The Impact of Land use on Bacterial Community Composition and Physicochemical Properties of Soil

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In order to determine the effects of land use on bacterial community composition and some physicochemical properties of soil, a study was carried out in three different land uses (orchard, farm and shrub) in arid-region soils located in southeast of Iran. The selected land uses were adjacent to each other and have not been changed in 50 recent years. Three samples of surface soil were collected from each land use and the bacterial community composition of soil was assessed using denaturing gradient gel electrophoresis (DGGE). Moreover, some soil physicochemical properties were investigated. The results showed that significant differences exist among all of the soil physicochemical properties in different land uses. Higher values of organic carbon, N, P, K, EC, clay, silt, $CaCO_3$, Fe, Mn, Zn and Cu and lower values of pH and sand occurred in the agricultural lands (orchard and farm) than shrub land. The results of principal component analysis showed that land use can affect soil properties. Furthermore, results of canonical correspondence analysis showed that the variability in bacterial community composition was related to the selected soil characteristics. Clay and $CaCO_3$, sand, and OC, total N and EC were shown to be the most significant properties affecting the bacterial community composition in farm, shrub and orchard land uses, respectively. Generally, the findings of the present study showed that agricultural activities not only did not cause a reduction in species richness, but also resulted in a significant increase in the mentioned indices in orchard and farm land uses compared to shrub land use

Kay words: Arid-region, Land use, soil bacteria, soil characteristics, DGGE, PCA, CCA.

Different soil particle sizes and their arrangement in soil are resulted in the creation of very diverse and heterogeneous physical environment. Microbial community composition has a close relationship with variety of soil characteristics such as chemical, physical and biological properties[1-3]. Jangid et al. (2008)[4] reported that pH and organic carbon content have the greatest impact on soil microbial community composition. In this regard, another study showed that changes in pH and soil texture greatly influenced bacterial community composition[5].

In addition to the inherent characteristics of soil, many factors like rhizosphere (root effect on microorganisms), soil pollutants and land use play significant roles on soil microbial community composition. Many researchers have reported that human activities such as changes of land use and management practices exert great influences on the community composition of soil microorganisms[4,6-9].

Recently, metagenomics methods have been frequently used for studying soil microbial communities. These methods eliminate the use of culture by providing access to genetic information

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of microorganisms existed in environmental samples. This issue can be a great help for comprehensive understanding of researchers of microbial community, and consequently plays a vital role in the progress of practical part of microbial research including agriculture[10-11].

One of the best PCR-based metagenomics methods used for assessing microbial community structure is PCR-DGGE method. The results of many researches have shown that molecular analysis using DGGE of 16S rDNA gene amplification is a powerful tool in the study of bacterial community composition in complex environments[12-16]. Therefore, the aim of this study was to evaluate the impact of land use on the bacterial community composition and some physicochemical properties of soil.

MATERIAL AND METHODS

Site and sampling

Jiroft plain with the elevation of 650 meters is one of the lowest plains located 28°28' 40" to 28°52' 6" North and 57°30' 8" to 58? 4' 27" East of Iran with hot and dry climate, mean precipitation of 140 mm, relative humidity of 55% and the maximum and minimum temperatures of 48 and 1 degree Celsius. Soil moisture and thermal regimes of the region are aridic and hyperthermic respectively.

In Jiroft plain, a dominant geomorphologic unit (alluvial plain, located in the village of Ali Abad) containing shrub land that has not been cultivated so far because of water scarcity (*Calligonumpersicum* and etc.), field crops (at least in the last 50 years under fall planting of potatoes and onions, and in some years the spring planting of corn and etc.) and horticultural crops (more than 50 years under citrus cultivation) was selected.

The order of studied soils in the three neighboring land uses was Aridisols. Three soil samples, each contains ten subsamples, were gathered from A horizon (0-10 cm) across each land use on February 2015.

Physicochemical analysis of soils

The soil samples were dried and finally passed through 2 mm sieve. Some important soil characteristics (pH, electrical conductivity (EC), calcium carbonate (CaCO$_3$) organic carbon (OC), clay, sand, silt, total nitrogen (N),available

phosphorus (P), potassium (K), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu)) affecting microbial community composition were determined using common laboratory methods.

