



Research article

Response of grass interplanting on bacterial and fungal communities in a jujube orchard in Ningxia, northwest China



Rui Wang, Bing Cao, Quan Sun, Lihua Song*

School of Agricultural, Ningxia University, Yinchuan, Ningxia 750021, PR China

ARTICLE INFO

Keywords:

Agricultural sciences
 Biochemistry
 Microbiology
 Plant biology
 Bacteria
 Fungi
 Jujube
 Network analysis
 Redundancy analysis

ABSTRACT

Orchard grass is an important soil management method that improves pest resistance in fruit trees and it reduces the usage of chemical fertilizer to protect the environment. In this study, we investigated the bacterial and fungi communities in the rhizosphere of *Ziziphus jujuba* Mill cv. 'lingwuchangzao' by high-throughput sequencing to test the effects of different sward types. The soil organic matter, available phosphorus, available potassium, and total nitrogen contents were higher with cleared tillage compared with the other planting grass treatments. Ascomycota, Basidiomycota, and Mortierellomycota were the dominant fungal groups. Most of the soil nutrient levels were lower in the treatments with grass (except for planting with ryegrass and pea grass) than cleared tillage, but there were no significant differences in the bacterial and fungi diversity. pH and total phosphorus were the main contributors to variations in the bacterial communities. The variations in the fungal communities were mainly attributed to the soil nutrient levels. The changes in the titratable acids and vitamin C contents were clearly correlated with the dynamics of the bacterial and fungi communities. Network analysis showed that 60% of the bacteria had close connections with fungi, including the dominant bacteria comprising Proteobacteria, Actinobacteria, and Bacteroidetes. Our findings demonstrated that different types of grass treatments affected the abundances of microbes rather than their composition.

1. Introduction

China has multiple climate types and different regions have diverse ecological environments. The Ningxia region is located far from the sea in the arid desert region of northwest China and it is a suitable area for jujube culture because it receives sufficient solar radiation, with large differences in the day and night temperatures, and annual precipitation of about 200 mm (Song et al., 2015). Jujube is an important fruit tree in China where it has been cultivated for over 7000 years. In recent decades, due to the development of the local economy, jujube has become a major farmland tree with high economic and ecological benefits. Based on the cultivated area and yield, jujube is the seventh most important cultivated fruit tree in China, with great development prospects (Yang, 2014). *Ziziphus jujuba* Mill cv. 'lingwuchangzao' is one of main economic jujube tree varieties grown in Ningxia because the fruit is large, delicious, and nutritious, and it is favored by consumers (Song et al., 2015). At present, planting *Ziziphus jujuba* Mill cv. 'lingwuchangzao' is important for increasing the income and agricultural efficiency for farmers.

The fragile ecological environment of the Ningxia region and the long-term misuse of chemical fertilizers and pesticides by farmers has

caused the loss of resources and severely polluted the environment (Song et al., 2015). Orchard grass is an important soil management method, which can improve the pest resistance of fruit trees as well as reducing the incidence of plant diseases and insect pests, thereby decreasing the usage of pesticides and chemical fertilizers to protect the environment and enhance the fruit quality (Liu et al., 2014). A previous study investigated the effects of orchard grass on the basic soil indicators related to improvements in the soil structure, optimizing the physical and chemical properties of soil (Li et al., 2008), and its effects on soil nutrients (Bugg, 1994). It is well known that soil microbial diversity is critical for maintaining the sustainability of an agricultural production system (Bending et al., 2004; Pimentel et al., 2005) because soil microbes respond rapidly to changes and they can adapt to different environmental conditions (Ros et al., 2006). However, our understanding of the effects of grass on the soil microbial community in orchards is still limited.

Microorganisms can respond rapidly to changing environmental conditions by modifying the microbial biomass and community composition (Krashevska et al., 2015). Bacteria and fungi are the two main types of soil microorganisms, and they generally comprise 90% of the total soil microorganisms, where they have different roles in soil

* Corresponding author.

E-mail address: songlihuanx@126.com (L. Song).

biogeochemical processes (Wang et al., 2014). These organisms have essential roles in agroecosystems such as promoting soil nutrient cycling, providing nutrition to plants, and inhibiting soil-borne plant diseases (Wang et al., 2013; Thomas et al., 2011; Fontaine et al., 2011). The application of grass has been shown to improve the soil and fruit quality (Li et al., 2008; Yang, 2014). Importantly, soil microorganisms can change the soil physical and chemical characteristics to provide important nutrient sources to support plant growth (Colvan et al., 2001; Florian et al., 2009). However, it is not clear whether alterations to the soil physical and chemical characteristics will inevitably lead to changes in the dominant population and the quantity of soil microorganisms. In addition, different types of grass may affect the soil characteristics in the rhizosphere. Thus, the relationships among the microbial community in the rhizosphere, soil properties, and nutrient levels require comprehensive analysis.

In this study, in order to prevent the grass sward from competing for water and nutrients with jujube Mill CV. 'lingwuchangzao' trees, we planted ryegrass, dandelion, and clover as shallow-rooted plants that do not require repeated sowing (they persist for many years after a single planting due to natural reproduction), and they are readily adapted to orchards. Peas were also grown because of their capacity for nitrogen fixation. We investigated the bacterial and fungal communities in the rhizosphere of *Ziziphus jujuba* Mill cv. 'lingwuchangzao' by high-throughput sequencing to determine the effects of growing different types of sward. The objectives of this study were: (1) to detect the changes in the nutrient levels in the soil rhizosphere; (2) to determine the diversity and abundances of bacteria and fungi; (3) to explore the relationships among the soil rhizosphere characteristics, bacterial communities, and fungal communities; and (4) to investigate the contributions of dynamic changes in the microbial community in the soil rhizosphere to the quality of the *Ziziphus jujuba* Mill cv. 'lingwuchangzao' plants. The results of this study may provide a reference to facilitate the production of *Ziziphus jujuba* Mill cv. 'lingwuchangzao' in the Ningxia region of China.

2. Experimental procedures

2.1. Site description

Field trials were conducted in Lingwu, Ningxia Hui Autonomous Region, P. R. China (38°2'59"N, 106°39'37"E; altitude, 1250 m above sea level). The study area has a typical continental monsoon climate and the average annual precipitation is 206.2–255.2 mm. The soil in the test area is an irrigation-silted soil classified as sandy loam and it is the main soil type found on the semiarid plains in China. Light energy is abundant in the test area with average sunshine hours of 7.8–8.3 h per day. The annual average number of sunshine hours is over 3,000 h. The annual average temperature is 8.8 °C, and the difference in the day and night temperatures is 10–15 °C. The frost-free period is 165–175 days and plant growth continues for 170 days.

