Original article

Effects of different cultivation strategies on soil nutrients and bacterial diversity in kiwifruit orchards

Li Tengfei, Liu Chongyi, Jin Xumei, Cao Xinyue, Lin Ziqi, Lu Qi, Long Mingxiu and He Shubin

College of Grassland Agriculture, Northwest A&F University, Yangling, Shaanxi, China

Summary

The basic principle underlying organic orchards is increased soil nutrient content, particularly organic matter content and increases soil bacterial activity and diversity. A standard organic management strategy significantly improved ($p < 0.05$) soil alkaline phosphatase, urease, and sucrase activities and soil organic matter, total nitrogen, total phosphorus, and nitrate nitrogen contents. The soils of two orchards mainly contained 32 bacterial phyla, which were dominated by *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Gemmatimonadetes*. Correlational analysis showed that the bacterial community was mainly influenced by catalase, sucrase, and soil organic matter. Catalase, urease, sucrase, and alkaline phosphatase activities and soil organic matter, total nitrogen, and nitrate-nitrogen contents promoted soil bacterial content. A comprehensive analysis showed that organic orchard management improved soil nutrients and enzyme activities and changed the soil bacterial diversity compared with those of conventionally managed soil.

Keywords

conventional orchard, community structure, organic orchard, soil bacterial diversity, soil nutrient content, 16S rDNA

Significance of this study

What is already known on this subject?

- Organic agriculture uses organic fertilizer to meet the nutritional needs of crops.
- The core of organic orchard system is to increase the soil nutrient content.

What are the new findings?

- In this study, the differences of soil physical and chemical properties with microbial community structure between organic and traditional orchard management were compared. The results showed that the activities of soil alkaline phosphatase, urease and sucrase in organic orchard management mode were significantly increased.

What is the expected impact on horticulture?

- It will help to improve the understanding of soil fertility in standard organic orchard, have important guiding significance for the management and regulation of soil ecological restoration and the improvement of organic fruit yield.

Introduction

Organic agriculture is also known as biological husbandry or eco-agriculture. This type of agriculture refers to crop production that uses organic fertilizers to meet the complete or basic nutritional demands of crops without the use of synthetic fertilizers, pesticides, growth regulators or livestock feed additives. Organic agriculture is a combination of traditional conservation agriculture and modern agricultural technology and can promote and strengthen the overall production management system of agro-ecosystem health, including biodiversity, biological cycles and soil biological activities (Hurtado-Barroso et al., 2017; Reganold and Wachter, 2016). By 2015, more than 50.9 million hectares of land in 179 countries were managed organically (Lernoud and Willer, 2017); among these regions, the European Union has set binding standards for the production of organic products (Mie et al., 2017).

The basis of the organic orchard system is to increase the soil nutrient content, particularly the organic matter content (Nielsen et al., 2009). An increase in soil organic matter helps to reduce atmospheric CO₂ levels (Stockmann et al., 2013) and increase soil bacterial activity and diver-

sity (Canali et al., 2009). Soil microorganisms are an important component of the soil ecosystem (Pankhurst et al., 1996) and are involved in the decomposition of organic matter, nutrient cycling and energy conversion (Averill and Hawkes, 2016). Microorganisms play an important role in maintaining the productivity, functionality and stability of the ecosystem (Felske et al., 2000); they are a key index to monitor soil quality and productivity (Kennedy and Smith, 1995); and there are differences in soil bacterial diversity among the different soil layers (Hinsinger et al., 2009). However, a comprehensive understanding of the mechanisms by which standard organic orchard management and traditional technology effect soil nutrients and bacterial communities is not clear. Therefore, studying the relationship between soil bacterial communities and soil nutrients under different management models can help advance an understanding of soil fertility in standard organic orchards and has significance for guiding the management and regulation of ecological restoration of organic orchard soil and the increase in organic fruit yields.

In this study, the soil of the Baiheng Organic Kiwi Orchard and that of conventionally managed kiwi orchards with similar site conditions were the research objects. The response of soil parameters to different agricultural technology management models was analysed by comparing the differences in soil physical and chemical properties with the bacterial community structure of different arable layers. Determining

the impact of different orchard management practices on soil physical and chemical properties and soil bacterial community structure provides a theoretical basis for promoting the development of organic agriculture and increasing the yield of organic products.

