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IMPACT OF SALINE WATER IRRIGATION ON DATE PALM (Phoenix dactylifera) ASSOCIATED BULK SOIL BACTERIAL COMMUNITIES IN OASES AGROECOSYSTEM OF UAE

Fardous Abdi Ibrahim Jibar Alhashmi

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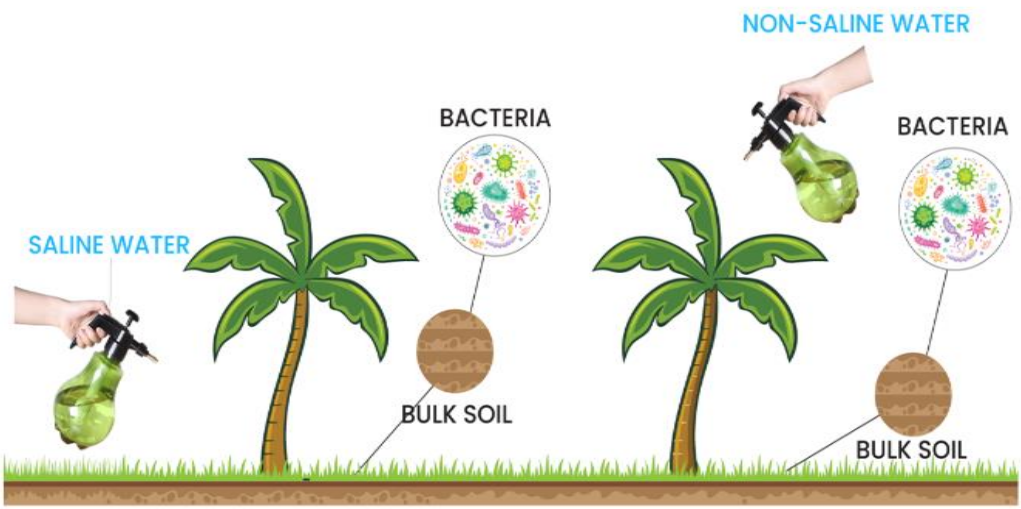

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Fardous Abdi Ibrahim Jibar Alhashmi

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Environmental Sciences

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Impact of saline water irrigation on date palm *(Phoenix dactylifera)* associated bulk soil bacterial communities in oases agroecosystems of UAE

(Photo: By Fardous Abdi Ibrahim Jibar AlHashmi)

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Declaration of Original Work

I, Fardous AlHashmi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Impact of Saline Water Irrigation on Date Palm (Phoenix dactylifera) Associated Bulk Soil Bacterial Communities in Oases Agroecosystems of UAE*", hereby, solemnly declare that this is the original research work done by me under the supervision of Dr. Sunil Mundra in the College of Science, at UAEU. This work has not previously formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

Irrigation of date palm (*Phoenix dactylifera*) with saline groundwater is routinely practiced in the arid agroecosystems of United Arab Emirates (UAE), due to freshwater scarcity. Saline groundwater irrigation is known to deposit salts in the top layers of soil and increase soil salinization. However, how increasing soil salinization affects the belowground bacterial communities, is not well investigated. Soil samples were collected from 14 different date farms where irrigation water source was either non–saline water or saline groundwater. Soil bacterial communities were identified using 16S rRNA gene metabarcoding. The results showed that bacterial diversity (including Shannon diversity, richness, and evenness) didn't vary between irrigation sources (non-saline water vs saline groundwater). However, distinct soil bacterial communities were observed between irrigation water sources, and they were significantly related to the irrigation water electrical conductivity. Of total 5155 OTUs, 21.3% were uniquely present in the soil while saline groundwater irrigation and 31.5% while non–saline water irrigation, and only 47.15% OTUs were shared. The abundance of Proteobacteria was higher in soil while saline groundwater irrigation, and pattern contrasted for Actinobacteriota. Compositional shift at genera level was also evident, wherein abundance of Subgroup_10, *Novibacillus, Bauldea* and *Mycobacterium* was higher while saline groundwater irrigation and *Microvirga, Marmoricola, Ammoniphilus* and *Lysinibacillus* abundance was low. *Mycobacterium* and *Steroidobacter* were the key indicator taxa while saline groundwater irrigation and *Solirubrobacter* and *Sorangium* were indicator of non–saline water irrigation. The results of this study indicate that soil determine colonization of bacterial communities under different irrigation water sources (non–saline water and saline groundwater irrigation) and it is influenced by salinity of irrigation water. The project

results revealed that salinity of irrigation water selects distinct bacterial communities in soil, which are essential for maintaining soil health in oases agroecosystem of arid environments.