Soil DNA extraction and PCR amplification

TheNucleoSpin® Soil kit (Macherey-Nagel, Duren, Germany) was used for extraction and purification of DNA from soil samples. Bacterial universal primer set of 341F (CCTACGGGAGGCAGCAG) and 534R (ATTACCGCGGCTGCTGG), which amplify the variable region V3 of bacterial 16S rDNA gene sequences, was used for amplification of 16S rDNA gene of soil bacteria. Besides, A GC-rich clamp (CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGGC ACGGGGGGCCTACGGGAGGCAGCAG) was added to the forward primer prevented the complete melting of the PCR amplicons during subsequent separation in DGGE[17].

Soil DNA was amplified in a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). PCR reaction mixture in a volume of 50 ìl contains: 50 ng of soil genomic DNA, 20 pmol of each of 341F-GC and 534R primers, 25 ìl of Taq DNA Polymerase 2X Master Mix Red (1.5mM MgCl$_2$) (Ampliqon, Skovlunde, Denmark) and Milli-Q water to the final volume of 50 ìl. PCR was conducted under the following conditions: initial denaturation at 95 °C for 5min, followed by35 cycles including 20 cycles of Touchdown program; denaturation step at 94 °C for 30 sec; annealing step at 55-65 °C for 30 sec (decreased 0.5 °C per cycle); elongation step at 72 °C for 30 sec, and 15 cycles including 94 °C for 30 sec, 55 °C for 30 sec and 72 °C for 30 sec, and completed by a final elongation step of 10 min at 72 °C. The amplified products (5 ìl) electrophoresed on a 2% agarose gel, stained with ethidium bromide, and finally imaging was performed using a UV transilluminator.

PCR-DGGE analysis

DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA) was used for DGGE analysis, so that equal amount (25 ìl) of amplified segments of 16S rDNA gene was run onto 8% polyacrylamide (37.5: 1, acrylamide: bisacrylamide) denaturing gel containing a 40-60% gradient(100% denaturant concentration was defined as 7 M urea and 40% v/v deionized formamide). Then, the gel was electrophoresed in 1X TAE buffer (40 mMTris

base, 20 mM acetic acid, and 1 mM disodium EDTA, pH 8.3) for 17 hours with a constant voltage of 100 V at 60 °C. After the run, the gel was silver-stained[18] and photographed with Camera.

Data analysis

DGGE image and bacterial species richness (the number of bands present) were analyzed using GelCompar II 6.6 software. The position and intensity of each band were determined automatically [19].

The data were subjected to a one-way ANOVA by using IBM SPSS software.Principal component analysis (PCA), performed by PAST3 software, was used for evaluating the soil properties in various land uses[20]. PAST3 software was also used for canonical correspondence analysis (CCA), which was used for assessing the influence of soil properties on the structure of the bacterial community (DGGE patterns)[21-22].

RESULTS

Soil physicochemical properties

The results related to the physicochemical properties measured from soils of different land uses are shown in Table 1. As the results showed, significant differencesat 5% level were observed in soil properties among land uses which continue to be listed separately.

Soil moisture content

Soil moisture content at the time of sampling in farm and orchard was significantly greater than shrub land use.

Soil texture

Results showed that doing agricultural practices for about half century on shrub land changed the soil texture, especially in the surface layer, thus the dominant sand texture in shrub land use (this land use is adjacent to orchard and farm land uses, but is not under cultivation due to water restrictions) has been changed to sandy loam texture in orchard and farm lands. In addition, a significant difference was found in the means of soil separates (clay, silt and sand) in various land uses. The mean percentage of sand in the soil of shrub land use was higher than that of the other two land uses. Silt and clay percentages were greater in orchard and farm land uses, respectively.

CaCO₃

CaCO₃ percentagein farm land use was significantly higher than the other two land uses, but no significant difference was found between shrub and orchard land uses at 5% level. The higher amount of $CaCO_3$ in farm land use may be associated to the role of agricultural practices like plowing, which occurs annually in the land use and leads to the transfer of $CaCO_3$ from subsurface horizons to soil surface.

Soil pH

Soil pH may change owing to the different land management methods.

The results showed that soil pH in shrub land use was higher than the two land uses of farm and orchard; however this difference was significant only with orchard land use.