Materials and methods

Study site and soil samples

Shaanxi Baiheng Organic Orchard Co., Ltd., located in Shangwan Village, Wuquan Town, Yangling, Shaanxi (107°59'E, 34°3'N), is a production enterprise for organic kiwi fruit. This company has successfully passed four international organic certifications: the Chinese GB/T19630, the European Union EOS, the American NOP, and the Japanese JAS. On September 26, 2019, five-point sampling was used to collect soil samples in organic orchards and traditional orchards with similar site conditions and basic fertility. Litter was removed from the surface of the soil, and soil samples were collected from the 0~10 cm, 10~20 cm and 20~30 cm layers with a soil drill.

Determination of the soil enzyme activity index

Soil enzyme activity analytical methods were based on Lin (2010). Catalase activity was determined by the potassium permanganate titration volume method and expressed as 0.1 mol L⁻¹ potassium permanganate in 1 g of soil after 30 min (mL g⁻¹ (30 min)⁻¹). Urease activity was determined by phenol sodium colorimetry and expressed as the mass of NH₃-N in 1 g of soil after 24 h (mg g⁻¹ d⁻¹). Sucrase activity was determined by 3,5-dinitrosalicylic acid colorimetric phenol sodium colorimetry and expressed as the mass of glucose in 1 g of soil after 24 h (mg g⁻¹ d⁻¹). Alkaline phosphatase activity was determined by the phenyl disodium phosphate colorimetric method and expressed as the mass of phenol released from 1 g of soil after 3 h (μg).

Determination of soil physical and chemical properties

Soil physiochemical analytical methods were based on Bao (2000). The organic matter content was determined by the potassium dichromate-external heating method. Total nitrogen (Total N) was determined by perchlorate-concentrated sulfuric acid digestion and the half trace Kelvin distillation method. Total phosphorus (Total P) was determined by perchloric acid-concentrated sulfuric acid digestion and the molybdenum blue colorimetric method. Total potassium (Total K) was determined by NaOH digestion and flame photometry. Available P was determined by sodium bicarbonate extraction and the molybdenum-antimony colorimetric method. Available K was determined by ammonium acetate extraction and flame photometry. NH₄⁺-N was determined by the indophenol blue colorimetric method.

Microbial diversity analysis

1. PCR amplification and 16S rDNA sequencing. The V4 region of the prokaryotic (bacterial and archaeal) small-subunit (16S) rRNA gene was amplified with versions of primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAA-3'). The PCR conditions to amplify the prokaryotic 16S fragments consisted of an initial denaturation at 98°C for 30 sec, 35 cycles of denaturation at 98°C for 10 sec, annealing at 54°C/52°C for 30 sec, extension at 72°C for 45 sec, and then final extension at 72°C for 10 min. The PCR products were confirmed

with 2% agarose gel electrophoresis. The PCR products were purified by AMPure XT beads (Beckman Coulter Genomics, U.S.A.) and quantified by Qubit (Invitrogen, U.S.A.). The amplicon pools were prepared for sequencing, and the size and quantity of the amplicon library were assessed on an Agilent 2100 Bioanalyzer (Agilent, U.S.A.) with the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, U.S.A.), respectively. The PhiX Control Library (v. 3) (Illumina) was combined with the amplicon library (expected at 30%).

Statistical analysis

The soil physicochemical properties and enzyme activity were analysed and the graphics were created using Excel 2010, R v. 3.5.2 and Origin 2018.

Results

Effects of different orchard management methods on soil enzyme activity

Figure 1 shows that under the both orchard management methods, the enzyme activity of the soil decreased with increasing soil depth. Compared with conventional orchard management, organic orchard management improved soil enzyme activity. Alkaline phosphatase, urease and invertase activities (Figure 1a, 1c, and 1d, respectively) in the soil were significantly increased in the organically managed orchard ($p < 0.05$) with the largest change observed in the 0 to 10 cm soil layer. As the depth of the soil increased, the increase in soil enzyme activities diminished with decreases of 23.84~46.22%, 13.90~46.18% and 43.66~87.91% for alkaline phosphatase, urease and invertase, respectively. Organic agricultural management of the orchard also influenced the catalase activity in soil (Figure 1b), but the difference was not significant.

Effects of different orchard management methods on soil physical and chemical properties

As shown in Table 1, compared with the conventionally managed orchard soil, the organically managed orchard soil exhibited significantly improved soil organic matter, total nitrogen, total phosphorus, and nitrate nitrogen contents in each soil layer ($p < 0.05$) with increases of 11.14~39.25%, 33.96~62.07%, 13.04~40.51%, 121.58~180.99%, respectively. The total potassium and ammonium nitrogen contents changed slightly but not significantly in each soil layer. Organic orchard management changed the contents of available potassium and available phosphorus in the different soil layers; the content of available potassium in the 0~10 cm soil layer increased significantly ($p < 0.05$). At 10~20 cm and 20~30 cm, the soil available potassium content decreased significantly ($p < 0.05$) by 17.24% and 21.13%, respectively.