Keywords: Bacterial communities, Date palm (*Phoenix dactylifera*), Irrigation sources, Metabarcoding, Oasis agroecosystem, Soil salinization.

Title and Abstract (in Arabic)

تأثير الري بالمياه المالحة على المجتمعات البكتيرية للتربة المرتبطة بنخيل التمر (*Phoenix dactylifera*) في النظم الزراعية البيئية في الإمارات العربية المتحدة

الملخص

يُمارس ري النخيل (*Phoenix dactylifera*) بإستخدام المياه الجوفية المالحة بشكل روتيني في النظم البيئية الزراعية القاحلة لدولة الإمارات العربية المُتحدة وذلك بسب ندرة المياه. ومن المعروف أن الري بالمياه الجوفية المالحة يُرسب الأملاح في الطبقات العليا من التربة ويزيد من ملوحة التربة. ومع ذلك، لم يتم التحقيق بشكل جيد في كيفية تأثير الملوحة على مجتمعات بكتيريا التربة تحت الأرض. قمنا بجمع عينات من التربة من 14 مزرعة تمور مختلفة حيث كانت مصادر مياه الري عبارة عن مياه جوفية مالحة ومياه جوفية غير مالحة وتم تحديد المجتمعات البكتيرية باستخدام عملية التمثيل الغذائي(16S rRNA gene metabarcoding). وجدنا أن التنوع البكتيري بما في ذلك تنوع شانون والثراء والتساوي لا يختلف بين مصادر الري المالحة مقابل مصادر الري الجوفية المالحة. ومع ذلك ، لوحظ وجود مجتمعات بكتيرية في التربة وكانت مرتبطة بشكل كبير بالتوصيل الكهربائي لمياه الري. وكان إجمالي وحدات التصنيف التشغيلية للمجتمعات البكتيرية 5155. حيث كانت 21.3٪ منها متواجدة بشكل فريد في عينات التربة التي كان مصدرها للري عبارة عن مياه جوفية مالحة و 31.5٪ كانت مجتمعات بكتيرية متواجدة بشكل فريد في عينات التربة التي تم سقيها بماء جوفي غير مالح، أما 47.15٪ من الإجمالي كانت مجتمعات بكتيرية متواجده في كل من مواقع الدراسة المالحة وغير المالحة. كانت وفرة *Proteobacteria* أعلى في التربة التي تم سقيها بالمياه الجوفية المالحة، وكان النمط متناقضًا مع *Actinobacteriota*. كان التحول التركيبي على مستوى الأجناس واضحًا أيضًا، حيث كانت وفرة *Subgroup_10* و *Novibacillus* و *Bauldea* و *Mycobacterium* أعلى وفرة في التربة التي تم سقيها بالمياه الجوفية المالحة، أما *Microvirga* و *Marmoricola* و *Ammoniphilus* و *Lysinibacillus* أظهرت وفرة منخفضة. كانتا *Mycobacterium* وSteroidobacter هما مؤشرا الأداء الرئيسي للنتائج التي أدت إلى تباين المجتمعات البكتيرية في التربة التي تم سقيها بالمياه الجوفية المالحة أما *Solirubrobacter* و *Sorangium* كانتا متواجدتان في التربة التي تم سقيها بالمياه الجوفية غير المالحة. تشير نتائج هذه الدراسة إلى أن التربة تحدد استعمار المجتمعات البكتيرية تحت مصادر مياه الري المختلفة بالمياه الجوفية المالحة وغير المالحة وتتأثر أيضاً بملوحة مياه الري.

مفاهيم البحث الرئيسية: نخيل التمر (*Phoenix dactylifera*)، Metabarcoding، النظام البيئي الزراعي في الواحة، مصادر الري، ملوحة التربة.

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Fardous Alhashmi is a diligent environmental scientist. In 2019, she got her Bachelor of Science in environmental science and sustainability from Zayed University, Abu Dhabi, UAE. In 2020, she enrolled a master's degree in environmental science engineering in United Arab Emirates University.

Fardous Alhashmi was nominated the Zayed University delegate to the USLS hosted by the Humanitarian Affairs Asia and the United Nations Development Program (UNDP) in Kuala Lumpur 2019, a yearly week-long event that acts as an international platform to provide the next generation of leaders with a vision of how to distinguish themselves as future leaders of the 21st century. Worked as research assistant with Dr. Yousef Nazzal (Professor, Chair of College of Natural & Health Sciences,) where she supported lab operations with accurate documentation and organized spaces.

