



Full Length Article

Factors Influencing Bacterial Diversity in the Rhizosphere of Cucumbers and Tomatoes in the Arabian Peninsula

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Abstract

The Arabian Peninsula is characterized by generally hot and dry conditions. Although limited studies addressed bacterial diversity in this part of the world, there is a lack of information about bacterial diversity under farming systems. This study investigated bacterial diversity across three farms in Oman, at the South Eastern part of the Arabian Peninsula. Pyrosequencing was used to analyze bacterial communities from the rhizosphere soil of tomatoes and cucumbers grown in the farms. Results revealed that bacterial diversity is variable among various farms. Chao 1 richness and Shannon diversity estimates demonstrated that soils from the three farms differed in the levels of bacterial diversity. *Proteobacteria* was the major phylum in the soil samples from all farms. *Gammaproteobacteria* was the main and most abundant class in the rhizosphere soil of cucumber, while *Gammaproteobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria*, *Bacilli*, *Actinobacteria*, *Cytophagia* and *Nitrospira* were common in the rhizosphere soil of tomatoes. The genera *Bacillus*, *Nitrospira*, *Sphingomonas*, *Gemmatimonas* and *Pseudomonas* were the most common in the rhizosphere of both crops in the three farms. Principle component analyses showed that bacterial diversity in the rhizosphere of cucumbers and tomatoes was found to be affected by the farming system but not the crop type. The study also presents information about the most common bacterial groups under farming systems in the Arabian Peninsula. Most of the bacterial taxa were saprophytic, suggesting that they play a role in cucumber and tomato growth and disease prevention. © 2021 Friends Science Publishers

Keywords: NGS; Pyrosequencing; Desert; Soil; Bacteria; Oman

Introduction

Soil microbial communities are essential indicators of soil health and their biodiversity influences ecosystem functioning (Xu *et al.* 2018; Nunan *et al.* 2020; Raza *et al.* 2020). Soil bacteria play crucial roles in biogeochemical cycles and nutrient turnover in terrestrial ecosystems (Gans *et al.* 2005; Krishna and Mohan 2017; Lehtovirta-Morley 2018). Changes in soil environment, water content, soil type, pH and plant diversity influence soil microbial composition, diversity and interaction with plants (Wu *et al.* 2008). Agricultural land use is defined as one of the most significant factors that alter soil physiochemical properties and biological processes (Jangid *et al.* 2008; Jesus *et al.* 2009).

Historically, it has been hypothesized that application of soil cattle manure, organic or inorganic fertilizers alters soil microbial structure, diversity and soil health. On the other hand, repeated application of fertilizers may cause environmental hazards. Soupir *et al.* (2006) proposed that frequent application of manure may introduce fecal microbes into the soil flora which have the potential to pose

environmental hazards by changing the endogenous microbial community.

Although a number of studies have demonstrated that shifts in land use can cause major changes in microbial communities, our understanding of how land use along with plant and soil properties may have impact on the abundance and presence of specific taxonomic groups is still unclear and the relationship is complex (Lauber *et al.* 2008).

Developments in sequencing technologies have allowed us to study the community structure of microbial communities (Bao *et al.* 2019; Eichmeier *et al.* 2019). Historical reports proved that only 0.1–1% of bacterial communities could be detected based on cultivation methods and will not give us true reflection of bacterial community and diversity (Sandaa *et al.* 2001; Smit *et al.* 2001). Nowadays, 16S rRNA sequencing has been applied in many studies, not only to examine bacterial community composition but also to understand to what extent changes in bacterial community composition gets influenced by external factors (Qin *et al.* 2018; Wang *et al.* 2018).

Oman is a dry country located at the South Eastern part of the Arabian Peninsula. Date palm is the most important fruit crop in the country, with a total production of 368,000 tons annually (FAO 2019). Tomato is the top vegetable crop in terms of production (199,000 tons annually). Other important crops include mangoes, acid limes, bananas and cucumbers. Most farms in Oman are in the coastal areas and they follow a conventional system in which they rely on growing different crops in the same farm. They also rely extensively on the use of pesticides and inorganic fertilizers. There are very few organic farms in the country. There are also some farms in desert areas where temperatures can reach 50°C in summer. Desert farms follow a conventional system, but environmental conditions determine the crops which can be grown in deserts.