Summary of 16S rDNA sequencing data

1. Bacterial community structure and management type. At the phylum level (Figure 2a), the organically managed soil and conventionally managed soil contained a total of 32 phyla in the three soil layers (0~10 cm, 10~20 cm, and 20~30 cm), and the following 12 phyla were present with an average relative abundance >1%: *Proteobacteria* (27.8~25.9%), *Acidobacteria* (25.43~18.54%), *Actinobacteria* (22.64~12.96%), *Chloroflexi* (9.96~6.89%), *Gemmatimonadetes* (9.55~4.25%), *Planctomycetes* (8.62~3.05%), *Rokubacteria* (5.54~1.37%), *Latescibacteria* (3.17~1.08%), *Verrucomicrobia* (2.27~1.17%),

Nitrospirae (2.64~0.83%), *Bacteroidetes* (1.93~0.91%), and *Firmicutes* (1.32~0.54%). Among these taxa, the dominant bacterial phyla *Acidobacteria* and *Chloroflexi* were significantly more abundant in the organic soil than in the conventional soil in the 0–10 cm soil layer ($p<0.05$). *Gemmatimonadetes* was significantly more abundant in the organic soil than in the conventional soil in the 10–20 cm soil layer ($p<0.05$). The non-dominant phylum *Elusimicrobia* was significantly more abundant in the 20–30 cm organic soil layer than in the 20–30 cm conventional soil layer ($p<0.05$).

At the genus level (Figure 2b), compared with the conventional soil (Figure 2c), the organic soil (Figure 2d) exhibited an increased number of soil microorganisms, particularly in the 0–10 cm and 10–20 cm layers. We recorded 32 genera from the organic and conventional soils that were distributed in all three soil layers (0–10 cm, 10–20 cm, and 20–30 cm). We classified genera with an average relative abundance <1% as “other”, which constituted more than 40% of the total number of genera. Many of the bacterial genera with large relative percentages such as MND1 (3.75~1.89%),

FIGURE 1. Changes of soil enzyme activity under different orchard management systems. Different lowercase letters indicate significant differences in enzyme activity in different soil types and depths ($P<0.05$). (a: Alkaline phosphatase activity; b: Catalase activity; c: Urease activity; d: Sucrase activity).

TABLE 1. Physical and chemical properties of soil under different orchard management.

Project	0–10 cm		10–20 cm		20–30 cm	
	OS	CT	OS	CT	OS	CT
Organic matter(g kg^{-1})	15.88 $\pm$ 0.53a	13.39 $\pm$ 0.53b	15.40 $\pm$ 0.09a	13.86 $\pm$ 0.21b	14.32 $\pm$ 0.15b	10.28 $\pm$ 0.58c
Total N (g kg^{-1})	1.87 $\pm$ 0.03a	1.16 $\pm$ 0.02b	1.28 $\pm$ 0.03b	0.95 $\pm$ 0.05c	1.23 $\pm$ 0.09b	0.84 $\pm$ 0.08c
Total K (g kg^{-1})	19.74 $\pm$ 0.28	20.23 $\pm$ 0.73	19.97 $\pm$ 0.34	20.55 $\pm$ 0.98	19.58 $\pm$ 0.09	20.74 $\pm$ 0.58
Total P (g kg^{-1})	1.65 $\pm$ 0.03a	1.46 $\pm$ 0.03b	1.46 $\pm$ 0.11b	1.22 $\pm$ 0.05c	1.28 $\pm$ 0.03c	0.91 $\pm$ 0.11d
$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	1.22 $\pm$ 0.04a	1.12 $\pm$ 0.08a	0.69 $\pm$ 0.03b	0.74 $\pm$ 0.10b	0.21 $\pm$ 0.03c	0.27 $\pm$ 0.07c
$\text{NO}_3^+\text{-N}$ (mg kg^{-1})	1.094 $\pm$ 0.04a	0.39 $\pm$ 0.02d	0.80 $\pm$ 0.03b	0.36 $\pm$ 0.02de	0.72 $\pm$ 0.03c	0.30 $\pm$ 0.02e
Available K (mg kg^{-1})	481.33 $\pm$ 10.70a	392.00 $\pm$ 10.58b	262.33 $\pm$ 7.50de	317.00 $\pm$ 5.19c	231.33 $\pm$ 15.01e	293.33 $\pm$ 30.07cd
Available P (mg kg^{-1})	47.00 $\pm$ 4.13a	43.47 $\pm$ 0.45a	19.03 $\pm$ 1.97c	24.70 $\pm$ 0.17b	14.33 $\pm$ 0.32d	18.97 $\pm$ 1.11c

Different lowercase letters indicate significant differences in soil physical and chemical properties of different soil types and depths ($P<0.05$).