According to the different types of sward grown around jujube trees located on the forest farm in Lingwu city, six treatments were established, i.e., CT: control treatment with clean tillage; NG: planted with natural grass; RG: planted with ryegrass (*Lolium* sp.); DG: planted with dandelion (*Taraxacum mongolicum* Hand.-Mazz.) and grass; CG: planted with clover (*Trifolium repens* L.) and grass; and RPG: planted with ryegrass and pea (*Pisum sativum* L.) grass. Each treatment was planted on three replicate plots measuring 144 m² (16 × 9 m) with jujube tree 13–15 strain (row spacing 2.5 × 3 m). All of the treatments were subjected to uniform cultivation management. The trials began in the middle of April 2016. Grass seed was sown artificially to a depth of 1–2.5 cm. The amounts of grass sown per hectare were 13.5 kg, 12 kg, 15 kg, and 22.5–25 kg for RG, DG, CG, and RPG, respectively. Each treatment was separated by a 60 cm cement border.

2.2. Sample collection

In October 2016 (in the jujube ripening stage), samples were collected from each plot via five-point sampling. Soil from the 0–20 cm soil layer was collected by earth drilling and the five samples were combined. Debris such as gravel and plant residues were removed, and all of the soil samples were transported to the laboratory in a small refrigerator, which was maintained at 4 °C. A subsample of each soil sample was stored at 4 °C for chemical analyses. Another subsample was stored at –80 °C for sequencing analyses. All of the analyses were performed within 2 weeks of sampling.

Four jujube trees with uniform growth were randomly selected for each treatment, and thus a total of 24 fruit trees were labeled. After ripening, 10 jujube fruit were picked randomly from four positions at the same height for each treatment, which were then labeled and placed in self-sealing bags. The fruit samples were returned to the laboratory to measure their vertical and horizontal diameters and quality.

2.3. Soil characteristics and jujube properties

The soil pH was determined with a glass electrode using a soil:water ratio of 1:5 (Mettler-Toledo GmbH, Greifensee, Switzerland). The soil organic matter (SOM) content was determined using the potassium dichromate method. The total N (TN) content was determined by Kjeldahl digestion. The available N (AN) content was determined with the alkaline hydrolysis diffusion method. The total P (TP) content was measured using sodium hydroxide fusion followed by colorimetric analysis, and the available P (AP) content was extracted with 0.5 mol L⁻¹ NaHCO₃ (pH 8.5). The available K (AK) content was extracted with NH₄OAc and determined by flame photometry.

The soluble solid (SS) content was measured using a handheld saccharimeter (Atago Co., Japan). The reduced vitamin C content (VC) was determined by molybdenum blue colorimetry. The titratable acid (AC) content was determined by NaOH titration. The soluble sugar (SSu) content was determined with the anthrone method. The vertical and horizontal diameters of the fruit were measured with Vernier calipers. The shape index was calculated as the ratio of the longitudinal diameter relative to the transverse diameter.

2.4. DNA extraction and high-throughput sequencing

Microbial DNA was extracted from each soil sample using a FastDNA® SPIN kit for Soil (Mpbio, CA, USA) according to the manufacturer's instructions. The quantity and purity of the extracted DNA were examined using a Nanodrop One spectrophotometer (Thermo Fisher Scientific, CA, USA). Using high-throughput technology, and 16S rDNA and internal transcribed spacer (ITS) sequencing, we conducted a comprehensive analysis of the soil quality under six different sward treatments and characterized their effects on the diversity of the microbial community (bacteria and fungi). In addition, we evaluated the connections between the bacterial and fungal communities by network analysis.

The 341F/806R primers (5'-CCTAYGGGRBGCASCAG-3'/5'-GGAC-TACHVGGGTWTCTAAT-3') were used to amplify the V3–V4 region of the bacterial 16S rRNA gene (Caporaso et al., 2012). The ITS3/ITS4 primers (5'-GCATCGATGAAGAACGAGC-3'/5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the ITS region of the fungal rRNA gene (White et al., 1994). The thermal cycling conditions and quality assessment procedure were described by Sutton et al. (2013) and Orgiazzi et al. (2012). PCR and tag-encoded high-throughput sequencing of the 16S and ITS genes were performed by Guangzhou Sagene Biotech Co. Ltd. using the Illumina HiSeq platform. The sequences obtained (bacterial and fungal) were then assigned to operational taxonomic units (OTUs) at 97% similarity (OTU0.03) and based on core diversity analyses.

2.5. Statistical analysis

Averages, standard deviations, and fold change values for the soil properties, as well as the fruit properties and microbial community diversity were compared by analysis of variance with Duncan's multiple range test. Pearson's correlation coefficients were calculated using SPSS 13.0. Bar charts were produced using SigmaPlot 12.5. Redundancy analysis (RDA) was performed with CANOCO 4.5. Network analysis based on the Pearson's correlation coefficients between bacterial and fungal taxa at the phylum level was conducted using Cytoscape 3.4.0.

3. Results

3.1. Physicochemical properties of soil samples

The soil properties in all of the treatments are shown in Table 1. The orchard soil was alkaline and the pH exceeded 8 in all of the treatments. Compared with the CT treatment, the orchard soil had a significantly higher pH where the grass was grown, except in the RG treatment. The soil total salt content decreased significantly under all treatments compared with CT, where the lowest total salt content was 0.39 g/kg in the DG treatment. These results suggest that the grass in the orchard clearly reduced the soil salt content, i.e., by 3.57%, 14.29%, 19.64%, 21.43%, and 30.36% in RPG, CG, RG, NG, and DG respectively. The AN, SOM, AP, AK, and TN levels were highest in CT, and the AN level was also high in RPG. The AN content was highest in the RPG treatment and it was 2.17% higher than that in CT. However, SOM and AP were lower in the RPG treatment than CT, i.e., by 46.39% and 66.08%, respectively. The AK levels were 44.44% and 42.06% lower in the CG and RPG treatments compared with CT, respectively.

3.2. Diversity of the microbial communities in the six treatments

We obtained 946,570 high-quality bacterial sequences (from 31,705 to 94,570 per sample) and 1,333,631 high-quality fungal sequences (from 30,246 to 59,293 per sample). The diversities of the bacterial and fungi communities were assessed (Table 2) and no significant effects of the six treatments on the OTU numbers were found in both the bacterial and fungal communities. The effects of the treatments on the bacterial samples in terms of two diversity indexes comprising Chao1 and Observed species were not significant ($p > 0.05$). Moreover, there were no significant differences in the bacterial communities ($p > 0.05$) between RG, CG, NG, and CT according to the Simpson and Shannon indexes. However, the RPG treatment significantly reduced ($p < 0.05$) the Simpson and Shannon indexes for the bacterial community.