We hypothesized that soil bacterial composition varies among locations or different crops. Using 454 pyrosequencing techniques, we examined bacterial diversity in the rhizosphere of cucumbers and tomatoes. This manuscript provides detailed information and statistical assessment of the soil bacterial communities at the class and genus level and their possible connection with soil properties, plant species and farming practices.

Materials and Methods

Sample collection and experimental design

The experiments focused on cucumber and tomato grown in three farms. A detailed description of the farms is shown in Table 1. Collection of soil samples was done during December–January (2013–2014), and randomized samples were taken from the rhizosphere of cucumber and tomato grown in the three farms.

Soil (1 kg) was collected from the rhizosphere of each crop. Sampling was carried out from five randomly selected plants in each farm. Soil samples were taken from three sections around the active feeder roots (< 1 cm). Sterile plastic containers were used to preserve soil samples. Each soil sample was thoroughly homogenized and passed through sieve to remove stone and plant debris. Part of each soil sample was ground using liquid nitrogen and then preserved at -80°C prior to DNA extraction. The rest of the soil samples were kept at 10°C for further soil physicochemical tests.

Determination of soil physicochemical properties

Soil samples were ground and sieved (2 mm sieve). Physicochemical properties of soil samples examined as described by Al-Ghaithi *et al.* (2016). Soil textural classification (Gee and Bauder 1986), electrical conductivity (EC) and pH measurements (Zhang *et al.* 2005), potassium (K), phosphorus, total inorganic and organic carbon, nitrogen, sulphate (SO₄) and organic matter were determined (Al-Ghaithi *et al.* 2016). Three replicates were used for each sample.

DNA extraction for PCR amplification

Total DNA was extracted from 0.05 g soil samples as described by the modified protocol of Volossiouk *et al.* (1995) as described by Kazeeroni and Al-Sadi (2016).

Pyrosequencing and bioinformatic analyses

The universal primer set 28F and 519R were used for the amplification of 16S rRNA bacterial gene (Liu *et al.* 2007). The amplified samples were submitted for high throughput 454-pyrosequencing to the Research and Testing Laboratory (RTL, Lubbock, TX, USA) (Dowd *et al.* 2008a).

The pyrosequencing raw data were edited to reduce the effect of sequencing error. RDP v. 9 was used to check high quality sequences (Cole *et al.* 2009). Sequences which were less than 300 bp or with low quality ends and tags were removed from the data set. Sequences were checked for chimeras using UCHIME chimera detection software (Edgar *et al.* 2011). The selected sequences were compared with high quality sequences obtained from NCBI and filtered at 97% similarity. Finally, outputs were validated based on taxonomic distance methods (Dowd *et al.* 2005; Dowd *et al.* 2008a, b). Further analysis of pyrosequencing data was conducted as explained by Kazeeroni and Al-Sadi (2016). Richness and Shannon diversity estimates and weighted UniFrac, unweighted UniFrac and Bray-Curtis analyses were carried out as explained by Al-Balushi *et al.* (2017).

Statistical analysis

Differences among soils in their physicochemical characteristics were analyzed using Tukeys' Studentized Range test (SAS, SAS Institute Inc., USA) at $P < 0.05$.

Results

Soil physicochemical characteristics

Soils obtained from farms differed in their physicochemical properties (Table 1). All soil types were loamy sand. The pH range was from 7.7 to 8.4, while the EC (salinity) ranged from 0.9 to 7.72 mS. The highest level of salinity was observed in DE and the lowest level was observed in OR ($P \leq 0.05$; Table 1). Total inorganic carbon (TIC), total organic carbon (TOC), nitrogen (N), phosphorus (P), potassium (K) and sulphate (SO₄) were different among the different farms (Table 1).