FIGURE 2. Community composition of soil microorganisms at phylum and genus levels (Relative abundance stacked bar plot). The number of microorganisms in different soil depths at genus levels.

TABLE 2. Analysis of bacterial diversity index of soil samples under different management modes.

Sample type		Bacterial diversity index			
		Shannon	Simpson	Chao1	Goods coverage
OS	0–10 cm	10.30±0.51a	0.99±0.00	2,618.11±285.09a	0.92±0.03
	10–20 cm	10.06±0.75ab	0.99±0.00	2,122.50±255.78b	0.92±0.05
	20–30 cm	10.02±0.45ab	0.99±0.00	2,313.00±367.46abc	0.94±0.01
CT	0–10 cm	10.29±0.11a	0.99±0.00	2,454.64±355.87ab	0.91±0.02
	10–20 cm	10.21±0.46ab	0.99±0.00	2,302.37±266.77ab	0.92±0.02
	20–30 cm	9.61±0.37b	0.99±0.00	1,869.78±149.90c	0.92±0.03

Different letters in the same column indicate significant ($P < 0.05$), while the same letter indicates no significance ($P > 0.05$).

RB41 (3.22~1.62%), *Gemmatimonas* (2.95~0.64%), *Nitrospira* (2.51~0.70%), *Haliangium* (1.43~0.96%), *Gaiella* (1.83~0.73%), *Blastococcus* (1.76~0.67%) and unclassified bacteria (11.91~10.09%) are known to be present in soils. The soil bacterial community structures of the conventional and organic soils also exhibited significant differences at the genus level; *Nitrospira*, *Blastococcus* and *Gemmatimonas* were the dominant flora exhibiting significant differences ($P < 0.05$).

2. Alpha diversity of the soil bacterial community. An alpha diversity analysis was conducted based on species annotation information. Table 2 shows that the Chao1 index under organic management was superior to that under conventional management at the 0–10 cm and 20–30 cm soil depths, indicating that organic management increased the number of soil microorganisms. In general, the Shannon index under organic management was higher than that of conventional management, whereas the Simpson index under organic management was smaller than under conventional management, indicating that the diversity of the soil microbial community of the organic orchard was generally better than that of the conventional orchard.

3. Beta diversity of the soil bacterial community. Based on the weighted UniFrac distance matrix, we used the vegan

package of R software to perform a principal coordinates analysis (PCoA) (Figure 3). The two first extracted principal components (PCoA1 and PCoA2) explained 55.58% of the variation, of which PCoA1 explained 39.49% and PCoA2 explained 16.09%, indicating that the soil bacterial community changed with different orchard management methods. The results showed that different orchard management methods resulted in significant structural differences in soil microorganisms in the 0–10 cm and 10–20 cm soil layers (Figure 3). At 20–30 cm (Figure 3), the soil bacterial composition structures were relatively similar, indicating that different orchard management systems had less effect on the deeper soil bacterial structure than on the more superficial structure.

Correlation between dominant bacterial flora and environmental factors

A Spearman correlation analysis of the dominant bacterial groups and soil environmental factors was conducted (Figure 4). At the phylum level, the average relative abundance of *Gemmatimonadetes* ($r = 0.36$) and *Chloroflexi* ($r = 0.38$) was positively correlated with catalase activity, whereas the relative abundance of *Latescibacteria* was negatively correlated with catalase ($r = -0.5$) and sucrose

FIGURE 3. Principal component analysis (PCoA) was used to analyze the Beta diversity of soil bacterial community. The red circle shows the structure of soil bacterial community under organic management.

FIGURE 4. Correlation coefficients between dominant bacteria groups and available edaphic factors. (a: Correlation between soil physical and chemical properties and phylum level dominant flora; b: Correlation between soil enzyme activity and phylum level dominant flora; c: Correlation between soil enzyme activity and physical and chemical properties).

