± 0.006^a

OS – orchard soil; CS – cropland soil; US – unused soil; AWCD – average well colour development; conditions labelled by different lowercase letters are significantly different ($P < 0.05$)

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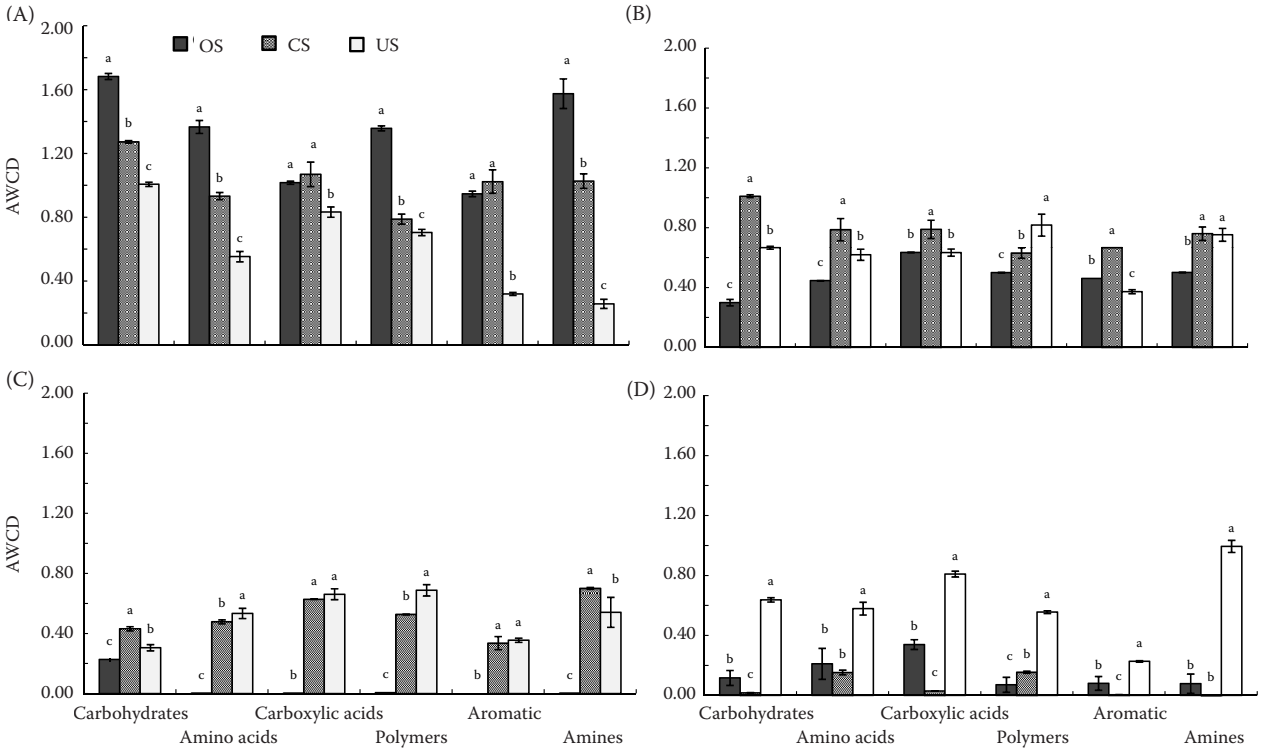

Figure 2. Use of six carbon sources by soil microbial communities under different land uses and soil depths: (A) 0–10 cm, (B) 10–20 cm, (C) 20–40 cm, and (D) 40–60 cm; bars labelled with different lowercase letters indicate a significant difference ($P < 0.05$); AWCD – average well colour development

PCA of soil microbial communities under different land use conditions. The results of our PCA analysis on the microbial use of 31 carbon sources revealed that the eigenvalues of the first four principal components (PCs; out of 31 PCs) were greater than one, contributing to 82.13% of cumulative variance (Table 3). We then analyzed microbial functional diversity using PC1 and PC2, which were collectively considered a single carbon source variable.