Bacterial richness, diversity and composition in the rhizosphere of cucumber

Differences were found between the three farms, designated OR, TR and DE, in the level of bacterial diversity. Cucumber grown in OR farm harbored a higher level of

Table 1: Physicochemical analysis of soil samples

Sample code	Farm#	pH	EC (mS)	% C(TC)	% TIC	% TOC	% N	P (mg/kg)	K (mg/kg)	SO ₄ (mg/kg)	% organic matter
ORCU	1	8.4 a	0.988 d	2.957 c	1.059 b	1.898 a	0.059 b	3.706 a	7.460 c	23.928 de	60.900 a
ORTO	1	8.4 a	1.211 d	2.905 c	0.015 c	2.900 a	0.050 b	3.246 a	24.575 b	33.499 d	60.362 a
TRCU	2	7.7 c	4.980 b	7.952 b	5.651 a	2.301 b	0.015 c	4.480 a	58.365 a	76.570 c	60.726 a
TRTO	2	8.0 b	2.670 c	12.000 a	5.736 a	6.264 a	0.003 d	3.720 a	9.216 c	47.856 d	60.416 a
DECU	3	8.0 b	6.240 a	8.036 b	5.181 a	2.855 a	0.259 a	0.095 b	51.783 a	124.426 b	60.472 a
DETO	3	7.8 c	7.720 a	6.900 b	4.132 a	2.768 a	0.020 c	3.272 a	45.639 a	193.817 a	60.804 a

Codes starting with the letters OR, TR and DE designate to different farms, while CU denotes for cucumber and TO denotes for tomato. Abbreviations denote to: EC = electrical conductivity, TIC = total inorganic carbon, TOC = total organic carbon, N = nitrogen, P = phosphorus, SO₄ = sulphate and K = potassium. Values with the same letter in the same column are not significantly different from each other at $P < 0.05$ (Tukey's Studentized Range test, S.A.S.)

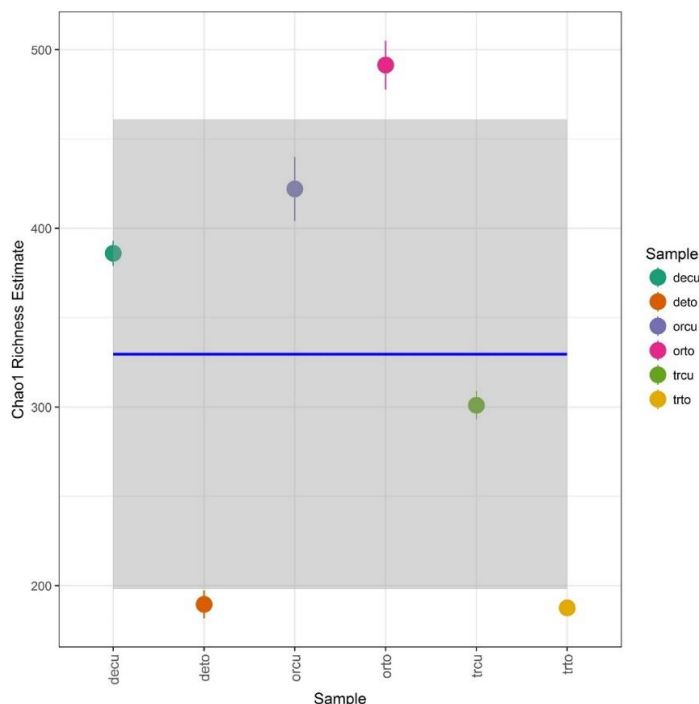


Fig. 1: Chao1 richness within the total microbiome data of soil samples obtained from the rhizosphere of cucumber and tomato grown in three farms. Sample codes are described in Table 1

bacterial diversity compared to TR and DE. The Chao1 richness values were 422 for cucumber soil from OR (ORCU) compared to 386 and 301 for soil from DE (DECU) and TR (TRCU) farms, respectively (Fig. 1). Similarly, Shannon diversity was highest in OR (Fig. 2).

Proteobacteria dominated phyla in soil samples from OR, DE and TR farms. Other common phyla included *Acidobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes* (Fig. 3). *Gammaproteobacteria* was the main and most abundant class in cucumber grown in OR, DE and TR (Fig. 4). Some classes such as *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Clostridia*, *Actinobacteria*, *Nitrospira*, *Planctomycetia*, *Acidobacteriia*, *Actinobacteria*, and *Bacilli* were present in the three farms (Fig. 4). Fluctuation in class distribution was observed under different farms. Several bacterial genera were detected in all farming systems. *Bacillus*, *Nitrospira*, *Sphingomonas*, *Gemmatimonas*, and *Pseudomonas* were shared between the three farms (Fig. 5).

