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METAGENOMIC ANALYSIS AND INVESTIGATION OF THE MICROBIOME IN DATE PALM AND THE EFFECT OF DIFFERENT FERTILIZERS ON MICROBIOME DIVERSITY

BY

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ABSTRACT

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Title: Metagenomic Analysis and Investigation of Bacteria in Date Palm Soil and The Effect of Different Fertilizers on Bacteria Diversity

Supervisor of Thesis: Juha, M, Alatalo.

Date palm "*Phoenix dactylifera* L." is considered a very important plant in the Middle East. This importance is due to its beneficial uses on agriculture, economy, and medicine. The study aimed to identify bacterial biodiversity in the soil of date palm and the effect of different fertilizers on bacterial diversity. Also, the relationship between cultivar type and biodiversity was identified. The metagenomic approach was used to analyze bacterial biodiversity. The biodiversity of bacteria in date palm soil was studied by OTUs (Operational Taxonomic Units). 6356 OTUs and 1164425 sequences were observed in total for all 27 samples. Similar cultivars from both farms did not share similar phylogeny except the Khalas cultivar. Higher relative abundance was shown in Actinobacteria and Alphaproteobacteria classes, followed by Gammaproteobacteria and ACidobacteroa_GP16. The wild date palm had a higher number of unique OTUs than the cultivars from the farms. Different fertilizer treatments had varying effects on bacterial biodiversity. Organic and bio-organic fertilizers positively affect delta-proteobacteria, acidobactria-Gp3, Anaerolinaea, and Clostridia. There was no noticeable effect on mixing other fertilizer types with a high concentration of chemical fertilizers, but classes including Bacilli, Nitrospira, Deltaproteobacteria, Spartobacteria, and Thermomacrobia classes almost have high relative abundance in treatments having high concentrations of chemical fertilizer. This study was the first to study wild date palm soil diversity.

DEDICATION

Giving my work to my family and friends that makes the life meaningful.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1: Introduction

Phoenix dactylifera L., known as "date palm," is one of the oldest plants that is important economically and environmentally, especially in the Middle East region. It is estimated that the oldest date palm records exist more than 7000 years ago (I. E. Hadrami & Hadrami, 2009). The annual production of date palm fruits is around 5.5 million metric tons. Date palm fruits can be consumed directly or used to produce other products such as juice, jam, and syrup. Also, its leaves are shaped into mats, baskets or fans, and other industrial materials. Furthermore, medical benefits from dates include recovering from cold, fever, sore throat, and coughing (Balick & Bek, 1990; A. E. Hadrami & Al-Khayri, 2012).

Discovering the microbiome underground may enhance the healthiness of plants and make agricultural production of crops more sustainable and consume less energy. However, some microbes have a pathogenic relationship with plants; plants that have a symbiosis with some endophyte bacteria can promote induced systemic resistance, which is like systemic-acquired resistance that induces mechanism of defense and protection toward pathogens (Ryan et al., 2008; Strobel et al., 2004). Ecosystem and its relation with the biodiversity of organisms, in general, have been brought to attention (Naeem et al., 2002). The changes in the last decades on the diversity and its decrease increase the need to know the effects of these changes on the ecosystem. Studies support the fact that changes in biodiversity affect many processes in the ecosystem, and primary production is one of them (Naeem et al., 2002). Having a more diverse microbiome can increase primary production, nutrient cycle, level of pollination, and the protection to pathogens (Naeem et al., 2002). The rhizosphere

layer is the layer of soil that surrounds the roots of plants, in which oxygen exchange and nutrients exchanges happen (Peiffer et al., 2013). Therefore, the rhizosphere layer makes a suitable environment for different microorganisms to grow.

Many techniques have been used to study the diversity of micro-organisms in the soil; scientists have used morphological and microscopical identification (Essarioui et al., 2020), ribosomal DNA (rDNA) sequencing (Al-Nadabi et al., 2020), internal transcribed spacer DNA (ITS-DNA) sequencing (Nishad & Ahmed, 2020) and metagenomic sequencing (Piombo et al., 2020). Using metagenomics to identify the microbiome in the soil, or other environments, by sequencing genomic DNA without culturing the microorganisms gives knowledge regarding the organisms living in the biome and its diversity (Cowan et al., 2005; Ramadan et al., 2021). During the last 30 years, DNA library formation by suitable DNA fragment size, fragment cloning into a vector, and gene screening have been used, and it worked well (Cowan et al., 2005). Metagenomic studies of soils can have many goals, and it allows investigating the diversity of microorganisms and studying their potentials and functions (Myrold et al., 2014). The aim of this study is to study bacterial community differences between wild populations and cultivars, and among cultivars. Also, to understand the relationship between soil chemical properties and the soil bacterial diversity in the date palm rhizosphere. Moreover, to study the effect of different fertilizers treatments on the diversity of bacteria in date palm within different cultivars. We hypotheses that bacterial diversity from soil samples in wild date palm will be lower than in cultivated samples and same cultivars from different sites will have similar diversity. Also, bio-organic fertilizer enhances bacterial diversity more than chemical and organic fertilizer.

Chapter 2: Literature review

Date palm history

It has been debated whether current date palm plants have been domesticated from two or more wild species, including *sylvestris* of the same genera *Phoenix*, or it was from the same species '*dactylifera'* (Barrow, 1998; Meyer et al., 2012; Pintaud et al., 2010; Tengberg, 2012). Comparing genetic data from an isolated area with a population of wild date palm without human interaction and selection in the mountains in Oman, and modern cultivated date palms. These data helped in understanding the ancestors of the domesticated date palm; it assumed that domestication involved at least two wild origins coming from Middle East and Africa suggesting that the first domestication event happened in the Middle East and the second domestication happened in Africa (Gros-Balthazard et al., 2017). The origin of the domestic date palm is believed to be starting from the nineteenth century (Tengberg, 2003). One of the activities that influenced that lifestyle in the Middle East is cultivating dates because date palm tree is adaptive to the desert conditions and provide a source of food that supported the human population increase. In the Assyrian and Babylonian tablets, date palm culture was depicted, and the Hammurabi famous Code had laws related to date sales and culture. Date palm is also mentioned in Egypt, Libya, Syria and Palestine (Nixon, 1951; Popenoe, 1973).

Date palm Use

Consumption

The fruits of date palm contain an average of 80% sugar, and it is considered a food that has high energy content (Ahmed, 1995). This percentage during an early stage named Khalas is mainly sucrose, and then the sucrose is reduced to fructose and glucose by hydrolysis in the ripening process. There are many nutrients found in the dates in

high amounts, such as iron, potassium, 16 amino acids, and (A, B_1, and $B_{2)}$ vitamins. It also contains calcium, chlorine, magnesium, sulphur, and chlorine. It contains phosphorus in low amounts as well (Ahmed, 1995; Nagy & Shaw, 1980).

Medical

There are different medical uses of date palm fruits. The date has different phenolic compounds such as tannins, which help relieve intestinal colic, cancer, and prevent pathogens and parasites (A. E. Hadrami & Al-Khayri, 2012). Moreover, the review showed that cold, cough and sore throat, fever, abdominal and liver aches, cystitis, edema, and gonorrhea had been treated using date syrup, paste, and other formulations like decoctions which is boiling some dates with water and infusions. Toothache is treated by roots of dates, and pollen is valuable because of the estrogenic compound content (A. E. Hadrami & Al-Khayri, 2012)

Other uses

Dates have many uses in public life. First, date palm wood and trunk are utilized as fuel, wood, and timber. Also, fruit stalks and leaf bases are used as fuel. The leaf fibre and trunk fibre are shaped into bags, baskets, fans, trays, paper, camel saddles, and to cover food as well (El-Mously & Darwish, 2020). The leaves dried bundle (Barusti) is used in making roof, shade, and wall separator (El-Mously et al., 2019). Also, leaf ribs are used for fishing traps or boats such as ‘Shasha’, a small boat for fishing.

Moreover, palm pith can be used to make date palm flour and palm heart which are terminal buds that can be eaten or cooked (Awofadeju et al., 2021). Date palm seeds have different uses. Soap can be made from manufacturing the oil of date palm seeds, the livestock feed on the seeds, and it can be used for decorations (Chao & Krueger, 2007).

Microbiome in soil and its role

Soil composition has been dramatically affected by the erosion coming from farming activities (Seitz et al., 2018). Agricultural activities and anthropogenic climate change have a high influence on the carbon content of the soil that affects plant inputs and decomposition of microbes (Friedlingstein et al., 2006) and affected by temperature and moisture. The microbiome has a symbiotic relationship with plants. It plays a vital role in fixing atmospheric nitrogen, making it bioavailable to plants, and substituting it with the Haber-Bosch process (Amundson et al., 2015).

Nitrogen mineralization is positively enhanced by the existence of microorganisms such as protozoa which increase the biomass of the plants without depending on the nutrients in the plant itself. Protozoa and nematode grazers are usually found to increase the nitrogen concentration in shoot and increase shoot biomass as well (Bonkowski, 2004). This process happens when protozoan consumes the competitors of nitrifying bacteria that grow faster. This causes nitrifying bacteria to be stimulated and increase the nitrate concentration and rhizosphere soil leachate (Verhagen et al., 1994). More than 70% of plant rhizosphere bacteria produce auxins (Cheryl & Bernard, 1996). In *Medicago sativa*, bacteria enhance the net carbon of the plant and the respiration of roots by a signal molecule that is a product of riboflavin breakdown named lumichrome (Phillips et al., 1999). Some bacterial populations apply quorum sensing processes to produce specific inducing signals to adjust their activity in the rhizosphere (Dunn & Handelsman, n.d.; Sturme et al., 2002).