There were also no significant differences among the fungal communities in all treatments according to the Simpson and Shannon indexes. There were no significant differences in the fungal communities in the RPG and CT treatments according to the Chao1 index ($p > 0.05$). There were also no significant differences ($p > 0.05$) among the other treatments (NG, RG, DG, and CG) based on the Chao1 index, but all were

significantly higher than that under the CT treatment. The Observed species indexes were significantly higher with the SYC and PGY treatments than CT, but there was no significant difference between those under the CT and NG treatments. There was no significant difference in the Observed species indexes for the RPG and CT treatments.

3.3. Compositions of soil microbial communities

The compositions of the microbial communities were also investigated in the different treatments (Figure 1). In all of the treatments, Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, and Bacteroidetes were the dominant bacterial phyla, where they accounted for 85–90% of the total OTUs, followed by minor phyla with average abundances $>1\%$, including Crenarchaeota, Nitrospirae, and Verrucomicrobia. Proteobacteria was the most abundant phylum in all of the treatments. The abundances of Proteobacteria, Acidobacteria, Chloroflexi, and Gemmatimonadetes did not differ greatly among the treatments. The abundance of Actinobacteria was only significantly higher in the CG treatment compared with that in CT. There were no obvious differences in the abundances of Bacteroidetes in the other treatments compared to CT. The abundances of fungi were low compared with those of bacteria. Ascomycota, Basidiomycota, and Mortierellomycota were the dominant fungi at the phylum level. The abundance of Ascomycota was significantly higher in the sward treatments compared with CT and highest in RPG. The abundance of Basidiomycota was significantly higher in RG and DG than CT, but lower in the other treatments, with the lowest abundance in RPG. The abundance of Mortierellomycota was lower in all of the treatments compared with CT, with the lowest abundance in DG. Cercozoa was not detected in the CT treatment but it was present in all of the other sward treatments. The different sward treatments affected the compositions of other fungi at the phylum level (average abundance $<1\%$) rather than their abundances. For example, Blastocladiomycota, Calcarisporiellomycota, Chytridiomycota, and Mucoromycota were detected in some sward treatments but not in the CT treatment. However, Olpidiomycota, Rozellomycota, and Zoopagomycota were detected in the CT treatment but not in some sward treatments.

3.4. Relationships among environmental variables and soil bacterial communities

The relationships among the bacterial communities, soil properties, and fruit quality (Table 3) were analyzed by RDA (Figure 2). The first and second RDA axes explained 62.8% and 17.2% of the total variation in the bacterial communities, respectively, according to the phylum level data. Among the soil properties, the total salt, pH, AP, MC (moisture content), TN, SOM, and TP were more strongly associated with the first RDA axis, whereas AK and AN were more strongly associated with the second RDA axis. pH and TP mainly affected the bacterial community structure. In terms of the fruit quality, AC and VC were more strongly associated with the first RDA axis, whereas SSu and SS were more strongly associated

Table 1. The soil properties in different treatments.

Treatment	pH	Moisture content	Total salt (g/kg)	Organic matter (g/kg)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	Total N (g/kg)	Total P (g/kg)
CT	8.42 ± 0.03 c	16.05 ± 0.61a	0.56 ± 0.03 a	14.81 ± 0.16 a	86.33 ± 1.82 ab	100.31 ± 0.24 a	556.67 ± 8.82 a	1.26 ± 0.01 a	0.87 ± 0.01 a
NG	8.50 ± 0.08 b	12.23 ± 0.42b	0.44 ± 0.00 e	8.93 ± 0.36 c	56.47 ± 1.02 d	64.30 ± 0.22 c	273.33 ± 6.67 c	0.70 ± 0.02 e	0.72 ± 0.01 a
RG	8.43 ± 0.23 c	12.28 ± 0.16b	0.45 ± 0.00 d	11.43 ± 0.44 b	78.40 ± 2.25 c	81.38 ± 0.40 b	283.33 ± 8.82 c	0.91 ± 0.01 c	0.55 ± 0.27 a
DG	8.54 ± 0.12 b	10.62 ± 0.37c	0.39 ± 0.00 f	12.12 ± 0.38 b	85.63 ± 1.23 ab	52.75 ± 0.27 d	340.00 ± 5.77 b	0.97 ± 0.01 b	0.77 ± 0.03 a
CG	8.56 ± 0.19 ab	9.94 ± 0.74c	0.48 ± 0.03 c	9.34 ± 0.87 c	81.90 ± 0.81 bc	44.99 ± 0.32 e	236.67 ± 3.33 d	0.78 ± 0.00 d	0.60 ± 0.003 a
RPG	8.60 ± 0.24 a	9.62 ± 0.81c	0.54 ± 0.00 b	7.94 ± 0.33 d	88.20 ± 1.46 a	34.03 ± 0.22 f	240.00 ± 5.77 d	0.73 ± 0.01 e	0.60 ± 0.01 a

Values (mean ± standard deviation) indicate the absolute amount of each characteristic. Data within the same column followed by the same lowercase letter are not significantly different at $P > 0.05$. Different letters in a column indicate a significant difference ($P < 0.05$). Analysis of variance (ANOVA) was performed and each treatment had three replicates.

Table 2. Bacterial and fungal community α -diversity at different treatments.

Treatments	Bacteria					Fungi				
	OTU	shannon	simpson	chao1	observed_species	OTU	shannon	simpson	chao1	observed_species
CT	44249 ± 1096a	6.92 ± 0.08a	0.99±0a	164 ± 0.5a	164±0a	44535 ± 12203a	5.97 ± 0.48a	0.95 ± 0.02a	1531 ± 279b	972 ± 179bc
NG	41027 ± 8131a	6.90 ± 0.06a	0.99±0a	164±0a	164±0a	48544 ± 3801a	6.22 ± 0.68a	0.95 ± 0.03a	2145 ± 674a	1323 ± 118ab
RG	40256 ± 6217a	6.70 ± 0.07ab	0.99±0a	162 ± 2.16a	162 ± 2.63a	41990 ± 7795a	6.26 ± 0.14a	0.97 ± 0.01a	2179 ± 258a	1247 ± 121ab
DG	50579	6.87	0.99	162	162	49674 ± 8826a	6.34 ± 0.39a	0.95 ± 0.03a	2336 ± 391a	1392 ± 260a
CG	48433 ± 833a	6.82 ± 0.08ab	0.99±0a	163 ± 2.55a	162 ± 1.48a	49341 ± 12752a	6.76 ± 0.24a	0.97 ± 0.01a	2447.02 ± 167a	1483 ± 159a
RPG	50569 ± 9504a	6.53 ± 0.31b	0.9 ± 0.01b	164 ± 0.82a	164 ± 0.5a	41041 ± 10358a	5.60 ± 1.62a	0.89 ± 0.17a	1368 ± 289b	802 ± 179c