Bacterial richness, diversity and composition in the rhizosphere of tomato

Bacterial diversity was higher in the rhizosphere of tomatoes grown in OR compared to TR and DE. The Chao1 richness values were 491, 189 and 187 for tomatoes grown in soil from the ORTO, DETO and TRTO in soils from the two other farms (Fig. 1). Shannon diversity was highest for ORTO (5.6) compared to DETO (4.4) and TRTO (4.3) (Fig. 2).

Pyrosequencing showed that the majority of bacterial taxa in the three farms belong to the *Proteobacteria* and *Firmicutes* phyla (Fig. 3). The other dominant phyla included *Actinobacteria*, *Acidobacteria*, *Cyanobacteria* and *Bacteroidetes* (Fig. 3). Our results detected 46 classes in OR compared to 27 classes in TR and 35 in DE. The most common classes in these farming systems were *Gammaproteobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria*, *Actinobacteria*, *Nitrospira*,

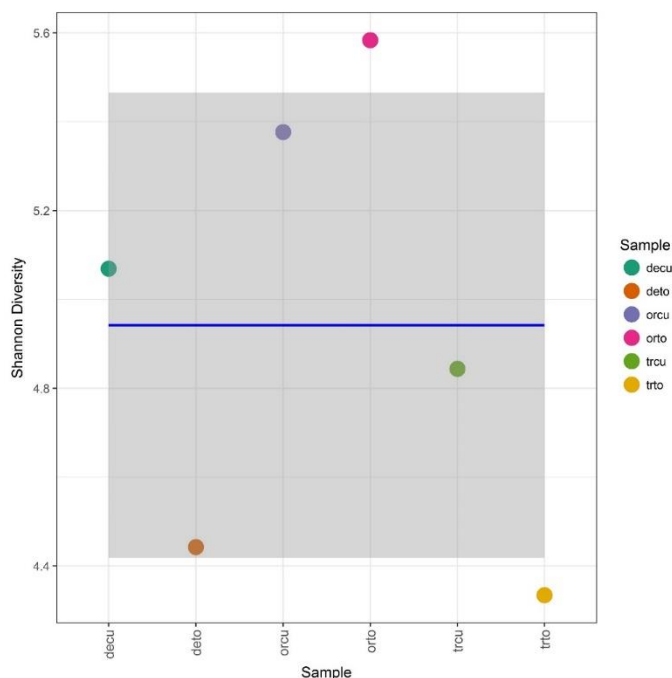


Fig. 2: Shannon diversity within the total microbiome data of soil samples obtained from the rhizosphere of cucumber and tomato

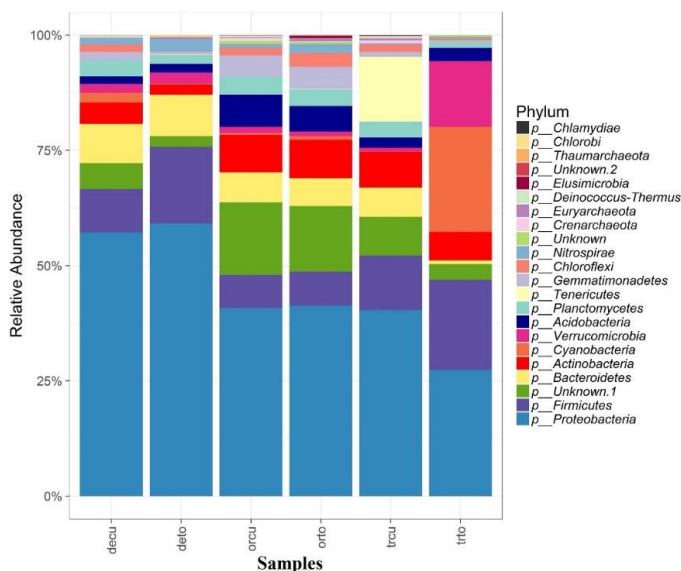


Fig. 3: Phylum-level relative abundance of bacterial communities in the rhizosphere of cucumber and tomato

Planctomycetia, *Sphingobacteriia*, and *Acidobacteriia* (Fig. 4). Some classes such as *Flavobacteriia* and *Anaerolineae* were not detected in TR (Fig. 4). Genera recovered from all farms included *Bacillus*, *Nitrospira*, *Sphingomonas*, *Gemmatimonas* and *Pseudomonas* (Fig. 5).