The microbiome of date palm

Gram-negative bacteria have been the dominant bacteria with 66% abundance from Gammaproteobacteria sub-classes, followed mainly by Alphaproteobacteria (7%) and Betaproteobacteria (1%) through the phylogenetic identification (Ferjani et

al., 2015b). The rest of the isolates were from Actinobacteria (26%), the Firmicutes (7%), and Bacteroidetes (2%) class. All isolates identified have been noticed to have an association with plants species which assure that these species occupy the soil as the main reservoir (Gürtler & Stanisich, 1996). Different genera, including *Pseudomonas*, *Pantoea*, *Microbacterium*, *Bacillus*, *Arthrobacter*, *Enterobacter*, *Salinicola*, *Rhizobium*, *Staphylococcus*, and *Labedella*, showed growth of population, drought stress resistance, and increase of IAA and siderophore (Ferjani et al., 2015a). Microorganism diversity in date palm under arid conditions was high, including 20 genera identified based on the agriculture management and root exudates, especially (Berg & Smalla, 2009). There were four species, including *Enterobacter*, *Salinicola*, *Rhizobium*, and *Staphylococcus*, that have been discovered and adapted to arid environments (Ferjani et al., 2015b). Only *Labedella* genera was detected as adapted to the oasis environment in plant roots. However, the diversity of protist and archaea associated with date palms have not been studied yet.

Plant growth-promoting rhizobacteria (PGPR) presented in the biofertilizer, including *Azotobacter*, *Azospirillum*, and *Bacillus genera*, increased the total microbial counts in soil, leaves length, and salinity resistance as well (EL-Sharabasy et al., 2018a). *Enterobacter* and *Microbacterium* produce aminocyclopropane-1-carboxylate (ACC) deaminase and indole acetic acid (IAA) (Jana et al., 2017, Yaish 2016). There were seven studies regarding the bacterial community of date palm soil. Three studies were done in Tunisia, two in Oman, one in Egypt, and the other in UAE (Table 1).

Table 1. Papers of Bacterial Biodiversity in Date Palm Soil

No	Species	Impact/function	Country	References
1	*Azotobacter chrococcum, Azospirillum brasilense, Bacillus megaterium, Bacillus circulans*	Growth promoting (PGPR) in various rates to increase microbial counts in soil. Significant increasing of leaves length of *Bartamuda* plants. Increase the plant resistance against salinity Chlorophyll a, b and carotene were significant increased. Bio fertilizers induced significant increases in the leaf content of potassium, phosphorous and nitrogen. Leaf mineral content increased with decrease *Bacillus megaterium* and *B. circulans*.	Egypt	(EL-Sharabasy et al., 2018a)
2	Gram-negative bacteria (66%), including: (Gammaproteobacteria (57%), Alphaproteobacteria (7%), and Betaproteobacteria (1%)), Firmicutes (7%), Actinobacteria (26%), and Bacteroidetes (2%)). *Genera: Pseudomonas*, *Pantoea*, *Microbacterium*, *Bacillus, Arthrobacter*, *Enterobacter*, *Salinicola*, *Rhizobium*, *Staphylococcus* and *Labedella*	IAA production. Siderophore-producing bacteria Drought stress resistance. Siderophore production	Tunisia	(Ferjani et al., 2015a)
3	*Pseudomonas*	Multiple PGP features. Produce a phytohormone amount of IAA. Phosphate solubilization. Protease activity. Biosurfactant production Siderophore production	Tunisia	(Mosqueira et al., 2019b)
4	*Enterobacter*	Solubilize the insoluble forms of potassium and zinc ions. Resist ten antibiotics of which Augmentin (AMC), Cefaclor (CF), and Cefadroxil (CFR) belong to the β-lactam antibiotic. Whereas, Azithromycin	Oman	(Jana & Yaish, 2020)

No	Species	Impact/function	Country	References
		(AZM), Clarithromycin (CLR), and Erythromycin (E), are classified as macrolide antibiotics; Clindamycin (CD) is a lincomycin class antibiotic, and Vancomycin (VA) is a glycopeptide class antibiotic. Produce mvocs to enhance growth of plant		
5	*Microbacterium* sp. Yaish 1	Aminocyclopropane-1-carboxylate (ACC) deaminase. Indole acetic acid (IAA	Oman	(Jana et al., 2017)
6	Classes: Alpha, Gamma and Deltaproteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Firmicutes and Chloroflexi. Genus: *Rhizobium*, *Sphingopyxis*, *Kaistobacter*, *Rhodoplanes*, *Chloroflexi*, *Acidimicrobiales*, *Pseudomonas*	Na	Tunisia	(Mosqueira et al., 2019a)
7	*Micromonospora*, *Rhodococcus*, *Str eptoverticillium*, and *Nocardia*	BCA showed fungicidal activities to *T. punctulata*,	United States of Emirates	(Saeed et al., 2017)

Na: Not available

Factors affecting microbiome in soil

Biotic factors

Microbial interaction

Metabolic webs between different species and kingdoms are related to the nutrients interactions that happens in soil (Heijden & Hartmann, 2016). Interconnected metabolic pathway, metabolic exchange, and cross-feeding interactions include complex nutritional interactions involving inter-connected metabolic pathways, with cross-feeding and metabolite exchange and cooperation between species in syntrophic relationships to compete on limiting nutrients access (Jansson & Hofmockel, 2018).

Physiological status

The physiological status of the microbiome in soil depends on the expression chain step (Mackelprang et al., 2016). Different metaphenomic responses such as cell to cell interaction and genetic regulation happens based on nutrient availability to the individual microorganisms in the community, and such response will result in alteration of specific protein production, reducing respiratory activity, and shifting in cell membrane fatty acids that correspond to physiological statues (Mackelprang et al., 2016). The response outcome from the change of environmental condition can be indicated through meta-transcriptomics, and metaproteomics can indicate the whole environmental state (Myrold et al., 2014). Using metagenomics to sequence the entire genome won't express all genes that encode proteins because some proteins are expressed only in certain environmental conditions (DeAngelis, 2016). In cold weather under zero conditions, different microorganisms apply different physiological responses such as increased carbon storage and osmolytes in the cell to maintain viability for the period with low levels of nutrients and resources (DeAngelis, 2016; Hultman et al., 2015). Moreover, salinity is considered a cause of sizeable

physiological stress alteration. It has been reported that *Humicola* sp. and *Aspergillus Niger* accumulated in different ways with and without salinity stress (Yaish et al., 2017). Microorganisms that become dormant or in low active mode decrease its interaction to the metaphenome compared to microorganisms that have an active metabolic rate (Jansson & Hofmockel, 2018).

Abiotic factors

Soil aggregation

Soil aggregation is defined as the dynamic process through time in which the individual microaggregates and macroaggregates particles are formed into larger particles and disintegrated to smaller particles by relative stability periods continuously (Rillig et al., 2017). In soil microbial communities, every single soil aggregate is considered a special environmental compartment that can isolate the surrounding and act as an incubator for some microbial evolution and changes (Rillig et al., 2017). Compared to source population to a specific phylotype enclosed population, they will be smaller and other populations may affect other enclosed populations. This effect happens when genetic diversity partially interferes with the small population, and the allele frequencies differ from the source population, and it may decrease the genetic diversity in the enclosed population (Rillig et al., 2017). However, it increases the genetic difference between populations in close aggregates. Aggregates can be made artificially by using a model bacterial system of *Bacillus subtilis* or *Pseudomonas aeruginosa*, for example and culture this strain or any other strain that have precise and known characteristics then evolve it and compare it with aggregate and without aggregate and isolated for genome sequencing (Rillig et al., 2017).

Fertilizers

Chemical fertilizer

During the 20th century, chemical fertilizer played an essential role in increasing crop productivity and more cheap food. Since 1870, using chemistry has decreased energy consumption and speed up many processes and scales, and this is what made chemical fertilizer a cheap material minimizing energy to be used (Dixon, 2018). Chemical fertilizer can enhance organic matter in the soil by applying it with a balanced quantity and increasing crop yield and soil fertility (Bhatt et al., 2019; Scholl & Nieuwenhuis, 2004). The reason behind the rapid growth of crops while using chemical fertilizer is that the nutrients are soluble and ready to be absorbed by the plants, and it requires little amount to produce much plant and in a short time (Han et al., 2016). On the contrary, chemical fertilizer can lead to soil degradation and decrease aggregation of soils in which nutrients can be degraded or moved out of the soil through leaching, gas emission, and fixation (Alimi et al., 2007; Bhatt et al., 2019). Also, excessive use of chemical fertilizer may decrease beneficial micro-organisms in the soil and the mycorrhizae in the roots (Yudelman, 2000).

Moreover, it can accumulate salt, increase the acidity of soil, and many pollute water reservoirs if rain washes them out after a short time of application (Bhatt et al., 2019; Ojeniyi, 2002). There are many negative impacts that chemical fertilizer has on water, soil, and humans. The accumulation of nitrate in the soil by converting nitrogen compounds coming from the chemical fertilizers by microorganisms to nitrate will leach in different concentrations until it reaches the bottom of the soil. The nitrate will move downstream until it reaches the groundwater because of its negative charge (Savci, 2012). There are different stages of nitrate toxicity in humans. In the primary effect, the bowel of the digestive and urinary system is noticed and inflammation when

the nitrate increases in the drinking water more than 50ml per liter. In the secondary effect, nitrate will cause infante methemoglobinemia disease, and as nitrate reacts with hemoglobin it will decrease the oxygen transport. Moreover, fertilizers with high concentrations of potassium and sodium decrease pH, destroy soil structure deterioration, and increase acid irrigation (Savci, 2012).