Values (mean ± standard deviation) were tested in Analysis of variance (ANOVA) with all replicates in each treatment. Data within the same column followed by the same lowercase letters are not significantly different at $P > 0.05$ level. Different letters in a column indicate a significant difference ($P < 0.05$).

with the second RDA axis. The changes in AC and VC were strongly correlated with the dynamic changes in the bacterial community. Moreover, VC was mainly affected by the pH. VC had strong relationships with soil properties such as AP, MC, TN, SOM, and TP. Figure 3 shows the relationships among the soil properties, fruit quality (Table 3), and fungal communities. The first and second RDA axes explained 69.8% and 23.3% of the total variation in the fungal communities, respectively, based on the phylum level data. Among the selected soil parameters, SOM explained 12.8% of the total variation, and AP, TP, TN, AK, pH, MC, and

total salt explained 4.8–11.9%, thereby suggesting that these variables had equally important effects on shaping the fungal communities. However, AN only explained 0.2% of the total variation in the fungal communities. The dynamic changes in fungi had the highest impact on the fruit quality according to the AC and VC results, which were similar to the results in Figure 2. VC also had a significant correlation with pH. Considering the effects of the different treatments on the microbial dynamics, we found that CT was not clearly separated from the other treatments in terms of the bacterial communities, whereas it was

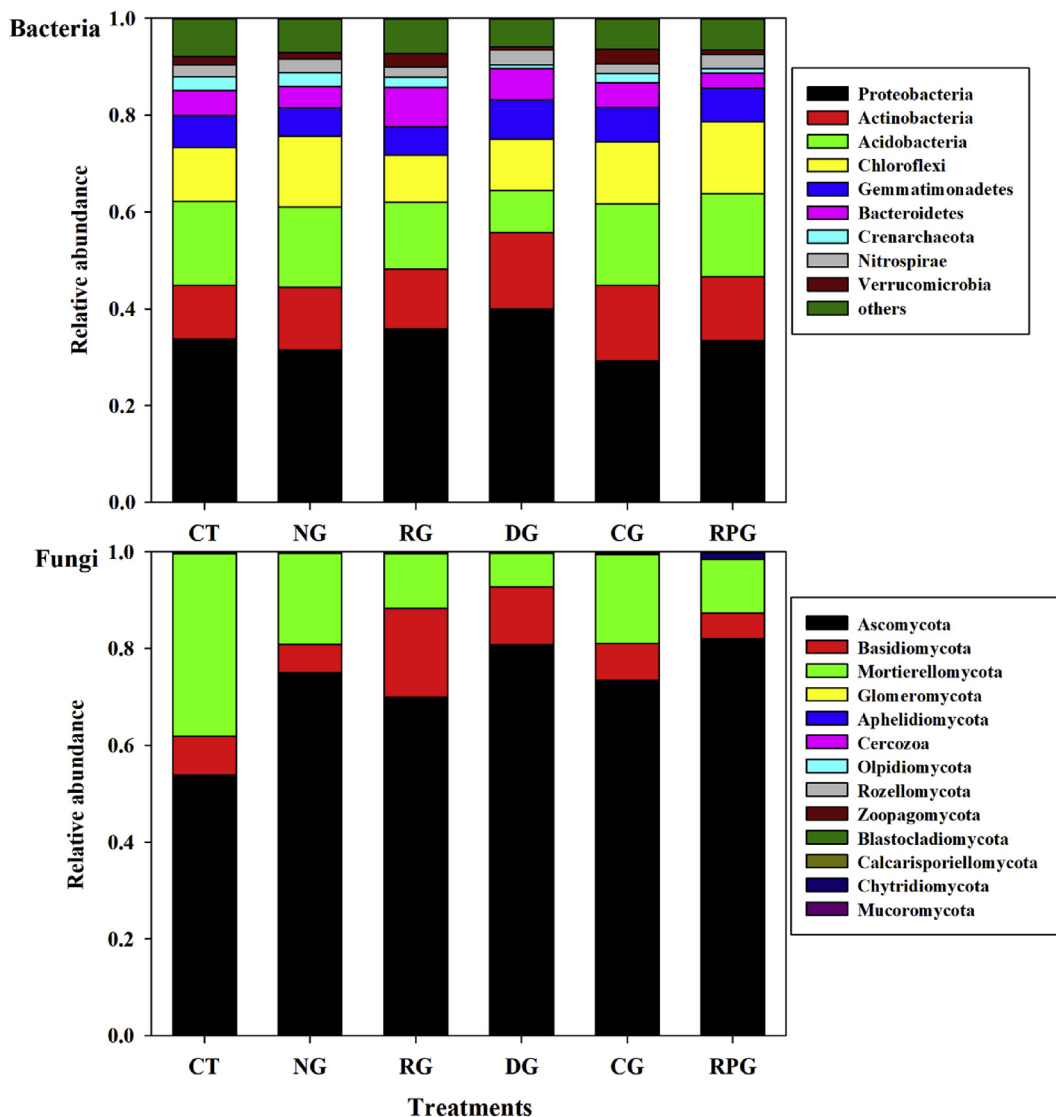


Figure 1. Composition of bacterial and fungal communities with the relative abundances at the phylum level.

Table 3. Fruit qualities in different treatments.

Treatments	Soluble solid content (%)	Vitamin C content (mg/100g)	Soluble sugar content (%)	Titrateable acid (%)
CT	33.10 ± 0.40a	67.95 ± 0.69c	16.20 ± 0.08d	0.20 ± 0.00cd
NG	30.23 ± 0.25c	68.08 ± 1.02c	22.72 ± 0.82b	0.19 ± 0.02cd
RG	31.13 ± 0.21b	54.54 ± 0.32d	17.13 ± 1.01d	0.22 ± 0.01ab
DG	33.30 ± 0.20a	66.99 ± 0.19c	32.84 ± 0.24a	0.23 ± 0.01a
CG	29.43 ± 0.40d	81.37 ± 0.13b	20.59 ± 0.47c	0.20 ± 0.00bc
RPG	31.33 ± 0.35b	109.17 ± 6.11a	20.49 ± 0.05c	0.18 ± 0.01d

Values (mean ± standard deviation) were tested in Analysis of variance (ANOVA) with all replicates in each treatment. Data within the same column followed by the same lowercase letters are not significantly different at $P > 0.05$ level. Different letters in a column indicate a significant difference ($P < 0.05$).

separated in terms of the fungal communities. However, the dynamics of the bacterial and fungal communities did not differ greatly among the treatments, which were similar to the results in Table 2.