Relationship between bacterial diversity and farming systems

Separation of soil samples from different farming systems

was depicted by using Bray-Curtis analysis, weighted UniFrac and unweighted UniFrac distances (Fig. 6). The analysis clearly separated samples from the OR farm from other samples. However, there was no clustering based on host crop.

Discussion

Our findings showed that bacterial community and diversity were high under the extreme dry conditions of Oman.

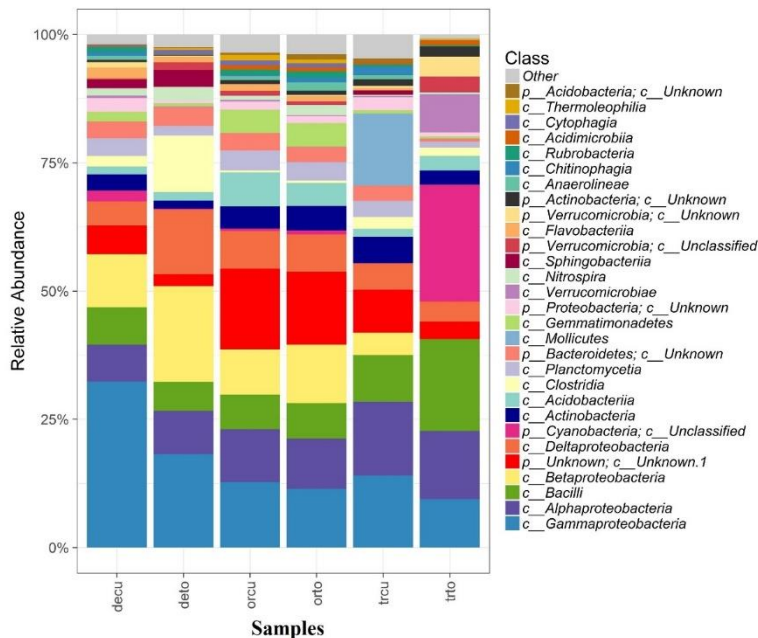


Fig. 4: Class-level relative abundance of bacterial communities in the rhizosphere of cucumber and tomato

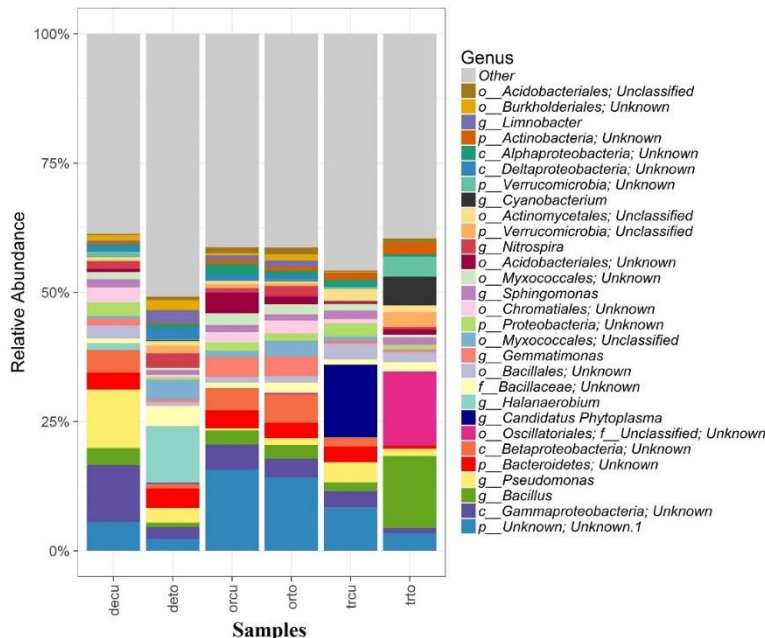


Fig. 5: Genus-level relative abundance of bacterial communities in the rhizosphere of cucumber and tomato