EC

EC rates in all the three land uses were low (less than 3 ds/m). A comparatively greater rate of this variable in orchard land use showed significant difference from the other two land uses, mainly due to the salinity resulting from the long-term use of chemical fertilizers.

OC

Orchard land use with an average of 0.086% OC showed significant differences at 5% level from farm and shrub land uses with 0.043 and 0.030%, respectively.

Total N

Orchard land use contained significantly a greater level of total N relative to farm and shrub land uses, which can be owing to the use of chemical fertilizers as well as greater content of organic material in orchard land use.

Available P

The amount of available P in orchard and farm land uses was significantly higher than shrub one, which might be mainly due to the use of phosphorous fertilizers in agricultural lands.

Available K

General comparison of the soil from the three land uses showed that the amount of K in shrub land use was significantly less than the other two land uses, which can be mainly owing to the consumption of potassium fertilizers in agricultural lands.

Available Fe, Mn, Zn and Cu

Unlike shrub land use, the means of four properties showed significant enhancement in agricultural land uses (orchard and farm). Climatic conditions, status of parent materials and

Table 1. The mean values of selected soil
physicochemical properties of different land uses

Physicochemical soil properties	Land uses		
	Orchard land use	Farm land use	Shrub land use
pH	7.70[a]	7.96[b]	8.17[b]
OC(%)	0.086[a]	0.043[b]	0.030[b]
N (mg/kg)	84.33[a]	48.59[b]	27.00[b]
P (mg/kg)	11.15[a]	9.87[a]	1.11[b]
K (mg/kg)	259[a]	273[a]	78[b]
EC (ds/m)	2.52[a]	0.89[b]	0.46[b]
Clay (%)	0.25[a]	3.71[b]	0.00[a]
Silt (%)	46.63[a]	39.00[a]	1.33[b]
Sand (%)	53.11[a]	60.61[a]	98.66[b]
CaCO$_3$ (%)	3.86[a]	5.50[b]	4.02[a]
Moisture (%)	13.06[a]	9.34[a]	1.23[b]
Fe(mg/kg)	3.69[a]	3.83[a]	1.14[b]
Zn(mg/kg)	0.57[a]	0.58[a]	0.12[b]
Mn(mg/kg)	2.56[a]	2.33[a]	1.15[b]
Cu(mg/kg)	0.36[a]	0.26[ab]	0.09[b]

Values with the same letters within a row are not significantly different at 5% level
among the land uses

topographic conditions in all the three adjacent land uses were the same; therefore, the main reason for the higher content of these micronutrients in agricultural lands could be the role of human and use of micronutrient-containing chemical fertilizers.

The PCA of soil physicochemical properties

Basically, if the number of properties is high, correlation coefficient increases and therefore they will be difficult to interpret. As a result, researchers have been looking for ways to reduce the data in a way that this reduced set of data shows the maximum variance in the data[23]. 15 soil physicochemical properties were used for the PCA. As the results of PCA analysis (Table 2) demonstrate, the first two components showed totally 89.39% (70.06% the first component and 19.33% the second component) of the variance among the investigated soils in different land uses.

If the two main components show totally the high variance with total data, properties can be distinguished well by plotting these two components and draw the biplot by this method. Biplot shows the position of properties. In the drawn biplot, the lesser distance of the vectors shows the higher correlation among properties.

The advantage of this method is that positive or negative relationships among properties can be easily observed[23].

Ordination biplot of the PCA of physicochemical soil properties in various land uses is shown in figure 1. Together the three soil samples of each land use in the diagram indicate that soil properties influenced by different land uses. Soil samples representing farm land use (FL1, FL2 and FL3) have the highest correlation with the positive side of the second component; while in the case of shrub land use, the samples (SL1, SL2

Table 2. The results of the components
obtained from the PCA

PC	Eigenvalue	% variance
1	10.51	70.06
2	2.89	19.33
3	0.75	5.05
4	0.44	2.98
5	0.24	1.62
6	0.08	0.58
7	0.04	0.31
8	0.00	0.03