Network analysis was performed to clarify the ecological relationships among the microbial communities under different sward treatments. All of the bacterial and fungal networks were constructed at the phylum level. Figure 4 shows the co-occurrence patterns for the bacterial and fungal communities based on the members with significant correlations. The overall network comprised 209 edges and 65 nodes, where the average number of neighbors was 6.43 and the clustering coefficient was 0.17. The network had a diameter of 6 and a characteristic path length of 2.60. Among the 65 nodes, 13 denoted fungi and 52 represented bacteria, where they comprised 98% of all the detected taxa. According to the network, 60% of the bacteria in the network had close connections

with fungi, including the dominant bacteria comprising Proteobacteria, Actinobacteria, and Bacteroidetes.

4. Discussion

Orchards may be planted with herbaceous plants to produce a sward throughout the whole orchard or between rows (except for the tree discs). This soil management model based on ground cover is an artificially constructed composite system, which involves multiple species, multiple levels, and multiple time series (Strasserf et al., 1995). Sward application in orchards is now recognized as a highly valuable management practice. However, the changes in the characteristic soil properties and microbial communities that occur in response to different types of sward in jujube orchards are not known.

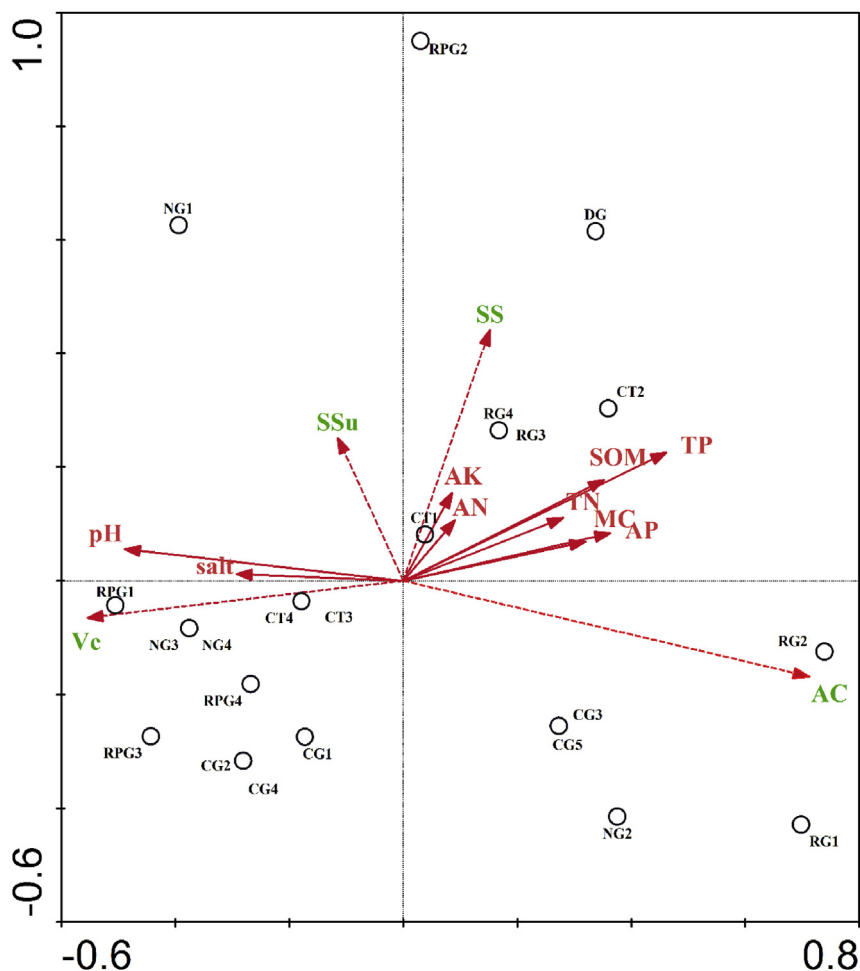


Figure 2. Redundancy analysis results showing the relationships between the soil physicochemical properties, fruit quality, and bacterial communities. Circles represent microbes in the treatments.