Figure 3 shows that land use and soil depth both had a significant effect on soil microbial functional diversity. The PC1 axis separated microbial communities in the OS treatment (at depths 0–10, 20–40, and 40–60 cm) and in the CS treatment (at 40–60 cm), while the PC2 axis mainly separated microbial communities in the US layers. Differing land uses had the greatest effect on the functional diversity of surface soil microorganisms, as indicated by the dispersed score values along the PC1 axis and the significant differences on the PC2 axis.

In terms of carbon sources that played a role in functional diversity (Table 4), six significantly contributed to PC1 (i.e. feature vector ≥ 0.20) and five significantly contributed to PC2. Our results demonstrate that carbohydrates and polymers are the primary carbon sources that differentiate PC1 and PC2. In other words, carbon source differences in soil layers under the three land use conditions can be mainly attributed to their carbohydrate and polymer composition.

Cluster analysis of soil microbial communities under different land use conditions. The results of the cluster analysis (Figure. 4) showed that the carbon use by microbial communities can be classified into three categories: (1) the 0–10 cm layers

Table 3. Characteristics of principal components

Principal components	Latent root	Percentage of variance (%)	Percentage of cumulative variance (%)
PC1	17.80	57.43	57.43
PC2	2.44	7.87	65.30
PC3	2.71	8.74	74.04
PC4	2.51	8.10	82.13

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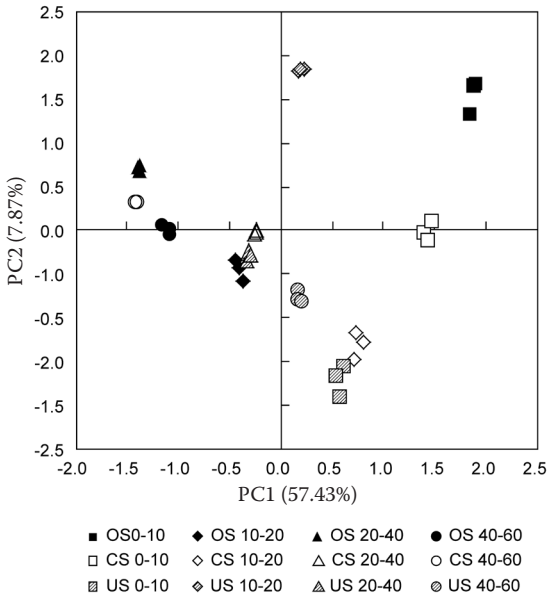

Figure 3. Principal component analysis (PCA) of carbon use by soil microbial communities under different land uses and soil depths

of OS and CS; (2) the 20–40 and 40–60 cm layers of OS plus the 40–60 cm layer of CS; (3) the remainder of the soil layer and land use combinations (notably including all layers of US).

DISCUSSION

In this study, we demonstrated that differing land use conditions, particularly orchards, had significant

Table 4. Carbon sources significantly contributing (absolute value of feature vector ≥ 0.20) to principal components PC1 and PC2

Functional guilds of carbon sources	Compound	Feature vector
PC1		
Carbohydrates	D-galactonic acid γ-lactone	0.202
	D-mannitol	0.221
Amino acids	L-asparagine	0.221
Polymers	Tween 80	0.202
Aromatic chemicals	4-hydroxy benzoic	0.203
Amines	putrescine	0.202
PC2		
Carbohydrates	ß-methyl-D-glucoside	–0.251
	D-xylose	0.312
	D-cellobiose	0.444
Polymers	α-cyclodextrin	0.265
	glycogen	0.271