Organic fertilizer

Organic fertilizer is a group of different materials from plant, animal wastes, manures, litters, and agriculture by-products. Organic fertilizers help the plants grow and develop through the utilization of nutrients from the fertilizers and the microorganisms that degrade complex materials with the aid of the fertilizer's substances. This type of fertilizer has been used for 6000 years by storing biodegradable waste in waste pits (Larramendy & Soloneski, 2019). Organic fertilizer increases the organics, air space, nitrogen content, availability and mobilization of nutrients, and water retention in soil (Roba, 2018). Also, it increases soil aggregation, cation exchange capacity, soil structure, and root growth (Lal, 2006). Organic fertilizer works as a buffering agent for soil pH and increases crop yield and quality (Bulluck et al., 2002; Olaniyi & Ajibola, 2008). However, organic fertilizers may have a negative impact by having pathogens in it as it comes from plant and animals matters that may be having these pathogens (Chen, 2006). Organic fertilizers are low in nutrients so a large amount of them is needed to grow plants efficiently, and it may not be available for farmers in small-scale (Bhatt et al., 2019; Vanlauwe et al., 2010). Microorganisms are needed to break organic matter in the fertilizer, and its composition varies based on the source of the fertilizer (Bhatt et al., 2019; Chen, 2006). Temperature and moisture of soil may affect the degradation of organics, and it can be degraded; however, the nutrient may not be needed by plants when it is released (Bhatt et al., 2019; Morris et al., 2007). The

excessive use of the fertilizer may contaminate the ground and surface water as it contains humus natter, nutrients, and fecal coliform (Bhatt et al., 2019; Mohammadi et al., 2009; Rees et al., 2011). Nitrogen and phosphorus content available in the organic fertilizer can increase the level of nitrate in groundwater and may cause surface water eutrophication (Bhatt et al., 2019). The nutrients might leach after application if there was rainfall (Bhatt et al., 2019; Mishra et al., 2006).

Bio-fertilizer

Bio-fertilizer is a type of fertilizer that contains different microorganism species that can transform the nutrients to available form to the plants (Itelima et al., 2018; Vessey, 2003). In 1895, the commercial history began with Nitragin, which is the fertilizer that includes nitrogen-fixing bacteria used by Nobbe and Hithler. It was followed by *Azotobacter* and blue-green algae (Itelima et al., 2018). Bio-fertilizer can be beneficial in increasing nutrient availability, killing pathogens and weeds by increasing degradation temperature, reducing odor and wastes, and transportation (Itelima et al., 2018). Bio-fertilizers have some disadvantages regarding their storage difficulty, inexperienced farmers, and lack of knowledge about this type of fertilizers. Also, carrier material and specific strains may not be available, and the abiotic conditions that may affect these strains (Itelima et al., 2018).

It has been reported that all biochar, synthetic fertilizer, and bio-organic fertilizer increased the growth of plant shoots by using it individually or in combination between each other compared to the control. However, the synthetic fertilizer alone killed the plant-based in the experiment. The bio-organic fertilizers contained one beneficial bacterial species '*Bacillus polymyxa*' which considered a plant growth-promoting rhizobacteria. This may be the reason behind having the highest biomass compared to biochar and synthetic fertilizer. Also, fertilizers may

induce the genes alteration of the nitrogen cycle and carbon degrading in the microbial community (Zhaoxiang et al., 2020).

Techniques used in soil microbiome investigation

There are different techniques to investigate micro-organisms in soil. There are dependent culture methods and independent culture methods. Dependent culture methods include: Dilution plating, plate counts, and Sole carbon source utilization patterns (Zhang & Xu, 2008), and the independent culture method includes molecular techniques such as metagenomics, 16S rRNA gene sequencing, and pyrosequencing (Bailón-Salas et al., 2017).

A study was conducted using both methods showed that in culture-dependent method by using seven different culture media gave a limited number of unique taxa with total species richness of 2% for bacteria and 5% for fungi. On the contrary, by using independent method which is 454-pyrosequencing a total species richness of 95% was obtained with less effort and time (Stefani et al., 2015).

Chapter 3: Methodology

Sample collection

A total of 103 soil samples was collected from the rhizosphere of the date palms (soils were collected roughly 10cm below surface with high abundance of fine date palm roots). 55 Soil samples in total were from wild date palm in Umm Bab (25°13'07.8"N 50°46'04.5"E) and two farms including Qatar University Farm (25°48'29.8"N, 51°20'47.0"E) and Rowdat Al-Faras Farm (25°49'22.3"N 51°19'58.1"E). Five date palm trees were randomly chosen in Umm Bab. Similalry, five date palm trees were selected from each of the five cultivars (Berhi, Shishi, Nabot Saif, Khalas and Khenezy) in both farms (Table 2). The collected samples were stored in paper bags during the transport to the laboratory. 48 samples in total were collected under date palm trees treated with 15 different fertilizer treatments, including organic fertilizer (fermented animal wastes) using 30 kg per tree per year, bio-organic fertilizer (ritual plus fertilizer obtained from BIOGEN company) using 85 g of fertilizer per 100 L of water per 15 days, and chemical fertilizer using N:P: K ration of 1.8:0.8:1 kg/date palm tree (numbers for the 100% treatment) with three replicates for each treatment and the control (Table 3). The soil samples were lifted to dry for 3-4 days to get rid of moisture. Then samples were grinded manually in the bag to prevent contamination.

Table 2. Abbreviation of Soil Sample from Different Culitvars

Sample ID	Sample name
RAB	Rowdat Al-Faras Berhi
QUB	Qatar University Berhi
RASH	Rowdat Al-Faras Shishi
QUSH	Qatar University Shishi
RAKH	Rowdat Al-Faras Khenezy
QUKH	Qatar University Khenezy

Sample ID	Sample name
RAK	Rowdat Al-Faras Khalas
QUK	Qatar University Khalas
RANB	Rowdat Al-Faras Nabot Saif
QUNB	Qatar University Nabot Saif
UB	Umm Bab (wild sample)

Table 3. Experimental Samples Fertilizer Treatment

Sample ID	Fertilizer treatment
T1	100 % chemical
T2	100 % organic
T3	100 % bio-organic
T4	100 % organic 100 % chemical
T5	100 % bio-organic 100 % chemical
T6	100 % organic 100 % bio-organic
T7	100 % of chemical, organic and bio-organic
T8	70 % chemical
T9	70 % chemical 100 % organic
T10	70 % chemical 100 % bio-organic
T11	70 % chemical, 100 % organic and 100 % bio-organic
T12	30 % chemical
T13	100 % organic 30 % chemical
T14	100 % bio-organic 30 % chemical
T15	30 % chemical, 100 % organic and 100 % bio-organic
T16	Control (No Fertilizer)

DNA extraction

A similar amount of soil from the five trees was combined into one bag for each cultivar, then follows the manual of DNeasy® PowerSoil® Kit (Qiagen GmbH, Hilden, Germany) to extract the DNA.

Gel electrophoresis

The quality of DNA extraction was evaluated using 1% TBE gel electrophoresis. 1 µl loading dye was mixed with 5 µl of extracted DNA from the samples.

PCR

30 ng of the sample DNA was used with 16S rRNA fusion (forward and reverse) primers of bacteria for V3-V4 region, including positive and negative control (Table 4). The condition of the PCR cycle was the following: 95 C˚ for 10 minutes, then 50 C˚ for 30 seconds, finally 72 C˚ for 1 minute, and then 10 minutes. The final sample temperature was 4 C for preservation tells doing the electrophoresis. The gel was made with 10% TBE and 1 g of agarose powder for 100 ml of a gel including 13.3 ml of safe cyber green loading dye. All the PCR products were purified by using Agencourt AMPure XP beads. Then they were dissolved in Elution Buffer and labeled to construct the library. Agilent 2100 Bioanalyzer was used to identify library size and concentration. Qualified libraries are sequenced on the HiSeq platform according to their insert size.

Table 4. Primers used for PCR and their sequence

Primer	Primer sequence
819 F	5′-ACTCCTACGGGAGGCAGCAG-3′
806 R	5′-GGACTACHVGGGTWTCTAAT-3′

Sequencing and Bioinformatics

Using the HiSeq 2500 platform with the sequencing strategy MiSeq-PE300 or PE250 (MiSeq Reagent Kit), the libraries were sequenced at Begin Genomics Institute

(Shenzhen, Guangdong, China). The raw data were filtered to obtain high-quality clean data by removing adaptors and low-quality ambiguous bases. Using Fast Length Adjustment of Short reads program (FLASH, v1.2.11), these paired-end reads were added to tags (Magoč & Salzberg, 2011). These tags overlap with each other and form clusters as operational taxonomic units (OUT) with 97% cutoff value using UPARSE software (v7 .0.1090) (Edgar, 2013), and chimera sequences were compared with the Gold database using UCHIME (v4.2.40) (Edgar et al., 2011). Ribosomal Database Project database was used to do taxonomic classifications to the OUT using Ribosomal Database Project (RDP) Classifier v.2.2 with a minimum confidence threshold of 0.6 and trained on the Greengenes database v201305 by QIIME v1.8.0 (Caporaso et al., 2010). The OUT abundance statistics stable for each sample was constructed by comparing all tags back to OUT using The USEARCH_global (Edgar, 2010). Based on the OTUs and taxonomic annotation results, alpha diversity, beta diversity, differential species analysis, and model prediction analysis were done. Alpha diversity at the OUT level was analyzed using MOTHUR (v1.31.2) (Schloss et al., 2009). Beta diversity at the OTU level was estimated by QIIME (v1.8.0) (Caporaso et al., 2010). The sample cluster was conducted by QIIME (v1.8.0) based on UPGMA. Barplot and heatmap of different classification levels were plotted with R package v3.4.1 and R package "gplots", respectively.