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I would like to thank my defense committee for their guidance, suggestions, enthusiasm, and constructive criticism.

Dedication

I am dedicating this thesis

to my beloved parents, without whom this thesis was almost impossible to complete.

to my family and friends for nursing me with affection, love, and their dedicated partnership for success in my life.

to myself, who spent the last eighteen years dreaming of becoming a scientist and never satisfied till I achieved.

أهدي رسالة الماجستير:

إلى الذي يحثني بالإصرار دائماً، أبي

وتمدني بالقوة أبداً، أمي

إلى طوق الحب والدعم، أخوتي وأصدقائي

إلى ذاتي التي قضت ثمانية عشر عاماً تحلم بأن تصبح عالمة، ولم تقبل إلا بالوصول.

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List of Abbreviations

ASV	Amplicon Sequence Variant
BSA	Bovine Serum Albumin
BWh	Subtropical Desert Climate
CI	Confidence Interval
E.Z.N.A.	Soil DNA kit
EC	Electrical conductivity
HF buffer	buffer High-Fidelity buffer
IMR	Integrated Microbiome Research
milliQ	Milli-Q water purification system
NMDS	Non–Metric Multidimensional Scaling
OM	Organic Matter
OTUs	Operational Taxonomic Units
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational analysis of variance
PGPR	Plant Growth Promoting Rhizobacteria
SOM	Soil Organic Matter
Tukey's HSD	Honestly Significant Difference
UAE	United Arab Emirates

Chapter 1

Chapter 1: Introduction

1.1 Overview

Increasing soil salinities due to natural processes in arid regions is known to raise groundwater salinity. Application of saline groundwater for irrigation in turn increases soil salinities through "secondary salinization" (Egamberdieva et al., 2010), secondary salinization is when natural salinity accumulates resulting from human activities, and the change of climate which can adversely affect soil productivity depending on salt concentrations and amount of irrigation water (Chen et al., 2019). Despite the drawbacks, cultivation of economically important plants (i.e. Date palms) using saline water irrigation is routinely practiced in arid agroecosystems of United Arab Emirates (UAE) due to water scarcity. Continuous crop management using saline water irrigation is known to cause insufficient percolation of water and accumulation of salts in the top veneer of soil, which severely affects soil productivity. Apart from salinity, the use of saline water also changes soil pH depending on cationic (sodium, calcium and magnesium) and anionic (chloride and carbonate) composition (Guo et al., 2020). Further, saline water irrigation is reported to decrease bulk density of soil, thereby affecting nutrient turnover and concentration of available nutrients (Yuan et al., 2018). Soil properties like flocculation (calcium) and dispersion (sodium) are also dependent on ionic composition and salinity of soil, which is critical for maintaining soil structure and facilitating water movement (Rengasamy, 2018). These adverse edaphic changes associated with saline water irrigation on soil chemistry and physical properties can further affect the belowground microbiota.

Soil bacteria play a vital role in biogeochemical processes of arid environments, thereby maintaining global ecosystem functioning. Soil

bacterial genes related to biogeochemical pathways such as, ammonia oxidation (Guo et al., 2020; Khan et al., 2020), nitrogen fixation (Khan et al., 2020), denitrification (Guo et al., 2021) and sulphate production were found to be strongly modulated under high salinities. In addition, soil salinity stimulated emission of nitrous oxide (greenhouse gas) in desert soil (Zhang et al., 2016), while another study reported inhibition of soil bacteria involved in nitrogen cycle (Li et al., 2021). Therefore, it is evident that salinity induced alterations in bacterial diversity and communities can potentially change ecosystem functioning.

Salinity dependent decrease in bacterial richness (Li et al., 2021; Zhang et al., 2019) and Shannon diversity index (Guo et al., 2021; Nan et al., 2022; Yu et al., 2021) were previously observed in saline soils. Similarly, irrigation water salinity levels decreased (Chen et al., 2019) as well as increased Shannon diversity index (Chen et al., 2017) in arid agroecosystems. Salinity was an important factor in structuring bacterial communities of saline soils (Guo et al., 2021; Li et al., 2021; Nan et al., 2022) and soil while saline groundwater irrigation (Chen et al., 2017; Chen et al., 2019) as well. These variation between bacterial diversity studies on with regard to electrical conductivity (EC) and pH were the major factors for saline soils (Nan et al., 2022; O'Brien et al., 2019), whereas saline groundwater irrigation related studies did not ascertain the factor responsible for structuring bacterial communities (Chen et al., 2017; Chen et al., 2019). Therefore, the inconsistencies in saline groundwater irrigation induced bacterial diversity and community structure of arid agroecosystems is urgently needed.