Table 3. Correlation of soil properties with the first two components of PCA

Soil properties	PC1	PC2
N	0.88	-0.41
OC	0.83	-0.52
P	0.89	0.11
K	0.89	0.26
Fe	0.82	0.30
Zn	0.93	0.22
Mn	0.93	0.09
Cu	0.85	-0.04
$CaCO_3$	0.17	0.94
pH	-0.89	0.35
EC	0.76	-0.54
Sand	-0.97	-0.12
Clay	0.30	0.92
Silt	0.97	0.11
Moisture	0.92	-0.01
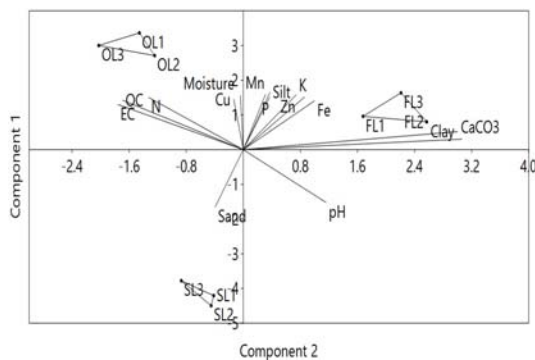
Fig. 1. The biplot diagram obtained from the PCA. Soil samples are plotted as symbols and soil properties are plotted as vectors. The codes for soil samples and soil properties are placed close to each symbol and vector, respectively, as listed in Table 1

and SL3) have the highest correlation with the negative side of the first component. Besides, in orchard land use, the soil samples (OL1, OL2 and OL3) were shown to have the highest correlation with the positive side of the first component.

Considering the correlation between the properties and PCA components (Table 3), total N, OC, P, K, Fe, Zn, Mn, Cu, EC, silt and soil moisture content are positively correlated with the first component. By contrast, properties of sand and pH have negative correlation with this component. The other two properties, the percentage of $CaCO_3$ and clay, have positive correlation with the second component.

Soil bacterial community analysis by DGGE

To begin with, DGGE banding patterns of V3 region of 16S rDNA amplified with primers 341F-GC and 534R were used to evaluate the bacterial species richness. As DGGE gel image clearly shows in Figure 2, some common bands with different relative intensity were observed in all profiles.

Furthermore, after attaining the bacterial species richness from DGGE banding patterns, one-way ANOVA was perform to evaluate as to whether or not there is significant difference among three land uses regarding species richness index. Based on the results, bacterial species richness (bands number) showed significant differences among different land uses, where the highest and lowest
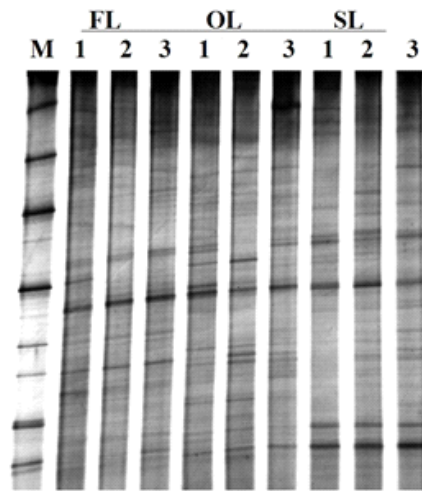
Fig. 2. DGGE profiles of PCR-amplified V3-16S rDNA variable region products generated from soil samples obtained from farm land (FL), orchard land (OL) and shrub land (SL). Numbers 1, 2 and 3 represent replicate samples. M (bacterial 16S rDNA marker): a mixture of PCR amplified 16S rDNA fragments from bacterial species of *Pseudomonas putida, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter baumannii* and *Salmonella entericaserovar* Typhi
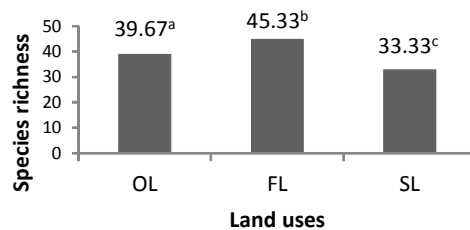
Fig. 3. The mean values of bacterial species richness of different land uses

species richness was seen in farm and shrub land uses with the average of 45.33 and 33.33 species, respectively. The number of bacterial species in orchard land use, on average, was 39.67 (figure 3).