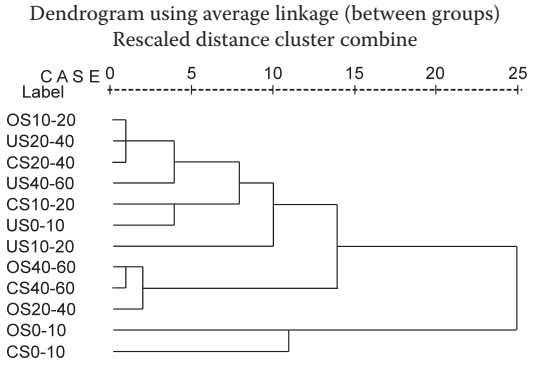

Figure 4. Cluster analysis of carbon use by soil microbial communities under different land uses and soil layers

effects on soil microbial distribution and functional diversity. Specifically, we found that near the surface (0–10 cm soil layer), the soil microbial abundance and diversity were significantly higher under OS conditions than under CS and US conditions, yet dropped in deeper soil layers (10–60 cm). These results corroborated previous research indicating that long-term planting of fruit trees significantly protects and improves surface soil structure, but caused compaction and hardening in deeper soil (Sun *et al.* 2011).

Additionally, our cluster analysis revealed that in the surface soil layer, the OS microbial communities had higher levels of carbon use than CS microbes, which used more carbon than US microbes. This result suggests that the two agricultural land uses exert a strong effect on soil microbes. However, the finding is at odds with previous studies demonstrating that orchard land had the lowest soil microbial carbon content in the top soil layer of all investigated soils (Wang *et al.* 2006), and unused land had the highest levels of soil bacteria and organic matter (Liu *et al.* 2013). We believe these differing outcomes can be explained by the use of a natural grass-growing management system in our OS condition. This management system resulted in a substantial, annual litter of leaves and weeds on the surface of the soil that provided a rich carbon source for soil microbes, in contrast to the other two land use conditions. Additionally, the natural grass-growing management system reduced the need for weeding, thus maintaining good soil structure, increasing surface soil water content, and creating favourable conditions for microbial growth. As for the CS condition, more straw and fertilizer were used in the 0–20 cm soil layer, which likely resulted in vigorous microbial metabolism, increasing microbial abundance and

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diversity above those of the US condition. However, the surface soils in the previous studies were subject to yearly, disruptive farming measures, such as tillage, which is one plausible reason why microbial flora altered and microbial numbers deceased. Alternatively, it is possible that the soil underwent frequently wet-dry cycles during cropland cultivation, making it difficult to maintain a large number of microbes. This could explain both the differences between our results and previous findings, as well as explain why CS microbial diversity was slightly lower than OS diversity in the 0–10 cm layer. Lastly, the relatively low microbial numbers in the surface layer of US could be due to the lack of human tillage and external input of carbon sources.

We note several issues that should be taken into account when considering our results. First, although BIOLOG technology yields a large amount of data, numerous factors can potentially influence the microwell plate colour in addition to microbial composition. For example, population composition, quantity and activity of microorganisms in the culture medium, sample pre-treatment, curing time and temperature, plus potential contamination of culture liquid can all introduce noise into the data. While we cannot fully exclude the possibility that these artifacts could have affected our results, we feel that we have tightly controlled all aspects of our experimental conditions and minimized the likelihood of confounds. Second, BIOLOG technology allows for the comparison and identification of microbial communities through information on metabolic function, but is unable to provide more details on community structure. Therefore, future studies will benefit from combining BIOLOG technology with other methods of community structure analysis, such as metabolic fingerprint analysis. The latter identifies specific microorganisms based on unique metabolic characteristics and makes possible a far more fine-tuned analysis of changes in community composition and dynamics. In turn, the relationship between community structure and function can be more clearly delineated.

CONCLUSION

In conclusion, out study demonstrated that orchards significantly changed the distribution of soil microorganisms at differing soil depths, and increased the richness and diversity of microbes in the surface layer. These results can be used to understand the mechanisms behind quality fluctuations of different soils, which have major agronomic significance. Our data may hopefully help decide on appropriate orchard management measures that improve soil quality, eventually leading to sustainable development of orchards and other agricultural land uses.

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