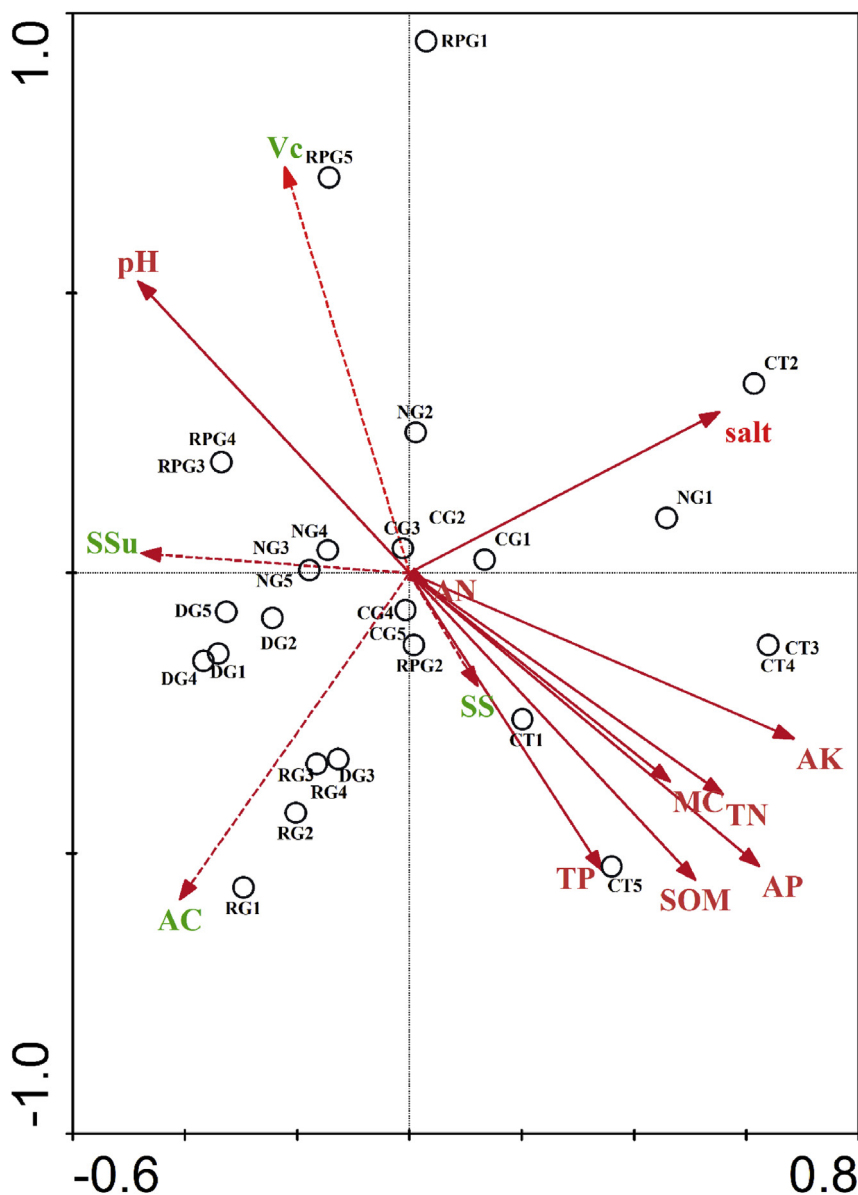


Figure 3. Redundancy analysis results showing the relationships between the soil physicochemical properties, fruit quality, and fungal communities. Circles represent microbes in the treatments.

In a previous study, [Sánchez et al. \(2007\)](#) investigated apple orchards in northern Patagonia and found that the SOM content in an orchard grown with grass for 6 years was 66.6% higher than that in a cleared orchard, and thus it significantly improved the soil environment in the orchard. Another study also investigated a pear orchard in the Yellow River delta region for 7 years and found that the AK content was increased by 79.7% under grass and the available nutrient contents in the surface soil increased significantly ([Wu et al., 2013](#)). In addition, many studies have shown that the daily variations in the soil temperature in orchards can be reduced by planting grasses, thereby improving the soil relative humidity ([Moreno et al., 2009](#)).

In our study, we found that the MC, AN, SOM, AP, AK, and TN levels were higher in the CT treatment than the other treatments planted with different grasses and other plants. Our results may be inconsistent with those obtained in previous studies because grasses require large amounts of nutrients in the early stages of growth and the topsoil is the direct source of these nutrients. Grasses compete for soil nutrients and this has been shown in many studies. For example, [Li et al. \(2007\)](#) found that in an apple orchard in an arid region, the consumption of soil nutrients was

greater than their accumulation in the presence of grass, and thus their levels declined in the early stage of grass growth. [Wang et al. \(2015\)](#) found that the soil nutrient levels tended to increase in mature orchards planted with grasses, whereas they decreased in young orchards. In our study, due to nitrogen fixation by the pea plants, the AN content was highest in the RPG treatment. However, this treatment consumed more AP and AK. Therefore, it is necessary to cut the grass regularly and supplement with appropriate fertilizer to reduce the competition for soil nutrients with grasses in young orchards.

Nutrients are the most important agricultural factors that significantly affect the abundance, diversity, and activity of soil microbes ([Chinnadurai et al., 2014](#); [Tamilselvi et al., 2015](#)). The RPG treatment significantly reduced ($p < 0.05$) the Simpson and Shannon indexes for bacteria, possibly due to the competition for soil AP and AK nutrients by pea plants. Interestingly, all of the diversity indicators for fungi did not differ significantly between the RPG and CT treatments. Most of the soil nutrient levels were lower than those in CT under the grass treatments (except for RPG), but there were no significant changes in the bacterial and fungi diversities. These results suggest that the competition for soil

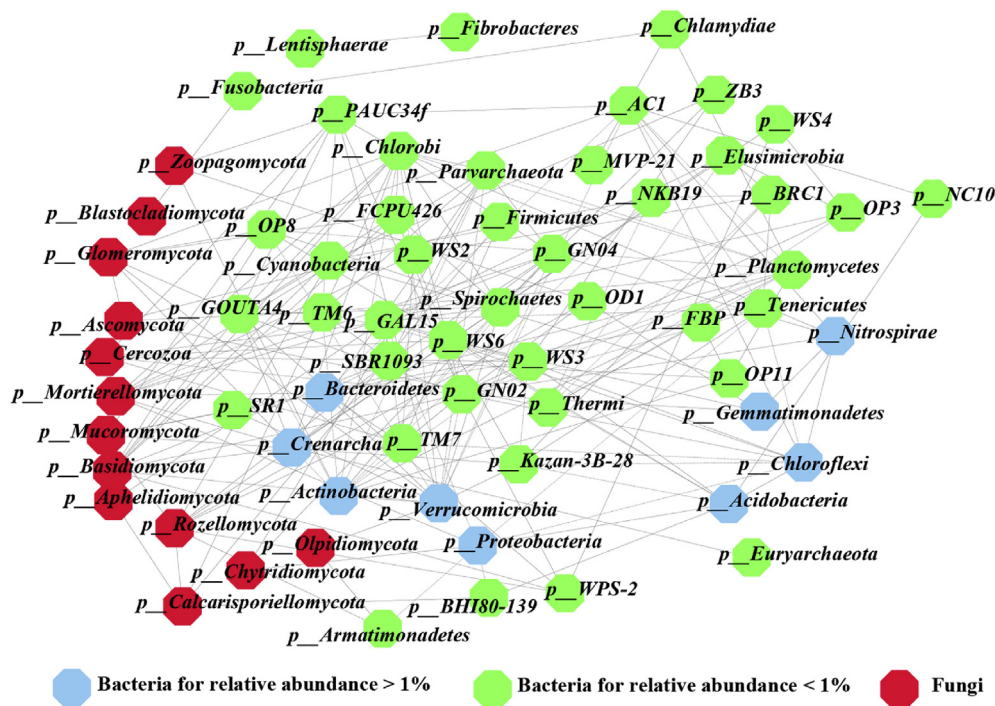


Figure 4. Network analysis showing co-occurrence of bacteria and fungi in different treatments.

nutrients by grasses did not affect the microbial richness. Similar to previous studies, we found that Proteobacteria, Actinobacteria, Acidobacteria, and Bacteroidetes were the main phyla in the bacterial communities (Zhang et al., 2017; Tripathi et al., 2014). The abundances of Proteobacteria and Acidobacteria did not differ greatly among the treatments. In addition, the abundances of Actinobacteria and Bacteroidetes did not differ significantly among most of the grass treatments and CT, thereby suggesting that the different type of grass treatments did not affect the structure of the main bacterial community. It should be noted that Firmicutes was also a major phylum detected in most previous investigations (Zhang et al., 2017; Tripathi et al., 2014; Banerjee et al., 2016; Ren et al., 2015) but not in the present study. Microbial populations may vary in terms of their preferred substrates and nutrient acquisition strategies (Goldfarb et al., 2011), where some have a lower capacity for nutrient acquisition, thereby affecting their likelihood of success in the community. Compared with bacteria, the fungal richness and diversity were very low and only 13 taxa were identified at the phylum level. The fungi were more susceptible to changes in the soil nutrient levels compared with the bacterial communities. Thus, the different grass treatments changed the fungal abundance and their composition (Figure 1). The relative abundances of the main groups (Ascomycota, Basidiomycota, and Mortierellomycota) varied among the different treatments. In addition, Olpidiomycota, Rozellomycota, and Zoopagomycota had low average abundances <1%, and they were detected in CT treatment but not in some of the grass treatments.