Soil Chemical analysis

For the chemical analysis of soil. pH, electric conductivity, total suspended solids and salinity were measured with prob with a ratio of 1:5 soil to distilled water. This was done after drying the samples in opened Petri-dishes for 48 hours under 60-62 C˚ oven and grinded for 40 min with three marble balls. Consequently, total carbon and nitrogen were analyzed using an elemental analyzer by direct input of 5 g of the

sample. Soil nutrients represented in nitrate and nitrite were measured using UV-visible spectrophotometry after different pretreatment for measuring nitrate and nitrite. After 2 g of sample was measured, it was mixed with 50 ml KCl and shacked for 1-hour minimum. The samples were centrifuged at 5000 rpm for 15 minutes, then 10 ml was taken into a new tube, and 0.5ml of nitrate reagent was mixed with the sample to give pink color representing the nitrate. For nitrite, 10 ml was taken into a new tube and diluted to 50 ml with distilled water. The sample was passed through cadmium column after adding 1 ml of ammonium chloride, then 20 ml was collected, and 0.5 ml of nitrate reagent was added.

Moreover, trace metals concentrations in soil were detected with ICP-OES 5300 DV after digestion. 0.25 g of soil was weighed into a Teflon tube and mixed with 9 ml of nitric acid. The tube is set for 30 minutes under 95 C hot block then add 9 ml HF and set for another 30 minutes. After that, the temperature is increased to 135 C for one hour then increased to 150 C for one more hour.

Water Chemical Analysis

Two water sources were obtained from Qatar University farm and Rowdat Al-Faras farm with four replicates for each source. All pH, conductivity, salinity, and TDS were measured directly using meters for all parameters. The procedure for nitrate and nitrite measurement of the soil was the same for the water.

Statical Analysis

The Shapiro-Wilk (S-W) was applied to analyze the obtained data to evaluate if the data is normally distributed. Minimum, Maximum, Mean and Standard Deviation was calculated for soil chemical characteristics and metals. T-test of two sample assuming equal variances was used for water samples at 95% confidence interval. Pearson Correlation Coefficient was obtained by (XLSTAT statistical software;

Addinsoft Inc., New York, USA) for soil chemical parameters to check the correlation between parameters. For the principal component, the PCA in OTUs was plotted with XLSTAT to visualize the linkages between the soil microbial communities and the main drivers, soil parameters and sites, and cultivars (farms with cultivated date palms, cultivars, and wild date palms). Alpha-diversity indices bacterial community based on OUT were also analyzed to find any patterns of distribution of the specific group using MOTHUR (v1.31.2). We applied Shannon and Simpson analyses to estimate bacterial species diversity and bacterial species richness. We estimated the relative abundance (frequency) for each class; this was done for each site sampled using XLSTAT. XLSTAT was also used to investigate relationships between the composition of different soil microorganisms' groupings, the chemical parameters in soils and different locations sampled. Also, to understand the relationship between different organic fertilizers and the diversity of the bacteria. The Venn plots in OTUs or in taxa were plotted with the R package “Venn Diagram” version (3.1.1). Heat map for different cultivars and different fertilizer treatments were done with relation to relative abundance of different bacterial classes using XLSTAT.

Chapter 4: Results and discussion

All samples showed band except the wild date palm sample, and further procedures were done to extract DNA from this sample. After extracting the DNA from the samples and running it in the electrophoresis gel and making sure there is DNA, PCR was done using specific primers from bacterial identification to amplify the sequenced DNA and the sequencing results showed the presence of total of 1164425.0. Biodiversity of bacteria in date palm soil was represented as OTUs (Operational Taxonomic Units), and 6356 OTUs in total for all 27 samples were obtained.

Bacterial community in date palm soil samples shared 474 OTUs in common after removing OTUs related to archaea and OTUs that were not annotated (Figure 1). The figure represents that the sample with the highest unique OTUs number reaching 165 OTUs is coming from the wild sample which is three times more than the unique number of other samples. This can be explained by drought stress of Qatari weather and salinity stress near seawater induce bacterial diversity in wild date palm soil. The other samples, including samples from different cultivars and samples from the fertilizer treatment, ranged between 1 to 49 unique OTUs for each sample.

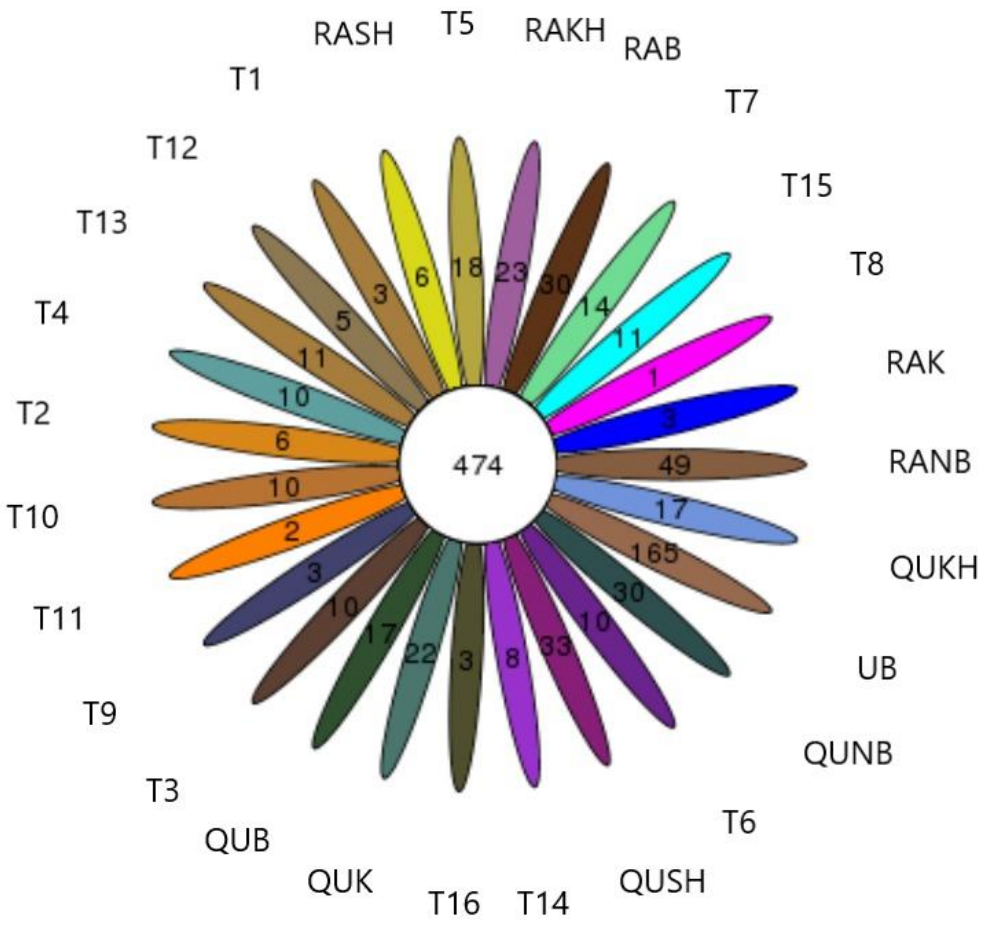

Figure 1. Core-Pan OTU Plot for soil samples. The middle circle indicates the number of shared OTUs in these samples or groups, and the ellipse outside the center circle indicates the number of OTUs that are peculiar in each sample.

The alpha diversity for each sample is presented along with Sobs, Chao, Shannon and ace indexes (Table 5). Samples with low sobs (OUT number) and high Shannon index indicates low diversity and vis versa. Almost all samples have values close to each other except the UB sample, which is the wild sample that has low Shannon index meaning low bacterial richness compared to other samples coming from different cultivars and the experimented samples. This result is consistent with

previously published in which salinity affect endophytic bacteria of date palm roots. Showing that salinity stress tend to decrease the OUT number comparing to the control (Yaish et al., 2017). The results showed that treatment 13 which is 100% organic and 30% chemical fertilizer have the highest richness followed by treatment 6 of (100% organic and 100% bio-organic) and treatment 7 (100% organic, 100% bio-organic and 100% chemical). This can indicate that organic and bio-organic fertilizer induce the bacterial richness more than the chemical fertilizer. This may refer to the fact that chemical fertilizer can alter the beneficial bacteria in soil negatively (Yudelman, 2000) Also, it affect soil aggregation negatively and lead soil degradation and this leads to leaching and degradation of nutrients (Bhatt et al., 2019). On the contrary, organic fertilizer and bio-organic increase the nutrient availability to micro-organisms that enhance their richness and biodiversity (Zhaoxiang et al., 2020).