($r=-0.380$) activities (Figure 4a). *Gemmatimonadetes* ($r=0.44$) and *Chloroflexi* ($r=0.60$) were positively correlated with soil organic matter, and *Latescibacteria* ($r=-0.52$) was negatively correlated with organic matter (Figure 4b). In the correlation analysis of soil enzyme activity and physical and chemical properties, the four measured enzymes (catalase, urease, sucrase and alkaline phosphatase) were positively correlated with soil organic matter, total nitrogen and nitrate and negatively correlated with total potassium (Figure 4c).

Discussion

Response of soil physical and chemical properties to different orchard management strategies

The physical and chemical properties of the soil directly affect the plant growth, bearing, yield and quality of orchards. The sustainable production of orchards is strongly associated with soil fertility (Chun et al., 2014). Standard organic kiwi orchard management significantly improved soil organic matter, total nitrogen, total phosphorus, nitrate nitrogen and other indicators, and these indicators were significantly different in different soil layers. These results indicate that organic management can meet the nutritional needs of fruit trees and maintain output without the use of external inputs such as pesticides and chemical fertilizers.

The long-term application of organic fertilizer, green manuring, etc., plays a key role in standard organic kiwi orchard management. Lin et al. (2019) found that the long-term application of organic fertilizers promoted the formation of soil organic matter and aggregates and changed the bacterial community structure and key groups of bacteria species. Escanhoela et al. (2019) found that organic agricultural management can increase nitrogen in the soil. Long-term planting of green manure plants between rows in organic orchards can increase soil organic matter and maintain fruit tree performance, improve soil organic matter, improve soil properties, and yield significantly higher levels of total nitrogen and total phosphorus in the fourth year compared with those in conventionally managed soils (Nielsen et al., 2014). In a study of weed management measures on the growth of citrus orchard fruit trees, fruit yield, and soil physical and chemical properties, it was found that compared with traditional tillage measures, returning weed cuttings and mulch to the field had a positive effect on soil nutrients (Hafeez-ur-Rahman et al., 2012).

Response of soil enzyme activity to different orchard management strategies

Soil enzymes are an important component of the soil ecosystem. The enzyme activity in the soil reflects the soil fertility status to some extent and is sensitive to changes in the external environment, acting as a sensitive early indicator of changes in the soil ecosystem (Ndèye et al., 2001). All biochemical reactions in the soil are accomplished with the participation of soil enzymes. (Mersi, 1991). Our results showed that the alkaline phosphatase, urease, and sucrase activities in the soil from a standard organic kiwi orchard were significantly increased in each soil layer compared with those in the soil from a conventionally managed kiwi orchard, and management strategy had the greatest influence on soil enzyme activity. As soil depth increased, the enzyme activity decreased. Under standard organic kiwi orchard management, green fertilizer crops are grown between the rows, and soil nutrient management is mainly accomplished by applying organic fertilizers. Li (2008) showed that in the 0~60 cm soil layer, the activities of catalase, urease and alkaline phosphatase in the orchard grassing area and the clear cultivation area were significantly higher in the upper soil layer than in the lower soil layer and decreased with increasing soil depth. Green fertilizer crops improved alkaline phosphatase, sucrase and catalase activities in pear orchard soil (Xu et al., 2010). A study evaluating the response of soil enzyme activity to alfalfa growth in the rows in a vineyard showed that planting alfalfa among the rows significantly increased the soil urease, phosphatase and cellulase activities compared with those of the other treatments, but the catalase activity did not change significantly in any treatment (Yan et al., 2007), which is consistent with the results of our study. No artificial synthetic substances (including pesticides, fertilizers, insecticides, bulking agents, etc.) were added during the entire organic orchard management process. However, the application of a large amount of pesticides in modern agricultural production will introduce pesticide residues and pollute the soil, affecting soil enzyme activity (Chen et al., 2017). Gianfreda et al. (1995) studied the effects of four pesticides (atrazine, carbaryl, glyphosate, and paraquat) on the catalytic behaviour of sucrase. It was found that glyphosate and paraquat increased the activity of invertase in kaolinite soil, whereas carbaryl decreased the activity of invertase in soil.