The influence of soil properties on the structure of bacterial community

CCA was used to identify the soil properties that best explain the pattern of DGGE profiles via Monte Carlo test. To perform CCA, firstly environmental data matrix (15 physicochemical soil properties) and zero one matrix (absence and presence of the species) of bacterial species derived from DGGE profiles were prepared, and then it was performed using PAST3 software.

The results of CCA are presented in Table 4. Three ordination axes showed 83.82% of the variance related to the relationship of the structure of bacterial community and soil properties. According to Table 4, the first and second axes with eigenvalues of 0.125 and 0.039, respectively, showed 56.25 and 17.62% of the variance between bacterial species and data obtained from physicochemical soil properties, respectively. The first and second axes, which have the highest eigenvalues, were used to display the results (Figure 4). In this diagram, more effective properties have longer vectors.

Table 5 shows the correlation coefficients for each of the soil properties in proportion to the ordination axes. The first axis has more correlation with properties of sand, silt, moisture, pH, N, K, P, Fe, Zn, Mn and Cu. The sand and pH properties are positively correlated with this axis and the rest of properties have negative correlation with it. Furthermore, the properties of OC and EC have negative correlation with the second axis and clay and CaCO$_3$ have positive correlation with it.

Figure 4 shows the ordination diagram of the soil samples of the three examined land uses based on the first and second ordination axes. The distance of soil samples representing each land use from axes represents the intensity of relationship, so that the longer the vectors length of soil properties and smaller their angles with axes, the greater the correlation between these properties and axes and stronger their relationship with the characteristics showing axes[21]. Moreover, the distance of the points showing soil samples on the diagram represents the degree of similarity or

difference in soil samples in terms of the studied factors (structure of bacterial species and soil properties).

Table 4. The results of the axes obtained by CCA

Axis	Eigenvalue	% Variance	P
1	0.125	56.25	0.04
2	0.039	17.62	0.04
3	0.022	9.95	0.76
4	0.013	6.20	0.60
5	0.009	4.16	0.69
6	0.006	2.71	0.90
7	0.005	2.47	0.95
8	0.001	0.62	0.53

Table 5. Correlation coefficients of soil properties with the first two axes of the CCA

Soil properties	Axis 1	Axis 2
N	-0.67	-0.65
OC	-0.6	-0.74
P	-0.9	-0.07
K	-0.9	-0.14
Fe	-0.83	-0.11
Zn	-0.94	-0.16
Mn	-0.93	-0.22
Cu	-0.8	-0.25
CaCO$_3$	-0.46	0.79
pH	0.72	0.59
EC	-0.57	-0.75
Sand	0.96	0.21
Clay	-0.56	0.72
Silt	-0.94	-0.26
Moisture	-0.86	-0.29
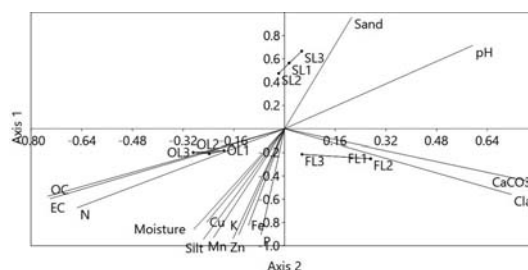
Fig. 4. The biplot diagram obtained from the CCA. Soil samples are plotted as symbols and soil properties are plotted as vectors. The codes for soil samples and soil properties are placed close to each symbol and vector, respectively, as listed in Table 1

As it is obvious in the diagram, three soil samples representing each land use are located in a separate location compared to soil samples in the two other land uses. The samples of shrub land use are located around the first axis in the first quarter (SL1 and SL3) and in the second quarter (SL2) of the diagram. The most effective soil variable in shrub land use is sand percentage. Samples of farm land use (FL1, FL2 and FL3) are located in the fourth quarter, and the most influential variable in isolation of these samples are $CaCO_3$ and Clay. Samples of orchard land (OL1, OL2 and OL3) use are situated in the third quarter, with OC, N and EC being the most effective properties in separating these samples.

DISCUSSION

The differences in the investigated land uses in the last 50 years in the study area have influenced the structure of soil bacterial communities as well as the soil physicochemical properties. The results of one-way ANOVA and comparison of means showed that all soil properties were significantly different at least in one land use compared to the two other land uses. In this regard, it was reported that land use changes (from forest to shrub) can result in significant and long-term impacts on soil properties such as nutrient elements, carbon amount, pH and texture[5].