In the first part of this study, we evaluated bacterial community present in the rhizosphere of cucumber grown in OR, TR and DE farms. In this investigation, *Proteobacteria* was the most dominant phylum in OR, TR and DE farms growing cucumber. There was fluctuation in the presence of classes in OR, TR and DE growing cucumber. *Alphaproteobacteria* was abundant in TRCU while more *Betaproteobacteria* and *Gammaproteobacteria* were detected in DECU. Moreover, a higher percentage of

Deltaproteobacteria was observed in ORCU. To interpret these results, the concept of oligotrophic and copiotrophic has been used by researchers (Meyer 1994; Fierer *et al.* 2007). A group of bacteria which predominate in soils with high nutrient availability are defined as fast growing or copiotrophic bacteria while slow growing or oligotrophic bacteria are defined as a group of bacteria that flourish in soils with low amount of nutrients. In our study we found higher levels of *Betaproteobacteria* in DE, followed by OR

and then TR. Total N was higher in ORCU compared to TRCU while DECU possesses higher N compared to ORCU. The higher abundance of *Betaproteobacteria* in OR compared to TR could be due to the higher amount of N. Moreover, the higher abundance of *Betaproteobacteria* in DE compared to OR and TR might be related to the high level of total C and total N. The percentage of the other common phyla such as *Acidobacteria* and *Actinobacteria* was higher in ORCU compared to TRCU and DECU. The highest percentage of *Bacteroidetes* and *Firmicutes* was observed in DECU and TRCU, respectively.

In the other part of this research, we investigated bacterial composition in the rhizosphere of tomato grown in OR, TR and DE farms. *Proteobacteria* was the most dominant phyla present in OR, TR and DE grown tomato. *Betaproteobacteria* class was dominant in DETO. *Betaproteobacteria* are considered as copiotrophic bacteria and it is expected to have their population to be in lower level in organic farms (Diepeningen *et al.* 2006; Fierer *et al.* 2007). Total N was higher in DETO compared to TRTO and ORTO and the lowest amount of N was observed in TRTO. The higher abundance of *Betaproteobacteria* in OR compared to TR could be due to the higher amount of N and pH. On the other hand, we observed a higher population of *Actinobacteria* in OR and this observation was consistent with a study by Grantina *et al.* (2011). The reason could be due to the presence of recalcitrant carbon sources. Fließbach *et al.* (2007) mentioned organic soils as rich sources of recalcitrant carbon. It has been reported that *Actinobacteria* are capable of decomposing recalcitrant carbon sources and play a role in carbon cycling and organic matter turnover (Acosta-Martínez *et al.* 2008; Jenkins *et al.* 2009). It would be expected to have higher diversity of *Actinobacteria* in organic fields than conventional fields. Moreover, our findings are consistent with other studies that indicated significant increase in *Actinobacteria* population with higher pH values (Jones *et al.* 2009; Nacke *et al.* 2011).

The effect of soil edaphic factors on the formation of microbial communities has been addressed by several studies (Yang *et al.* 2017; Jin *et al.* 2019; Bickel and Or 2020). He *et al.* (2012) indicated nitrogen, pH and soil organic carbon as the most important factors that have influence on soil microbial function and composition. Previous reports noted that besides total nitrogen, pH, EC and organic matter, specific plant groups such as legumes and forb can be considered as important factors affecting soil microbial composition (Li *et al.* 2014; Qu *et al.* 2016).

Long term N fertilization can have a positive impact on *Gammaproteobacteria* and a negative impact on *Deltaproteobacteria* (Zhang *et al.* 2014; Zhou *et al.* 2017). Our finding followed the same trend for cucumber farms but not for tomato farms. In cucumber farms, the highest percentage of *Gammaproteobacteria* and the lowest percentage of *Deltaproteobacteria* were detected in DECU which had the highest amount of nitrogen (N: 0.2%). In tomato farms, the highest percentage of