Recently, several ecosystem studies have shown that the soil pH strongly affects the compositions of microbial communities (Geisseler and Scow, 2014; Ding et al., 2016) and it is a good predictor of the composition of bacterial populations. In the present study, RDA (Figure 2) based on the soil properties and bacteria taxa showed that the soil pH was the most important contributor to the variations in the microbial communities. TP mainly affected the bacterial community structure, as also shown by Kumar et al. (2017). In addition, the RDA results (Figure 3) demonstrated that the structure of the fungal communities was closely correlated with most of the soil properties, where the soil characteristics had equal effects on shaping the fungal communities. The dynamic changes in bacteria strongly affected the fruit quality

in terms of AC and VC, and similar results were also obtained for fungi. These results indicate that the bacterial and fungal communities played important roles in the soil ecosystem, but they also determined the quality of the jujube fruit. Network analysis based on co-occurrence measured according to the correlations in the abundances of microbial taxa can help to understand complex microbial communities and provide more meaningful information than a simple sample level comparison (Barberan et al., 2012; Gilbert et al., 2012; Fuhrman, 2009). In the networks determined in our study, the bacterial communities were more compact and clustered than the fungal communities, possibly due to the greater richness of the bacterial community structure. Therefore, the less abundant taxa also need to be considered. We found that many of the low abundance bacterial taxa at the phylum level were the key links between bacteria and fungi (Figure 4).

The main limitation of our study is that we only explored the microbial community structure in one year of fruit production while intercropping with different sward types. In future research, longer sampling periods will be investigated. In particular, more samples will be collected at specific time intervals during experiments to obtain a more comprehensive understanding of the changes in the soil properties, soil microbial communities, and the fruit production during the summer, autumn, and ripening period. In addition, it is important that jujube farmers should consider the changes in microorganisms over a period of many years. Reasonable fertilizer application is essential for jujube fruit and tree development. Thus, understanding the relationships among growth, fruit quality, and fruit production indicators, and the soil microbial community with different fertilizer treatments such as organic fertilizer, compound fertilizer, and inorganic fertilizer is also essential. These analyses would provide a sound theoretical basis for the sustainable development of agriculture.

5. Conclusion

The competition for soil nutrients by grasses is unavoidable, and thus we found that the MC, AN, SOM, AP, AK, and TN levels were higher in the CT treatment than the different treatments with grasses. Due to the fixation of nitrogen by peas, the AN content was highest in the RPG

treatment, but it also consumed more AP and AK. The competition for soil nutrients by grasses did not affect the microbial richness (bacteria and fungi). In addition, the different types of grass treatments did not affect the composition of the major bacteria in the communities. However, the different grass treatments changed the abundances of fungi, as well as their composition at the phylum level. The bacterial and fungal communities played important roles in the soil ecosystem, but they also determined the quality of the jujube fruit. Many low abundance bacterial taxa at the phylum level were the key links between bacteria and fungi, which requires further research. This study only investigated the effects of interplanting on the bacterial and fungal communities in a jujube orchard during one year. Thus, future studies should investigate the effects over many years.

Declarations

Author contribution statement

R. Wang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

B. Cao: Contributed reagents, materials, analysis tools or data.

Q. Sun: Contributed reagents, materials, analysis tools or data.

L. Song: Conceived and designed the experiments.

Funding statement

This work was supported by the Major Innovation Projects of Western Top Universities (ZKZD2017008) and the Key Research and Development Program of Ningxia (2017BN0102).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We thank Dr Duncan E. Jackson for editing and improving the manuscript.