Response of soil bacterial diversity to different orchard management strategies

Individual species and communities of soil microbes play key roles in the many functions of the soil system (Canali et al., 2009). These functional processes include obtaining and recycling nutrients needed for plant growth, maintaining soil structure, degrading pesticides, and biological control of animal and plant pests (Parkinson and Coleman, 1991). Shishido et al. (2008) found that the bacterial community structure of orchards could be changed by different utilization methods. The results of our study showed that organic management increased the number of soil microorganisms and that there were obvious structural differences between the conventionally and organically managed orchards in the soil bacterial structures of the 0–10 cm and 10–20 cm soil layers. These differences may be related to the application of organic fertilizers in organic orchard management and the planting of green manure. St. Laurent et al. (2008) demonstrated that orchard grass production contributes to an increase in soil bacterial populations. The composition of the soil bacterial community structure also differed in soil samples subjected to different orchard management strategies. *Proteobacteria*, *Acidobacteria*, *Actinobacteria* and *Chloroflexi* were the dominant phyla in the orchard soil. Studies have shown that *Proteobacteria* and *Actinobacteria* are mainly involved in organic matter decomposition (Li et al., 2016; Štursová et al., 2012), and *Chloroflexi* and *Gemmatimonadetes* are mainly involved in carbon and nitrogen fixation (DeBruyn et al., 2011; Huang et al., 2011; Zhang et al., 2016). *Proteobacteria* and *Acidobacteria* are often used as indicators of soil nutrient status due to their different mechanisms of survival (Hartman et al., 2008). Previous studies have shown that the relative abundance of *Proteobacteria* is positively correlated with soil organic matter (Fazi et al., 2005; Fierer et al., 2007), and the abundance of *Proteobacteria* is higher in nutrient-rich soils than in soils with nutrient deficiencies (McCaig et al., 1999). Although our study failed to confirm this point, *Proteobacteria* was the dominant phylum in all treatments and was regarded as the main functional bacteria for the decomposition and transformation of organic matter. The results of our study are consistent with previous results (Mander et al., 2012).

Conclusions

Different orchard management measures had different effects on soil nutrients, enzyme activities and bacterial community diversity. An organic orchard management strategy can improve soil nutrient content and soil enzyme activity. By increasing soil bacterial diversity and changing community structure, standard organic management improved the stability of the soil ecological environment, which can also promote the stabilization of soil nutrients.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Averill, C., and Hawkes, C.V. (2016). Ectomycorrhizal fungi slow soil carbon cycling. *Ecol. Lett.* 19, 937–947. <https://doi.org/10.1111/ele.12631>.

Bao, S.D. (2000). *Soil and Agricultural Chemistry Analysis* (Beijing, China: Chinese Agric. Press).

Canali, S., Bartolomeo, E.D., Trinchera, A., Nisini, L., Tittarelli, F., Intrigliolo, F., Roccuzzo, G., and Calabretta, M.L. (2009). Effect of different management strategies on soil quality of citrus orchards in Southern Italy. *Soil Use Mgt.* 25, 34–42. <https://doi.org/10.1111/j.1475-2743.2008.00191.x>.

Chen, J., Zhou, S., Rong, Y., Zhu, X., Zhao, X., and Cai, Z. (2017). Pyrosequencing reveals bacterial communities and enzyme activities differences after application of novel chiral insecticide Paichongding in aerobic soils. *Appl. Soil Ecol.* 112, 18–27. <https://doi.org/10.1016/j.apsoil.2016.12.007>.

Chun-Hui, H., Xue-Yan, Q.U., Liu, K.-P., et al. (2014). Analysis of soil physicochemical properties, leaf nutrients and fruit qualities in the orchards of 'Jinkui' kiwifruit (*Actinidia deliciosa*). *J. Fruit Sci.* 31, 1091–1099.

DeBruyn, J.M., Nixon, L.T., Fawaz, M.N., Johnson, A.M., and Radoosevich, M. (2011). Global biogeography and quantitative seasonal dynamics of *Gemmatimonadetes* in soil. *Appl. Environm. Microbiol.* 77, 6295–6300. <https://doi.org/10.1128/AEM.05005-11>.

Escanhoela, A.S.B., Pitombo, L.M., Brandani, C.B., Navarrete, A.A., Bento, C.B., and do Carmo, J.B. (2019). Organic management increases soil nitrogen but not carbon content in a tropical citrus orchard with pronounced N₂O emissions. *J. Environm. Mgt.* 234, 326–335. <https://doi.org/10.1016/j.jenvman.2018.11.109>.

Fazi, S., Amalfitano, S., Pernthaler, J., and Puddu, A. (2005). Bacterial communities associated with benthic organic matter in headwater stream microhabitats. *Environm. Microbiol.* 7, 1633–1640. <https://doi.org/10.1111/j.1462-2920.2005.00857.x>.

Felske, A., Wolterink, A., Van Lis, R., De Vos, W.M., and Akkermans, A.D. (2000). Response of a soil bacterial community to grassland succession as monitored by 16S rRNA levels of the predominant ribotypes. *Appl. Environm. Microbiol.* 66, 3998–4003. <https://doi.org/10.1128/AEM.66.9.3998-4003.2000>.