Table 5. Alpha Diversity Statistical Table for Soil Samples

Sample ID	sobs	chao	ace	Shannon	Simpson	coverage
RAB	3283	3923.8	3949.094	6.890539	0.002432	0.982521
QUB	2673	3232.507	3169.284	6.603998	0.003402	0.98589
RASH	2765	3434.193	3377.223	6.56371	0.004581	0.984159
QUSH	2851	3472.938	3418.266	6.717943	0.00295	0.982974
RAKH	3040	3733.046	3724.189	6.69123	0.003736	0.981047
QUKH	2595	3118.565	3142.816	6.436159	0.005924	0.984834
RAK	2837	3395.324	3431.507	6.516131	0.006064	0.983674
QUK	2810	3382.212	3385.979	6.729371	0.002782	0.982017
RANB	3176	3724.629	3755.691	6.833336	0.002921	0.984084
QUNB	2913	3452.793	3453.922	6.740199	0.003033	0.983538
UB	1850	2202.07	2175.651	5.773418	0.014684	0.989615
T1	2929	3688.242	3653.701	6.570165	0.004517	0.981608
T2	3066	3783.935	3728.129	6.776364	0.003682	0.980286
T3	3174	3813.212	3842.381	6.856839	0.002996	0.980241
T4	3207	3923.723	3924.587	6.83918	0.003061	0.978833
T5	2978	3842.082	3740.258	6.656328	0.003478	0.981195
T6	3292	4052.158	4069.555	6.78985	0.003295	0.981096

Sample ID	sobs	chao	ace	Shannon	Simpson	coverage
T7	3291	4076.164	4060.803	6.751209	0.003903	0.980648
T8	2999	3691.696	3747.255	6.528027	0.004874	0.983186
T9	2952	3610.929	3651.133	6.579999	0.005806	0.981097
T10	2899	3585.327	3631.112	6.58999	0.004084	0.98074
T11	3053	3835.846	3828.928	6.55996	0.005788	0.979829
T12	3039	3830.865	3792.652	6.771292	0.003402	0.978134
T13	3326	4055.338	4032.515	6.806947	0.00385	0.98154
T14	3166	3904.764	3872.007	6.747487	0.003968	0.982084
T15	3168	3867.863	3886.671	6.801862	0.003147	0.982104
T16	3117	3824.08	3813.223	6.707876	0.003899	0.982945

Relative abundance in the class level for the sample showed that all samples except the wild sample have almost the same relative abundance (Figure 2). Actinobacteria and Alphaproteobacteria have the highest relative abundance between the other classes, followed by Gammaproteobacteria and ACidobacteroa_GP16. Nearly 20% of the relative abundance was other classes that were not classified. These results were similar to other study showing that Actinobacteria and Alphaproteobacteria had the highest relative abundance in date palm soil, followed by Gammaproteobacteria and Chloroflexi (Mosqueira et al., 2019b). Also, the study shared similar classes, including *Deltaproteobacteria*, Chloroflexi, Cytophagia, Acidobacteria and Bacilli with different percentages. The reason behind these similar results is that the identification of bacteria were both from date palm soil as the bacterial diversity in roots was very different than soil (Mosqueira et al., 2019b). Another study was showed that Gammproteobacteria had the highest percentage of relative abundance of 57%, followed by Actinobacteria and Alphaproteobacteria with 26% and 7%, respectively (Ferjani et al., 2015b). The results can assume that the classes with high relative abundance are able to tolerate abiotic conditions such as temperature and moisture.

Moreover, these classes composition and classes' relative abundance can be possibly found specifically for date palm soil.

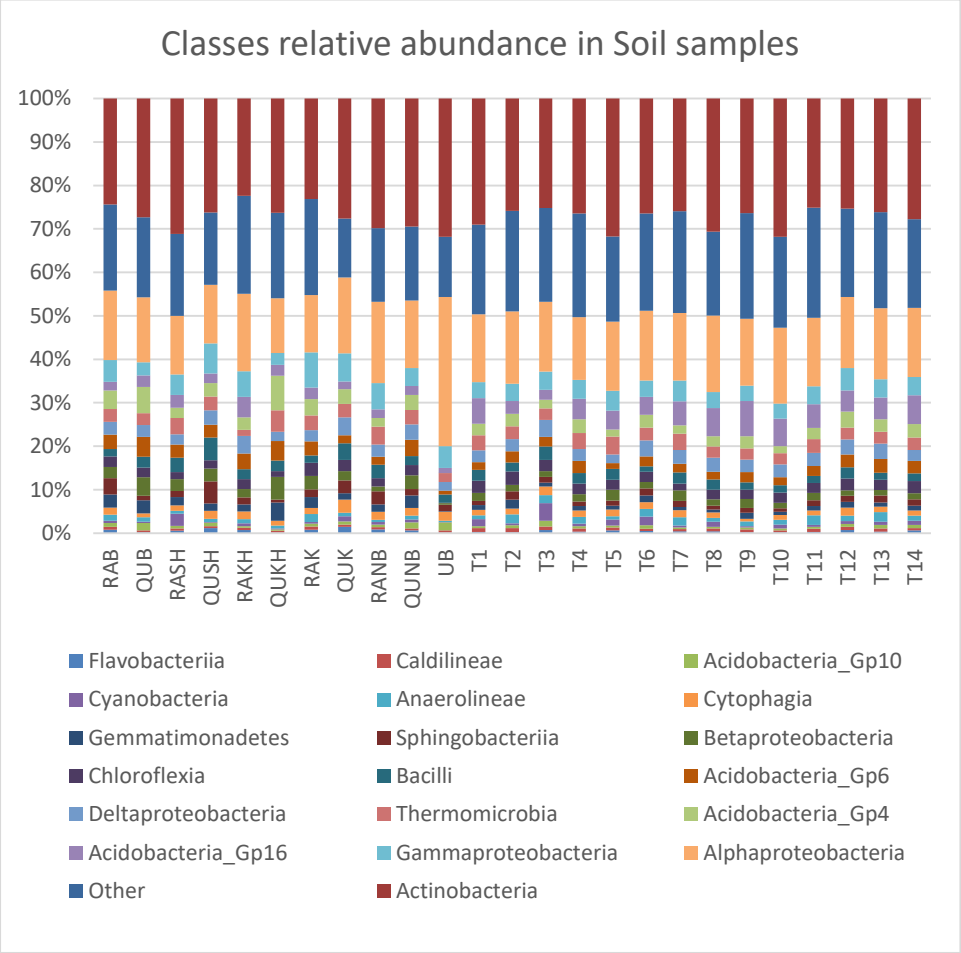

Figure 2. Class relative abundance of soil samples.

A biplot of PCA analysis that includes the samples, soil properties, and bacterial classes showed a correlation between samples of the two farms and all of salinity, total suspended solids, and conductivity (Figure 3). F1 and F2 showed higher eigenvalues for PCA (Table 6). The experimental samples showed a high correlation with pH and

nitrate concentrations. As they are clustered close to each other, they are highly correlated as they are from the same cultivar, 'Khalas.' Moreover, the wild sample (UB) showed no correlation with other samples but had the highest calcium concentration and total carbon percentage. Also, it is noticed that Alphaproteobacteria and actinobacteria had a higher correlation with the wild sample. There might be some relation between the high calcium concentration and the high Alphproteobacteria relative abundance.

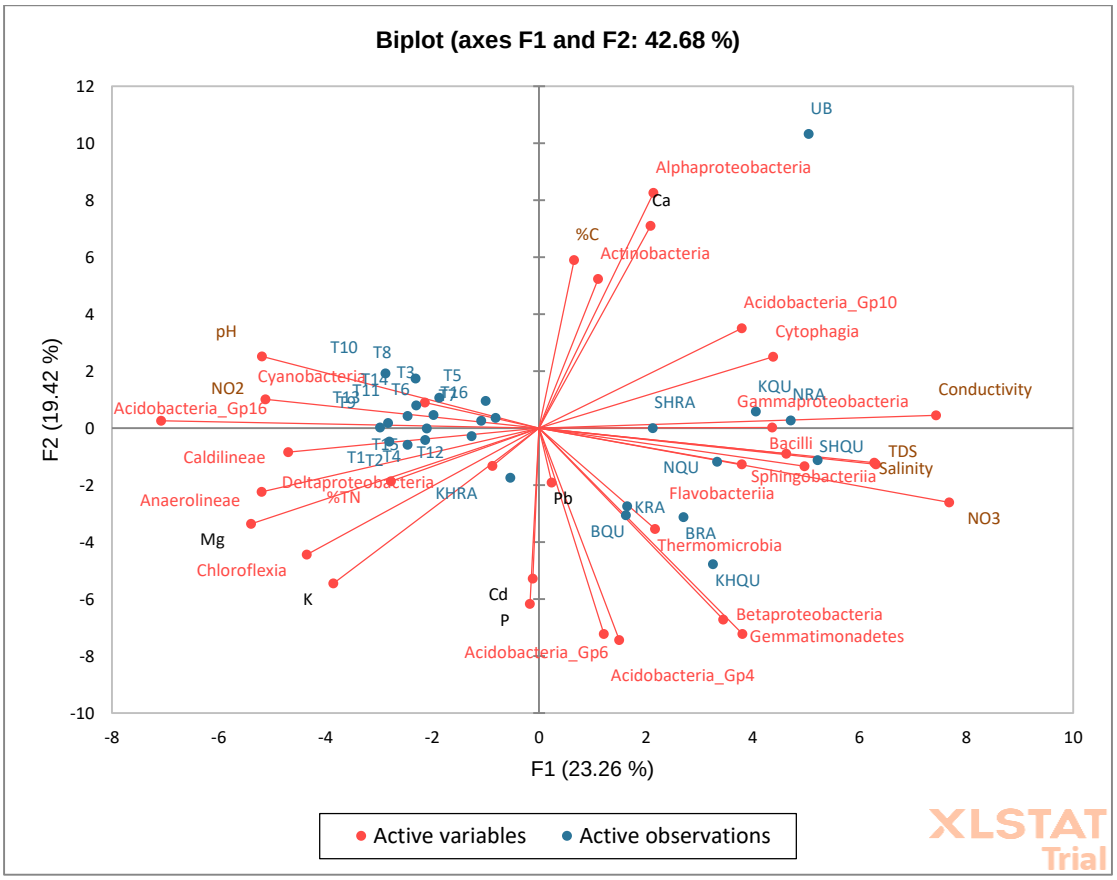

Figure 3. PCA analysis of soil samples with soil properties and classes diversity.