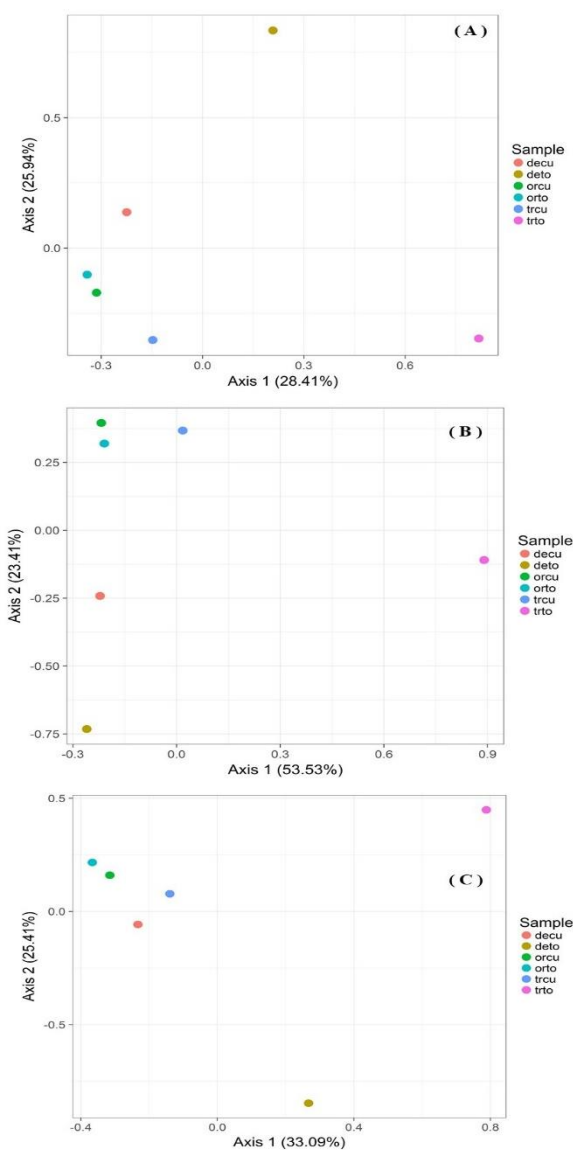


Fig. 6: Principal component analysis of the relative abundances of bacterial communities in the rhizosphere of cucumber and tomato based on Bray-Curtis analysis (A), weighted UniFrac distances (B) and unweighted UniFrac distances (C). The rhizosphere soils samples were from cucumber and tomato

Gammaproteobacteria and *Deltaproteobacteria* was observed in DETO (N: 0.02%). Some studies reported the negative impact of nitrogen addition on reduction of recalcitrant carbon decomposition (Craine *et al.* 2007) and this may affect *Actinobacteria* which play a role in the carbon cycling (Ventura *et al.* 2007). Pan *et al.* (2014) indicated the presence of correlation between *Actinobacteria*, *Acidobacteria*, *Verrucomicrobia* and K, AL, and Ni. In our study the highest percentage of K (58.3%) was detected in TR (TRCU) and in this farm the population of *Actinobacteria* was high.

Previous studies reported soil pH as a primary factor

that has an influence on the richness and diversity of bacteria (Cline and Zak 2015; Ling *et al.* 2016; Xue *et al.* 2017). It indicated that the highest richness and diversity were observed when the pH was near the neutral or alkaline levels (Rousk *et al.* 2009; Ramirez *et al.* 2010). Our findings agree with this, as generally bacterial diversity was higher in soils with higher pH values.

Conclusion

Our findings suggest that farming systems in the Arabian Peninsula have a relatively high level of bacterial diversity. This bacterial abundance and composition may lead to a change in soil quality and fertility. Different types of farming systems can support various groups of beneficial bacteria and this is crucial for improving soil quality and fertility. Further investigation is required to evaluate the impact of farming systems on the abundance of beneficial and pathogenic bacteria in the long run.

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Author Contributions

E.A. Kazerooni: planned work; conducted experiments, analyzed data and wrote the manuscript; A.M. Al-Sadi: planned work; supervised work, analyzed data, and proof read the manuscript.

Conflict of Interest

The authors declare that they have no known conflict of interest.

Data Availability

All data related to this work are presented in the manuscript.

Ethics Approval

Not applicable.

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