Fierer, N., Bradford, M.A., and Jackson, R.B. (2007). Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364. <https://doi.org/10.1890/05-1839>.

Gianfreda, L., Sannino, F., and Violante, A. (1995). Pesticide effects on the activity of free, immobilized and soil invertase. *Soil Biol. Biochem.* 27, 1201–1208. [https://doi.org/10.1016/0038-0717\(95\)00034-C](https://doi.org/10.1016/0038-0717(95)00034-C).

Hafeez-ur-Rahman, Ghulam Nabi, Ijaz Ali, Tauseef Tahir, and Mukhtar Ahmed (2012). Growth and yield of Kinnow (*Citrus reticulata* Blanco) and soil physical properties as affected by orchard floor management practices in Punjab, Pakistan. *Soil Environm.* 31, 163–170.

Hartman, W.H., Richardson, C.J., Vilgalys, R., and Bruland, G.L. (2008). Environmental and anthropogenic controls over bacterial communities in wetland soils. *Proc. Nat. Acad. Sci. U.S.A.* 105, 17842–17847. <https://doi.org/10.1073/pnas.0808254105>.

Hinsinger, P., Bengough, A.G., Vetterlein, D., and Young, I.M. (2009). Rhizosphere: Biophysics, biogeochemistry and ecological relevance. *Plant Soil* 321, 117–152. <https://doi.org/10.1007/s11104-008-9885-9>.

Huang, L.N., Tang, F.Z., Song, Y.S., Wan, C.Y., Wang, S.L., Liu, W.Q., and Shu, W.S. (2011). Biodiversity, abundance, and activity of nitrogen-fixing bacteria during primary succession on a copper mine tailings. *FEMS Microbiol. Ecol.* 78, 439–450. <https://doi.org/10.1111/j.1574-6941.2011.01178.x>.

Hurtado-Barroso, S., Tresserra-Rimbau, A., Vallverdú-Queralt, A., and Lamuela-Raventós, R.M. (2017). Organic food and the impact on human health. *Critical Rev. Food Sci. Nutr.* 59, 704–714. <https://doi.org/10.1080/10408398.2017.1394815>.