Table 6. Principal Component Analysis Eigenvalues

	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**
Eigenvalue	7.676	6.409	4.084	3.022	2.252	1.56	1.414	1.226	1.049	0.913	0.657	0.6	0.454
Variability (%)	23.262	19.423	12.376	9.158	6.823	4.728	4.286	3.715	3.178	2.767	1.991	1.818	1.375
Cumulative %	23.262	42.684	55.06	64.218	71.042	75.77	80.055	83.77	86.948	89.715	91.706	93.524	94.899
	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24	F25	F26
Eigenvalue	0.359	0.338	0.255	0.215	0.167	0.135	0.07	0.064	0.029	0.022	0.014	0.01	0.004
Variability (%)	1.088	1.024	0.774	0.651	0.507	0.408	0.213	0.193	0.088	0.068	0.043	0.03	0.013
Cumulative %	95.987	97.011	97.785	98.436	98.943	99.352	99.565	99.758	99.846	99.914	99.957	99.987	100

In (Figure 4), the heatmap explains the phylogeny of the species in each experimental sample, how they are related to each other through their ancestors, and the relation between the samples themselves. T8 (70% chemical), T1 (100% chemical), T5 (100% bio-organic 100% chemical), T7 100% chemical, organic and bio-organic), T9 (70% chemical 100% organic), T10 (70% chemical 100% bio-organic) are shown to be closely related to each other drawing an assumption that there is no high difference or effect on mixing other fertilizer types with a high concentration of chemical fertilizers. Bacilli, Nitrospira, Deltaproteobacteria, Spartobacteria and Thermomacrobia classes almost have high relative abundance mentioned treatments above. The other experimental samples with high organic or bio-organic concentrations are related to each other. In the control sample, the closely related classes delta-proteobacteria, acidobactria-Gp3, Anaerolinaea, and Clostridia have low relative abundance compared to the sampling sharing the same phylogeny is estimated that the organic and bio-organic fertilizers may affect these classes positively. Similarly, bio-organic fertilizer, including four active strains; *Azotobacter chrococcum* as source of diazotrophs, *Azospirillum brasilense* as a source of nitrogen, *Bacillus megaterium* as a source of phosphorus, and *Bacillus circulans* as a source of potassium, increased the bacteria number in soil compared to control treatment (EL-Sharabasy et al., 2018b).

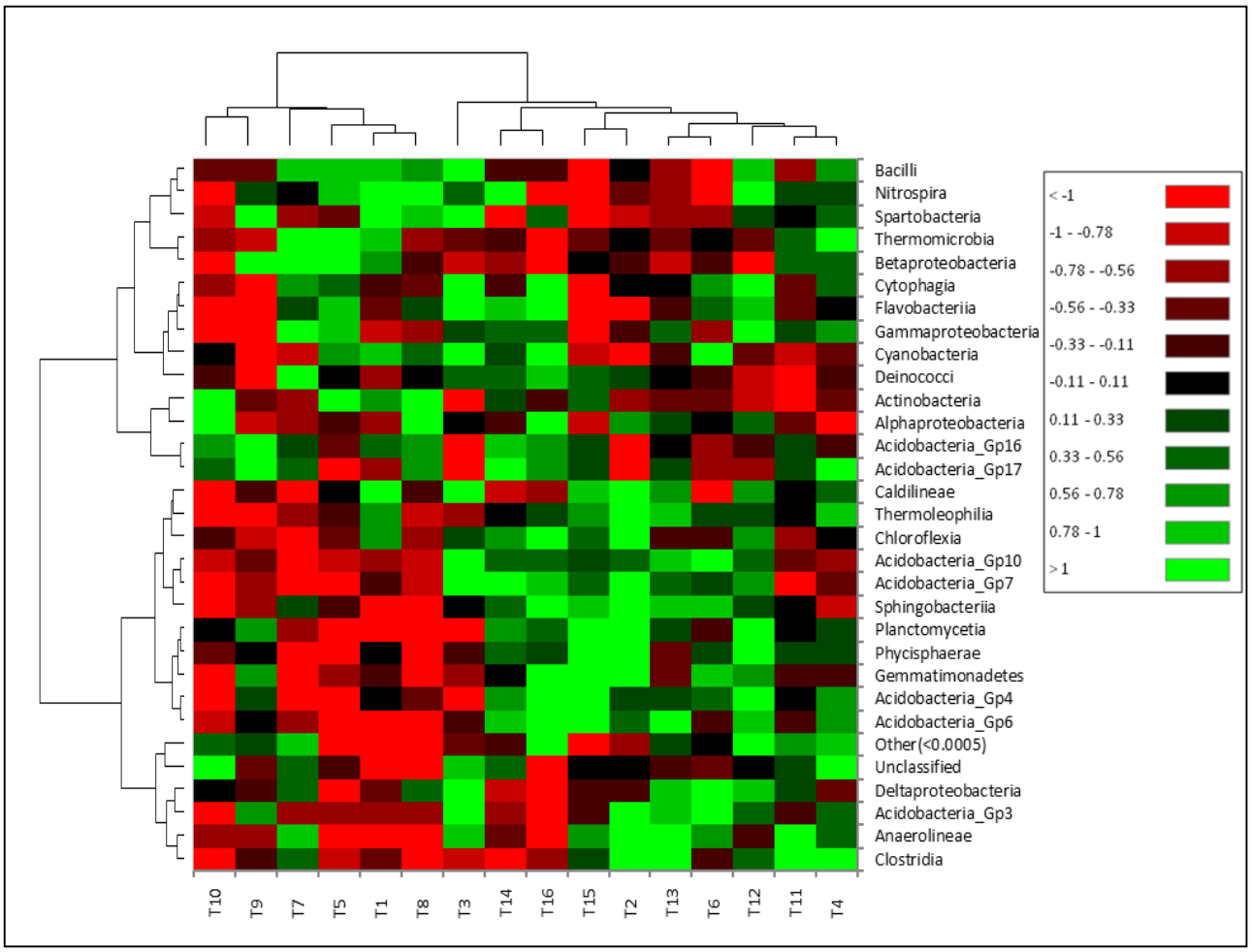

Figure 4. Heatmap of experimental samples of different fertilizers.

For different cultivars samples from two farms, the wild sample is coming from a very different phylogeny and all other cultivars are coming from similar phylogeny (Figure 5). Wild sample have the highest abundance of Actinobacteria, Acidobacteria-Gp10, Deltaproteobacteria, and Cytophagia. The other classes had low relative abundance in the sample. Berhi, Naboot Saif and Khenezy cultivars from QU farm share close phylogeny to each other with Shishi from RA farm and share high relative abundance of Nitrospira, Actinobacteria_Gp3, Actinobacteria_Gp4, Actinobacteria_Gp6, Actinobacteria_Gp7, Actinobacteria_Gp16, Actinobacteria_Gp17, Thermoleophilia, Chloroflexia and Betaproteobacteria classes. Berhi, Naboot Saif and Khalas from RA farm share close phylogeny with Shishi and Khalas from QU farm. It was expected to

have similar cultivar from different farms to share close phylogeny but only Khalas cultivar from both farms shared similar phylogeny. These results can conclude that date palm location have higher correlation with bacterial biodiversity than the cultivar type.