The resiliency of soil bacteria against increased salinity in arid ecosystems is dependent on the colonization and enrichment of specific bacterial taxa. Increased soil salinities enhanced abundance of Proteobacteria (Nan et al., 2022) and its classes Gamma- and Alphaproteobacteria (Zhang et al., 2019),

while reducing Actinobacteroidota (Guo et al., 2021) Chloroflexi, Acidobacteria and Planctomycetes in saline soils (Li et al., 2021). Whereas saline groundwater irrigation reduced abundance of Actinobacteria, Gemmatimonadetes and Acidobacteria in cotton field soil (Chen et al., 2019). Another study involving irrigation water sources with different salinities showed increased (*Proteobacteria* and *Actinobacteria*) and decreased (*Planctomycetes* and *Bacteroidetes*) abundance of certain taxa in cotton field soil (Chen et al., 2017). Soil bacteria from these taxa withstand salinity–stress by producing spores, extracellular polysaccharides, antioxidant enzymes and osmolytes for survival under extreme environmental conditions. Soil salinity also induced colonization of specific bacterial genera in maize (*Halobacteria* and *Nitriliruptoria*) (Li et al., 2021) and barley (*Rhodanobacter, Acidobacterium, Candidatus Nitrosotalea,* and *Candidatus Koribacter*) field soils (Li et al., 2021). The possible mechanisms behind selection of specific bacterial taxa due to irrigation water salinity influences is unknown.

1.2 Statement of the Problem

The current knowledge on secondary salinization effect on bacterial diversity and community showed inconsistencies arising from limited sampling locations and salinity ranges. Therefore, there is a need to perform comprehensive study with multiple locations and different salinity ranges to test the effect of saline groundwater irrigation on soil bacterial populations especially in arid agricultural soil, therefore accounting spatial variability and representing salinity ranges prevailing in arid agricultural settings. Bacterial communities of soil associated with date palms receiving different irrigation water sources (non–saline water and saline groundwater) were investigated from fourteen different sites across UAE.

1.3 Research Objective

The aim of the study was to assess relationship between soil and irrigation water salinity with bacterial diversity, communities, structuring factors, key taxa specific saline groundwater irrigation and potential ecosystem functions in arid agricultural soil. However, Salinity filtering should be the key factor structuring bacterial communities and diversity of soil.

1. Does irrigation with saline water affect bacterial diversity, communities, and structuring factors?

2. What are the key taxa specific saline groundwater irrigation?

1.4 Relevant Literature

1.4.1 Importance and adaptation of date palm plantation in arid and saline environment

The Middle East is the world's leading producer of dates, owning 70% of the world's resources of date palms (*Phoenix dactylifera L.)* and having an estimated 120 million trees (Cybulska et al., 2017). Date palm (*Phoenix dactylifera* L.) has for some time been one of the main characteristic item crops inside the dry districts of the Middle East, for example, Arabian Peninsula, center East, and North Africa, since it can adjust dry season, heat and generally significant levels of soil saltiness.

It is assessed that around 62 million hectares (20%) of the world's flooded land is antagonistically influenced by salinity (Egamberdieva et al., 2019). The date palm tree development and their creation are likewise experienced the exorbitant measures of salts that have collected in soil because of anthropogenic exercises, for example, over-water system utilizing underground saline water and the ascending of pungent water tables because of the evacuation of local vegetation (Gavrichkova et al., 2020). Soil saltiness

is a worldwide farming issue: around 20 % of developed grounds and 50 % of inundated zones are influenced by saltiness (Yaish, 2015). Specifically, high soil saltiness causes a serious misfortune in the amount and nature of yields. Notwithstanding the way that some date palm assortments can adjust to moderately high saltiness levels up to 12.8 dS(m-1) (Yaish, 2015).