- Kennedy, A.C., and Smith, K.L. (1995). Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil* 170, 75–86. <https://doi.org/10.1007/BF02183056>.
- Lernoud, J., and Willer, H. (2017). *The World of Organic Agriculture. Statistics and Emerging Trends.* (Frick, Switzerland: Research Institute of Organic Agric. (FiBL)).
- Li, H.K. (2008). Eco-environmental effect and integrated technical system of green cover in apple orchard in Weibei dryland farming areas. Ph.D. thesis (Shanxi, China: Northwest A&F Univ.) (in Chinese).
- Li, X., Sun, M., Zhang, H., Xu, N., and Sun, G. (2016). Use of mulberry-soybean intercropping in salt-alkali soil impacts the diversity of the soil bacterial community. *Microb. Biotechnol.* 9, 293–304. <https://doi.org/10.1111/1751-7915.12342>.
- Lin, X.G. (2010). *Principles and Methods of Soil Microbiology Research* (Beijing, China: Higher Education Press).
- Lin, Y., Ye, G., Kuzyakov, Y., Liu, D., Fan, J., and Ding, W. (2019). Long-term manure application increases soil organic matter and aggregation, and alters microbial community structure and keystone taxa. *Soil Biol. Biochem.* 134, 187–196. <https://doi.org/10.1016/j.soilbio.2019.03.030>.
- Mander, C., Wakelin, S., Young, S., Condrón, L., and O’Callaghan, M. (2012). Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. *Soil Biol. Biochem.* 44, 93–101. <https://doi.org/10.1016/j.soilbio.2011.09.009>.
- McCaig, A.E., Glover, L.A., and Ji, P. (1999). Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. *Appl. Environ. Microbiol.* 65, 1721–1730. <https://doi.org/10.1128/AEM.65.4.1721-1730.1999>.
- Mersi, W. von, and Schinner, F. (1991). An improved and accurate method for determining the dehydrogenase activity of soils with iodinitrotetrazolium chloride. *Biol. Fertil. Soils* 11, 216–220. <https://doi.org/10.1007/BF00335770>.
- Mie, A., Andersen, H.R., Gunnarsson, S., Kahl, J., Kesse-Guyot, E., Rembiałkowska, E., Quaglio, G., and Grandjean, P. (2017). Human health implications of organic food and organic agriculture: A comprehensive review. *Environ. Health* 16. <https://doi.org/10.1186/s12940-017-0315-4>.
- Ndèye Ndour Yacine Badiane, Chotte, J.L., Masse, D., and Rouland, C. (2001). Use of soil enzyme activities to monitor soil quality in natural and improved fallows in semi-arid tropical regions. *Appl. Soil Ecol.* 18, 229–238. [https://doi.org/10.1016/S0929-1393\(01\)00159-7](https://doi.org/10.1016/S0929-1393(01)00159-7).
- Neilsen, G., Forge, T., Angers, D., Neilsen, D., and Hogue, E. (2014). Suitable orchard floor management strategies in organic apple orchards that augment soil organic matter and maintain tree performance. *Plant Soil* 378, 325–335. <https://doi.org/10.1007/s11104-014-2034-8>.
- Neilsen, G.H., Lowery, D.T., Forge, T.A., and Neilsen, D. (2009). Organic fruit production in British Columbia. *Canadian J. Plant Sci.* 89, 677–692. <https://doi.org/10.4141/CJPS08167>.
- Pankhurst, C.E., Ophel-Keller, K., Doube, B.M., and Gupta, V.V.S.R. (1996). Biodiversity of soil microbial communities in agricultural systems. *Biodiv. Conserv.* 5, 197–209. <https://doi.org/10.1007/BF00055830>.
- Parkinson, D., and Coleman, D.C. (1991). Microbial communities, activity and biomass. *Agric. Ecosyst. Environm.* 34, 3–33. [https://doi.org/10.1016/0167-8809\(91\)90090-K](https://doi.org/10.1016/0167-8809(91)90090-K).
- Reganold, J.P., and Wachter, J.M. (2016). Organic agriculture in the twenty-first century. *Nature Plants* 2, 1–8. <https://doi.org/10.1038/nplants.2015.221>.
- Shishido, M., Sakamoto, K., Yokoyama, H., Momma, N., and Miyashita, S.-I. (2008). Changes in microbial communities in an apple orchard and its adjacent bush soil in response to season, land-use, and violet root rot infestation. *Soil Biol. Biochem.* 40, 1460–1473. <https://doi.org/10.1016/j.soilbio.2007.12.024>.
- St. Laurent, A., Merwin, I.A., and Thies, J.E. (2008). Long-term orchard groundcover management systems affect soil microbial communities and apple replant disease severity. *Plant Soil* 304, 209–225. <https://doi.org/10.1007/s11104-008-9541-4>.
- Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.B., de Remy de Courcelles, V., Singh, K., et al. (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agric. Ecosyst. Environm.* 164, 80–99. <https://doi.org/10.1016/j.agee.2012.10.001>.
- Štursová, M., Žifčáková, L., Leigh, M.B., Burgess, R., and Baldrian, P. (2012). Cellulose utilization in forest litter and soil: Identification of bacterial and fungal decomposers. *FEMS Microbiol. Ecol.* 80, 735–746. <https://doi.org/10.1111/j.1574-6941.2012.01343.x>.
- Xu, L., Han, Q.F., Wu, Z.-Y., et al. (2010). Spatial variability of soil enzyme activity in pear orchard under clean and sod cultivation models. *Sci. Agric. Sinica* 43, 4977–4982 (in Chinese).
- Yan, L., Xi, Z., and Cheng, J.M. (2007). Ecological distributing of soil microorganisms and activity of soil enzymes in vineyard green covering. *J. Northwest A&F Univ. (Nat. Sci. Edn.)* 35, 99–103 (in Chinese).
- Yongxin Lin, Guiping Ye, Yakov Kuzyakov, Deyan Liu, Jianbo Fan, and Weixin Ding (2019). Long-term manure application increases soil organic matter and aggregation, and alters microbial community structure and keystone taxa. *Soil Biol. Biochem.* 134, 187–196. <https://doi.org/10.1016/j.soilbio.2019.03.030>.
- Zhang, B., Kong, W., Wu, N., and Zhang, Y. (2016). Bacterial diversity and community along the succession of biological soil crusts in the Gurbantunggut Desert, Northern China. *J. Basic Microbiol.* 56, 670–679. <https://doi.org/10.1002/jobm.201500751>.

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Address of authors:

Li Tengfei, Liu Chongyi, Jin Xumei, Cao Xinyue, Lin Ziqi,
Lu Qi, Long Mingxiu* and He Shubin
College of Grassland Agriculture, Northwest A&F University,
Yangling, Shaanxi, China

* Corresponding author;

E-mail: longmingxiu@nwsuaf.edu.cn