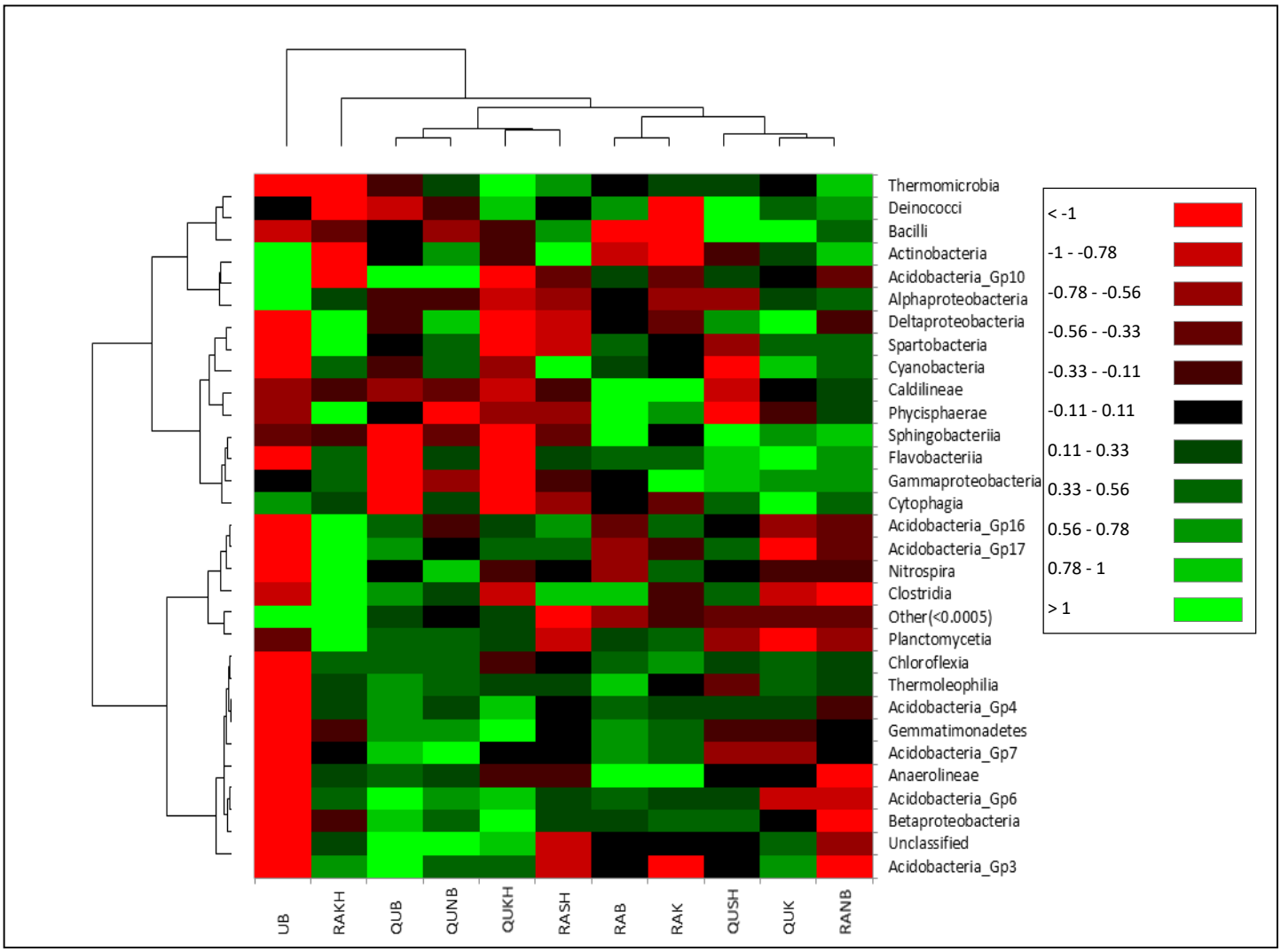

Figure 5. Heatmap of different cultivars and wild sample.

Among the pH of all composite samples, the Khalas sample at Qatar University farm was the lowest (7.18), and treatment 10 in the fertilizer experiment was the highest

(7.89). However, samples pH, in general, ranged from (7.18-7.89), which is weak alkaline, and it is suitable for plant growth as well as the microorganism growth. This result were lower than a study conducted in showing range of 7.5 to 8.1 (Mlih et al., 2019)

Salinity between samples from the fertilizer experiment had low variation and it ranged between 0.4 and 0.6 (Table 7). However, other samples, including Khalas and Naboot Saif in RA, had high salinity of 3.13 and 2.55 ppt, respectively. Total suspended solids ranged from 0.59 to 4.59 g/L. Umm Bab sample had a salinity of 1.5. TDS had a positive relationship with salinity, samples with higher salinity had higher TDS, and samples with low salinity had low TDS. The conductivity ranged from 0.74 to 5.44 mS. And it follows the same relationship with TDS and salinity. Total carbon in the soil samples ranged from 3.34 %C to 7.38%C. The interesting result is that the wild date palm in Umm Bab had the highest carbon percentage of 7.38, which is not expected. The total carbon percentage were higher compared to a study conducted in Tunisia (Mlih et al., 2019). Total nitrogen ranged from 0.045 to 0.810 % which is lower compared to the same study. Total nitrogen percentage ranged from 0.045 in UB to 0.307 in T2, excluding the outlier in samples T1 that has a percentage of 0.810 %TN. Nitrite concentration in experimental samples was higher compared to the samples coming from different cultivars, and this is expected as they are supplied with fertilizers. Compared to the nitrate and nitrite levels, total nitrogen showed low concentration in all soil. BRA sample had the highest nitrite concentration of 328.4 mg/Kg, and the lowest was 14.19 mg/Kg in T2. On the contrary, nitrate concentrations were extremely higher in different cultivars and wild samples than in the experimented samples except Khenezy cultivar from RA because of the fertilizer contents. The concentration of nitrate in the soil ranged from 2.4 in T4 to 0.29 in

SHRA. In Table 8, salinity and TDS show high correlation between each other as well as salinity and TDS with nitrite.

Heavy metals including (Mg, Ca, Cd, Pd, K, P) in soil were analyzed. Calcium concentration in Umm Bab sample was the highest of all samples because Umm Bab soil composition consist of limestone (Al-Saad, 2005) (Table 7). Cadmium concentration varied among samples with a mean of 0.386 ppm (Table 8). There was high correlation between phosphorus and cadmium (Table 9). There was zero concentration of cadmium in the Umm Bab sample, and the highest concentration was in the Khenezy cultivar of RA. Magnesium concentration varied from 7264.1 in the Umm Bab sample, and the rest of the samples ranged from 16078.4 32098.7 ppm. Phosphorus concentration was exceptionally low in the wild sample with 107 ppm compared to the rest of the samples ranging from 1612.1 to 8349.3 ppm. Potassium had a similar phosphorus curve with low concentration in wild samples and close range between other samples. Lead concentration was high in Naboot Saif cultivar, Khenezy of RA, and fertilizer treatment 13 of 30% chemical, 100% organic, and 100% bio-organic fertilizer. However it was high in treatment 13, the highest bacterial diversity was shown in this samples and possible explanation is that lead concentration did not reach to the level that inhibit bacterial species and their enzymatic functions (Khan et al., 2010).

Table 7. Chemical properties and heavy metals concentration in soil samples

Sample ID	pH	Conductivity (mS)	Salinity (ppt)	TDS (g/L)	mg NO3-N/ Kg soil	mg NO2-N/ Kg soil	%C	%TN	Ca (ppm)	Cd (ppm)	Mg (ppm)	P (ppm)	Pb (ppm)	K (ppm)
BQU	7.72	1.57	0.83	1.31	283.8268	0.5732	4.587012	0.198013	106218.9	0.9	21084.9	7333.2	1.21	11216.1
BRA	7.33	2.25	1.20	1.81	328.3818	0.3932	4.032556	0.127123	107987.3	0.98	23908.2	7596	0	12064.9
KHQU	7.38	2.45	1.28	1.94	322.2848	0.8652	3.335226	0.125165	80696.2	0.42	16078.4	3991.2	0.28	11294.7
KHRA	7.59	0.82	0.40	0.66	23.2368	0.4132	4.708676	0.191128	103416.8	1.05	22603.2	8349.3	2.32	12769.8
KQU	7.16	5.44	0.50	0.76	194.5918	0.4332	5.374143	0.271384	113735.1	0.5	23068.6	2905.2	0.25	11478.2
KRA	7.83	0.95	3.13	4.59	147.1398	0.3852	5.475009	0.291105	94994.4	0.48	25751.4	4911.1	1.8	12115.3
NQU	7.27	2.97	1.53	2.36	230.1528	0.4972	4.182298	0.090797	86618.8	0.39	22004.8	3096.9	0	10562.8
NRA	7.48	4.64	2.55	3.70	232.6173	0.8452	3.70745	0.153697	90999.8	0.25	18756.1	4497	3.28	10851.4
SHQU	7.37	3.65	1.98	2.92	296.9408	1.2092	4.797426	0.161632	92108.5	0.16	24961.3	2523.9	0.95	12008.7
SHRA	7.54	2.58	1.45	2.14	226.2943	0.2932	4.032154	0.1257	97749.9	0.08	24204.4	1612.1	0.01	12579.4
T1	7.69	1.18	0.50	0.73	27.5478	0.4772	3.741354	0.810297	100804	0.43	26913.9	3994.2	1.15	13310.4
T10	7.97	0.74	0.40	0.59	33.7568	1.8932	4.194647	0.050884	106991.7	0.42	27050.4	3774.8	0	12181.3
T11	7.63	0.95	0.50	0.76	34.7438	2.2812	5.160939	0.204796	84266.6	0.3	23393.5	2942.8	0.74	10771.5
T12	7.45	1.16	0.60	0.93	38.3318	2.1932	4.146712	0.155264	96870.2	0.23	25574	3345.3	0	12591.8
T13	7.58	0.85	0.43	0.70	39.3398	2.1852	6.025739	0.285701	104773.6	0.44	29517.6	3990.5	0.42	13210.6
T14	7.91	0.89	0.45	0.71	38.9808	2.1692	4.600432	0.128207	108556.8	0.25	26266.3	3159.2	0.08	12092.4
T15	7.40	1.16	0.60	0.91	40.6558	2.3692	5.445394	0.251394	75749.2	0.16	21593.5	2950.9	2.64	9136.2
T16	7.62	0.94	0.50	0.75	29.9708	0.4292	4.39202	0.161872	90588.7	0.29	21731.7	2883.9	0	10536.9
T2	7.80	0.92	0.50	0.79	14.1938	0.5812	4.774213	0.30697	107140.7	0.44	29583	3151.2	1.4	12812.4
T3	7.83	0.99	0.60	0.95	36.6138	2.1612	4.460814	0.235863	107994.7	0.29	26108.5	2557.4	1.48	12678.5
T4	7.64	0.96	0.50	0.77	56.9988	2.4012	4.679375	0.212452	100292.9	0.28	27037.7	3804.9	1.66	12865.4
T5	7.67	1.17	0.60	0.94	26.0998	0.4252	3.975786	0.153778	108540.3	0.26	32098.7	2596	0.12	13503.5
T6	7.72	0.98	0.50	0.79	40.0968	1.5532	4.816762	0.208879	101121.2	0.36	28213.8	2962.2	0	12583.1
T7	7.55	1.17	0.60	0.94	32.7408	0.4092	4.318589	0.154672	103480.2	0.26	29589.2	2495.6	0.65	13128