References

- Bugg, R.L., 1994. Waddington C. Using cover crops to manage arthropod pests of orchards: a review. *Agric. Ecosyst. Environ.* 50, 11–28.
- Bending, G.D., Turner, M.K., Rayns, F., Marx, M.C., Wood, M., 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. *Soil Biol. Biochem.* 36, 1785–1792.
- Banerjee, S., Kirkby, C.A., Schmutter, D., Bissett, A., Kirkegaard, J.A., Richardson, A.E., 2016. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil Biol. Biochem.* 97, 188–198.
- Barberan, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* 6, 343–351.
- Colvan, S., Syers, J., O'Donnell, A., 2001. Effect of long-term fertiliser use on acid and alkaline phosphomonoesterase and phosphodiesterase activities in managed grassland. *Biol. Fertil. Soils* 34, 258–263.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berglyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1621–1624.
- Chinnadurai, C., Gopalaswamy, G., Balachandar, D., 2014. Impact of long-term organic and inorganic nutrient managements on the biological properties and eubacterial community diversity of the Indian semi-arid. *Alfisol. Arch. Agron. Soil Sci.* 60, 531–548.
- Ding, J., Jiang, X., Ma, M., Zhou, B., Guan, D., Zhao, B., Li, J., 2016. Effect of 35 years inorganic fertilizer and manure amendment on structure of bacterial and archaeal communities in black soil of northeast China. *Appl. Soil Ecol.* 105, 187–195.
- Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revalliot, S., Maron, P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biol. Biochem.* 431, 86–96.
- Florian, C., Antoine, F., Christian, G., 2009. Competition for nitrogen in an unfertilized intercropping system: the case of an association of grapevine and grass cover in a Mediterranean climate. *Eur. J. Agron.* 30, 41–51.
- Fuhrman, J.A., 2009. Microbial community structure and its functional implications. *Nature* 459, 193–199.
- Goldfarb, K.C., Karaoz, U., Hanson, C. a, Santee, C. a, Bradford, M. a, Treseder, K.K., Wallenstein, M.D., Brodie, E.L., 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. *Front. Microbiol.* 2, 94.
- Gilbert, J.A., Steele, J.A., Caporaso, J.G., Steinbruck, L., Reeder, J., Temperton, B., Huse, S., McHardy, A.C., Knight, R., Joint, I., Somerfield, P., Fuhrman, J.A., Field, D., 2012. Defining seasonal marine microbial community dynamics. *ISME J.* 6, 298–308.
- Krashevskaya, V., Klamer, B., Widyastuti, R., Maraun, M., Scheu, S., 2015. Impact of tropical lowland rainforest conversion into rubber and oil palm plantations on soil microbial communities. *Biol. Fertil. Soils* 51, 697–705.
- Kumar, U., Shahid, M., Tripathi, R., Mohanty, S., Kumar, A., Bhattacharyya, P., Lal, B., Gautam, P., Raja, R., Panda, P.B., Jambhulkar, N.N., Shukla, K., Nayak, A.K., 2017. Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization. *Ecol. Indic.* 73 (73), 536–543.
- Liu, F.T., Zhang, L.S., Li, X.W., Li, B.Z., Han, M.Y., Gu, J., Wang, X.L., 2014. Effects of inter-row planting grasses on soil organic carbon fractions and soil microbial community of apple orchard in Weibei dryland. *Plant Nutr. Fert. Sci.* 20, 355–363 (in Chinese).
- Li, H.K., Zhang, G.J., Zhao, Z.Y., Li, K.R., 2008. Effects of different herbage on soil quality characteristics of non-irrigated apple orchard in Weibei Loess Plateau. *Sci. Agric. Sin.* 41, 2070–2076 (in Chinese).
- Li, H.K., Zhang, G.J., Zhao, Z.Y., 2007. Effects of interplanting of herbage on soil nutrient of non-irrigated apple orchard in the Loess Plateau. *Acta Hortic. Sin.* 34, 477–480 (in Chinese).
- Moreno, B., Garcíarodríguez, S., Cañizares, R., Castro, J., Benítez, E., 2009. Rainfed olive farming in south-eastern Spain: long-term effect of soil management on biological indicators of soil quality. *Agric. Ecosyst. Environ.* 131, 333–339.
- Orgiazzi, A., Lumini, E., Nilsson, R.H., Giralanda, M., Vizzini, A., Bonfante, P., Bianciotto, V., 2012. Unravelling soil fungal communities from different Mediterranean land-use backgrounds. *PLoS One* 7, e34847.
- Pimentel, D., Hepperly, P., Hanson, J., Douds, D., Seidel, R., 2005. Environmental, energetic, and economic comparisons of organic and conventional farming systems. *Bioscience* 55, 573–582.
- Ros, M., Goberna, M., Moreno, J., Hernandez, T., Garcia, C., Insam, H., Pascual, J.A., 2006. Molecular and physiological bacterial diversity of a semi-arid soil contaminated with different levels of formulated atrazine. *Appl. Soil Ecol.* 34, 93–102.
- Ren, W., Ren, G., Teng, Y., Li, Z., Li, L., 2015. Time-dependent effect of graphene on the structure, abundance, and function of the soil bacterial community. *J. Hazard Mater.* 297, 286–294.
- Song, L.H., Qin, F., Bai, X., 2015. Effect of elevated temperature and drought stress on fruit setting rate and fruit quality of Lingwu Long jujuba. *J. Northwest For. Univ.* 30, 129–133 (in Chinese).
- Sutton, N.B., Maphosa, F., Morillo, J.A., Al-Soud, W.A., Langenhoff, A.A., Grotenhuis, T., Rijnaarts, H.H., Smidt, H., 2013. Impact of long-term diesel contamination on soil microbial community structure. *Appl. Environ. Microbiol.* 79, 619–630.
- Strasserf, R.J., Srivastava, A., Govindjee, 1995. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochem. Photobiol.* 61, 32–42.
- Sánchez, E.E., Giayetto, A., Cichón, L., Fernández, D., Aruani, M.C., Curetti, M., 2007. Cover crops influence soil properties and tree performance in an organic apple (*Malus domestica*, Borkh) orchard in northern Patagonia. *Plant Soil* 292, 193–203.
- Thomas, F., Hehemann, J.H., Rebuffet, E., Czjzek, M., Michel, G., 2011. Environmental and gut Bacteroidetes: the food connection. *Front. Microbiol.* 2, 93.
- Tamilselvi, S.M., Chinnadurai, C., Ilamurugu, K., Arulmozhiselvan, K., Balachandar, D., 2015. Effect of long-term nutrient managements on biological and biochemical properties of semi-arid tropical Alfisol during maize crop development stages. *Ecol. Indic.* 48, 76–87.
- Tripathi, B.M., Kumari, P., Weber, K.P., Saxena, A.K., Arora, D.K., Kaushik, R., 2014. Influence of long term irrigation with pulp and paper mill effluent on the bacterial community structure and catabolic function in soil. *Indian J. Microbiol.* 54, 65–73.
- Wang, J., Zhang, H., Zhang, X., Qin, S., Tan, H., Li, X., 2013. Effects of long-term chlorimuron-ethyl application on the diversity and antifungal activity of soil *Pseudomonas* spp. in a soybean field in Northeast China. *Ann. Microbiol.* 63, 335–341.
- White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J.L., 1994. 38-Amplicon and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols* 38, 315–322.
- Wu, Y.S., Zhang, Y.M., Ji, X.H., Zhang, R., Liu, D.L., Zhang, Z.Y., Li, W.Y., Chen, X.S., 2013. Effects of natural grass on soil nutrient, enzyme activity and fruit quality of pear orchard in Yellow River Delta. *Sci. Agric. Sin.* 46, 99–108 (in Chinese).

- Wang, J., Zhang, H., Li, X., Su, Z., Li, X., Xu, M., 2014. Effects of tillage and residue incorporation on composition and abundance of microbial communities of a fluvo-aquic soil. *Eur. J. Soil Biol.* 65, 70–78.
- Wang, Y.Y., Ji, X.H., Wu, Y.S., Mao, Z.Q., Jiang, Y.M., Peng, F.T., Wang, Z.Q., Chen, X.S., 2015. Research progress of cover crop in Chinese orchard. *Chin. J. Appl. Ecol.* 26, 1892–1900 (in Chinese).
- Yang, Y., 2014. Research progress of genetic improvement of *Ziziphus jujube* in China. *J. Jilin For. Sci. Technol.* 43, 43–46 (in Chinese).
- Zhang, X., Zhang, R., Gao, J., Wang, X., Fan, F., Ma, X., Yin, H., Zhang, C., Feng, K., Deng, Y., 2017. Thirty-one years of rice-rice-green manure rotations shape the rhizosphere microbial community and enrich beneficial bacteria. *Soil Biol. Biochem.* 104, 208–217.