The most important soil characteristic which was different between shrub land use and the other land uses was soil texture, which changed from sand (in shrub land use) to sandy loam in both orchard and farm land uses as the result of agricultural activities during the past half-century.

Moreover, the difference among land uses was significant in terms of soil organic carbon. Climatic conditions such as temperature and rainfall have great impacts on the amount of soil organic carbon. Naturally, accumulation of organic matter under the conditions of higher precipitation and cooler temperatures is higher; but, by contrast, decomposition of organic matter is higher under warmer and drier conditions[24]. In Jiroft plain, decomposition of organic matter is higher due to the low rainfall and hot and dry conditions, while production of organic matter is lower owing to the unfavorable climatic conditions. Therefore, soil organic carbon content in all the three land uses is low. Fertilization and irrigation caused organic carbon content to be higher in orchard and farm land uses than shrub one. Through affecting production, fertilization increases plant residue and consequently soil organic matter. On the other hand, irrigation delays the decomposition of organic matter by increasing soil moisture content[24-25].

Furthermore, cultivation of farm crops and establishment of citrus orchards in the region as well as consumption of nutrients as fertilizer led to the increase in the concentration of nutrient elements (N, P, K, Fe, Mn, Zn and Cu) in agricultural land uses compared to shrub land use.

The results obtained from PCA indicated that agricultural land use has had a significant impact on soil physicochemical properties. In figure 1, the position of vectors indicates how the intended properties in the studied land uses were changed. Considering the length and angel of vectors, sand percentage can be known as the effective factor in isolation of the soil samples representing shrub land. Besides, clay and $CaCO_3$ content were effective in separating of soil samples representing farm land. As is evident in figure 1, more soil properties were involved in isolation of soil samples representing farm land, among which OC, total N and EC were the most important properties.

As the findings of this study represent, there was lower bacterial species richness in shrub land use compared to the two other land uses; the possible reason can be low average of rainfall in the study area, which consequently leads to unfavorable soil moisture conditions for growth and reproduction of bacteria.

By contrast, favorable moisture conditions in soil and application of nutrients in the form of fertilizer in agricultural lands provided better conditions for bacteria growth and reproduction compared to the shrub land[26-27].

CCA technique was used for investigating some soil physicochemical properties influencing the structure of soil bacterial communities in various land uses. Ordination diagram obtained from CCA showed the position of the soil samples representing each land use in relation to the studied soil characteristics. Our results showed that differences in the structure of bacterial communities in the studied soils were

significantly correlated to changes in soil properties. In farm land use, clay and $CaCO_3$ content had the greatest impact on the structure of bacterial communities; in shrub land use, the most important factor was sand percentage; and more factors were involved in orchard land use, where according to the length of the vectors, OC, N and EC can be said to be more important. Because soil type was the same for the three studied land uses in 50 years ago, it can be assumed that changes in soil properties associated with land use change from shrub to agriculture[27-28].

In general, in line with the results of other studies, our results also showed that agricultural practices have strong impacts on soil physicochemical properties which will lead to shifts in the structure of soil microbial communities[28]. For example, researchers concluded that land use and soil properties were found as important factors determining the diversity and structure of bacterial communities[29-30].

Furthermore, other researches also showed that the application of mineral fertilizers can significantly affect structural shifts in the soil microbial communities[31-34].

CONCLUSION

This study, for the first time, reports that agricultural activities during the last 50 years in arid and semi-arid regions of southeast Iran, Jiroft County, caused a change in the structure of soil bacterial communities and some soil physicochemical properties of former shrub lands, which may permanently affect the natural soil ecosystem. Based on the results of the present study, it can be said that bacterial species richness in shrub land use was lower than agricultural land uses (orchard and farm); mainly because of hot weather and unfavorable soil moisture conditions. Therefore, it can be concluded that agricultural practices such as irrigation and fertilization improved the species richness of soil bacteria in agricultural land uses (orchard and farm) compared to shrub land use.

Finally, it is notable that generalizing and transferring of findings obtained at our studied region with specific soil properties cannot be accurate for other regions with different soil properties.

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