Sample ID	pH	Conductivity	Salinity	TDS	mg NO3-N/ Kg soil	mg NO2-N/ Kg soil	%C	%TN	Ca (ppm)	Cd (ppm)	Mg (ppm)	P (ppm)	Pb (ppm)	K (ppm)
T8	7.79	0.82	0.40	0.65	40.7228	2.1772	4.574154	0.151625	84266.6	0.3	23392.5	2942.8	0.74	10771.5
T9	7.68	0.80	0.40	0.67	35.6828	2.2172	4.595529	0.190115	103425.4	0.5	25279.3	4367.5	0	12336.9
UB	7.67	2.81	1.50	2.25	197.3488	0.4012	7.38177	0.044757	187782.6	0	7264.1	107	0	5726.3

Table 8. Soil Characteristics Minimum, Maximum, Mean and Standard Deviation Values

Variable	Observations	Minimum	Maximum	Mean	Std. deviation
pH	27	7.155	7.968	7.600	0.200
Conductivity	27	0.737	5.441	1.697	1.255
Salinity	27	0.400	3.125	0.904	0.710
TDS	27	0.589	4.585	1.371	1.029
mg NO3-N/ Kg soil	27	14.194	328.382	112.937	110.004
mg NO2-N/ Kg soil	27	0.293	2.401	1.194	0.837
%C	27	3.335	7.382	4.649	0.811
%TN	27	0.045	0.810	0.202	0.139
Ca	27	75749.200	187782.600	101747.078	19798.387
Cd	27	0.000	1.050	0.386	0.247
Mg	27	7264.100	32098.700	24186.259	4872.740
P	27	107.000	8349.300	3660.819	1754.484
Pb	27	0.000	3.280	0.784	0.924
K	27	5726.300	13503.500	11747.333	1594.956

Table 9. Proximity Matrix (Pearson Correlation Coefficient)

	pH	Conductivity	Salinity	TDS	NO3-N	NO2-N	%C	%TN	Ca	Cd	Mg	P	Pb	K
pH		-0.697	-0.204	-0.208	-0.553	0.277	0.087	0.085	0.194	-0.059	0.269	-0.043	0.022	0.159
Conductivity			0.489	0.485	0.712	-0.417	0.003	-0.147	0.132	-0.111	-0.447	-0.137	0.058	-0.312
Salinity				1.000	0.635	-0.411	0.035	-0.142	0.003	-0.124	-0.353	0.017	0.297	-0.246
TDS					0.642	-0.413	0.037	-0.151	0.004	-0.117	-0.356	0.021	0.289	-0.247
mg NO3-N/ Kg soil						-0.461	-0.110	-0.278	0.058	0.172	-0.524	0.185	-0.051	-0.300
mg NO2-N/ Kg soil							0.117	-0.076	-0.254	-0.266	0.213	-0.147	0.042	0.064
%C								-0.085	0.607	-0.186	-0.328	-0.291	0.001	-0.556
%TN									-0.145	0.125	0.305	0.119	0.281	0.343
Ca										-0.096	-0.393	-0.253	-0.268	-0.423
Cd											0.100	0.930	0.119	0.300
Mg												0.096	-0.061	0.874
P													0.324	0.325
Pb														-0.014
K														

Heavy metals in water were analyzed as well (Figure 6). Noticeably, there was no cadmium detected concentration in both water samples. All other metals were higher in Rowdat Al-Faras water, beside lead, it was higher in Qatar university farm water. Testing heavy metal in water was done to know if the heavy metal concentration in soil comes from the watering source. The concentration of magnesium, calcium, and potassium between the two water sources showed high significance. However, their concentrations in the soil in different cultivars were almost similar, and it is expected as they are watered from the same source. Phosphorus showed no significant difference between both samples. However, lead concentration varied a lot between different cultivars from different farms. The lead concentrations of QU and RA showed no significant difference with p-value of 0.684. It was expected to have a higher lead concentration in QU farm as the watering source had a higher concentration than RA farm. Still, RA farm cultivars showed higher lead concentration leading to other contamination possibilities.

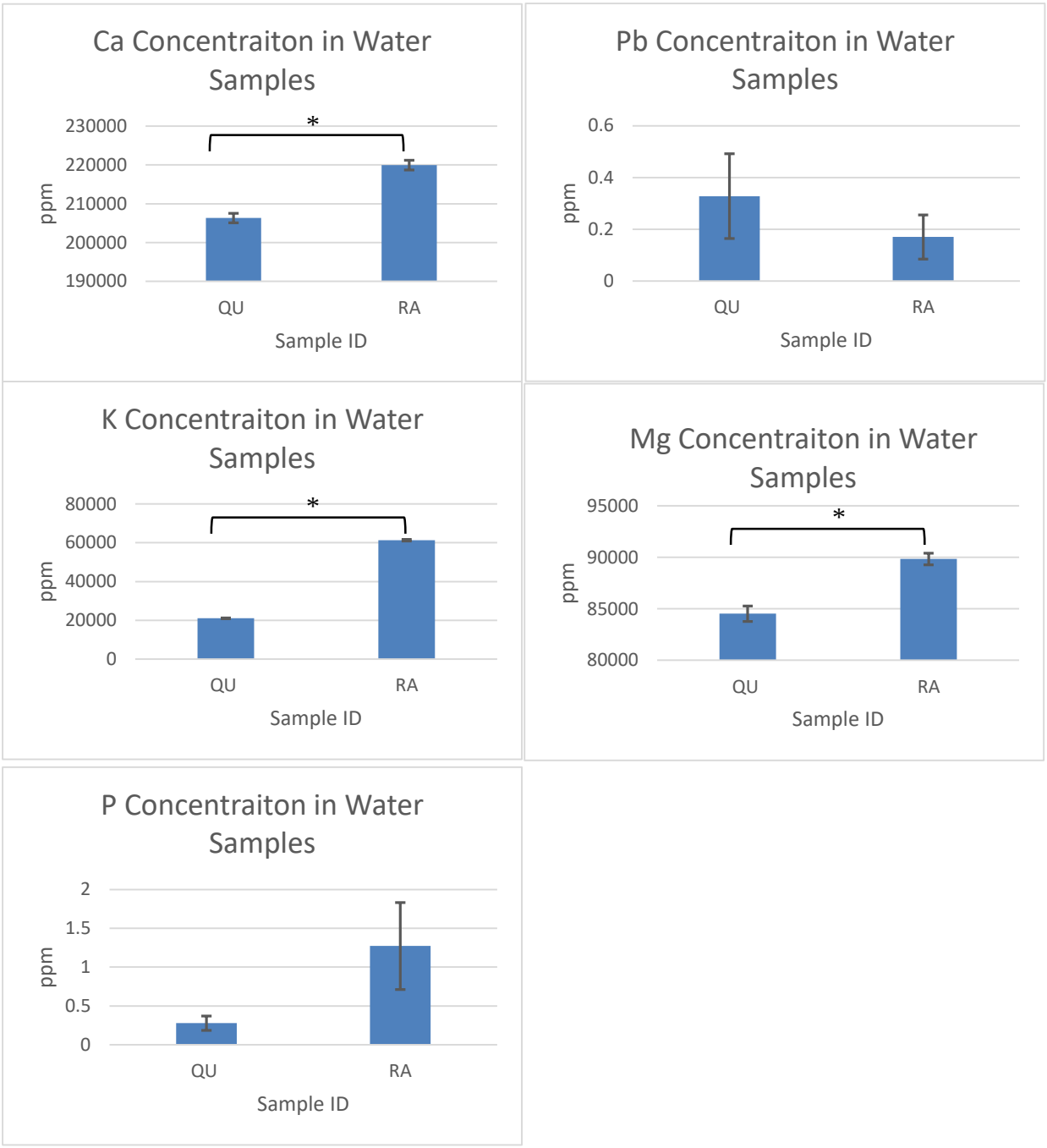

Figure 6. Metal concentrations in water samples. Bars represent mean ±SE (n=4). (*) represent significant difference between samples ($p \leq 0.05$).

Chapter 5: Conclusion

To conclude, date palm is considered an important plant in the gulf region for its numerous benefits. The wild date palm showed a lot of unique OTUs related to bacteria indicating higher diversity in the wild species. The addition of organic and bio-organic fertilizer with 100% chemical fertilizer did not affect the bacterial biodiversity. However, 100% of organic and bio-fertilizer showed positive effective on bacterial relative abundance of different classes. Correlation between cultivars type and bacterial diversity was very weak except for Khalas cultivar from both farms shared similar phylogeny indicating higher correlation of date palm location with bacterial biodiversity. Chemical parameters of soil such as salinity, TDS and Conductivity showed high correlation. Beneficial Bacterial classes for date palm production can be extracted and used to enhance quality and quantity of dates. Also, functions of wild sample classes and bacteria from classes having high relative abundance can be used to enhance date palm tolerance to salinity and drought. Different fertilizer treatments can be suggested to farmers based on bacterial diversity needed to enhance yield and quality of date palm trees. Further studies should be undertaken regarding bacterial diversity in date palm soil and date palm yield in Qatar and worldwide to gain more knowledge on microbiome diversity and its role in date palm soil and improve yield quality and quantity.

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