1.4.2 Plant – Microbe interaction and their contribution in plant growth under stressful environment

Plants give a huge number of specialties to the development and multiplication of a variety of microorganisms, including bacteria, fungi and viruses (in general called holobiont). It has a developmental potential to manage biotic and abiotic stress than the plant itself. Plant-related microbiomes are found as endophytes inside the plant, as epiphytes appended on plant surfaces and in the close by the soil around the roots. These microorganisms can have useful, unbiased, or impeding consequences for plant wellbeing and advancement (Knief, 2014). Plant-related microbiomes give wellness preferences to the plant have, including development advancement, supplement take-up, stress resilience, and protection from microorganisms (Trivedi et al., 2020). Plant-related microorganisms (rhizo-microbes and endophytes) advantage the host by emphatically influencing paedogenesis and supplement accessibility, animating development, stifling illnesses, prompting abiotic stress resilience, and affecting harvest yield and quality (Cherif et al., 2015). In addition, they additionally upgrade plant wellbeing and execution under various pressure conditions (Kumar et al., 2020). Different salt tolerant microorganisms, preferring plant development have been disconnected from extraordinary soluble, saline, and sodic soils (Egamberdieva et al., 2019). Plant microbiomes present wellness focal points to the plant have, including development advancement, supplement take-up, stress resilience, and protection from microorganisms (Trivedi et al., 2020).

Several biotic and abiotic factors shape the bacterial communities of roots and encompassing soil.

Next generation sequencing (NGS) technologies have impressively accelerated research in biological science during the last years by enabling the production of large volumes of sequence data to a drastically lower price per base, compared to traditional sequencing methods. The ongoing developments in the 16S based metabarcoding studies allow addressing research questions in plant-microbial interaction and are increasing our knowledge about microbiota and their drivers, in globally collected samples (Thompson et al., 2017; Bahram et al., 2018).

Chapter 2

Chapter 2: Methods

2.1 Study site description and sample collection

The bulk soil (hereafter referred as soil) samples were collected across fourteen sites of date palm farms located in the oasis ecosystem of Al Ain, Abu Dhabi, UAE in March 2019 (Table 1).

Table 1: The geographical locations of soil sample collection locations (NS-Non–saline and S- Saline groundwater irrigation).

Location	Location name	Latitude	Longitude	Mean annual temperature (ºC)	Mean annual precipitation (mm)	Temperature of warmest quarter (ºC)
Town_ceter	NS1	24°12'58.20"N	55°45'9.80"E	27.7	75	32.8
Nahel_1	NS2	24°54'29.05" N	55°62'08.33"E	25.3	109	31.4
Nahel_2	NS3	24°52'01.5" N	55°65'89.06"E	25.3	109	31.4
Nahel_3	NS4	24°53'51.95" N	55°60'58.67" E	25.3	109	31.4
Al_rawda	NS5	24°06'03.9"N	55°32'08.6"E	27.8	75	33.0
Seah_sharkiya	NS6	24°12'12.66"N	55°48'53.40"E	27.7	75	32.8
Nabbagh	NS7	24°18'06.0"N	55°43'08.9"E	27.7	75	32.8
Sarooj	S1	24°12'08.0"N	55°47'18.1"E	27.7	75	32.8
Nahel_1	S2	24°52'02.13" N	55°64'43.09" E	25.3	109	31.4
Nahel_2	S3	24°52'25.56" N	55°65'45.12" E	25.3	109	31.4
Nahel_3	S4	24°52'02.13" N	55°64'43.09" E	25.3	109	31.4
Nahshala	S5	24°24'38.49" N	55°23'53.55"E	27.4	83	33.0
Seah_salem_east	S6	24°20'44.2"N	55°27'39.9"E	27.4	83	33.0
Seah_salem_west	S7	24°20'45.5"N	55°26'24.4"E	27.4	83	33.0

The sampling sites recorded mean annual rainfall of 75–109 mm and mean annual temperature of 25.3–27.8ºC based on past 50 years data (www.worldclim.org). The climate of sampling sites classified as "Bwh" (Subtropical Desert Climate) according to Koeppen climate classification. Soil samples were collected from two different types of irrigation water sources namely, non–saline freshwater (hereafter referred as non–saline water) and saline groundwater. The grouping of samples into non–saline water (<4 ds m^{-1}) or saline groundwater (>4 ds m^{-1}) categories was carried out based on previous studies (Chen et al., 2019; Zhang et al., 2016). At each site, three replicates of root–free soil samples were collected near date palms at a depth of approximately 20–30 cm. In total, 42 soil samples were collected (seven farms x two types of irrigation x three replicates per farm) for both chemistry and molecular analyses. Irrigation water samples for chemistry analyses were collected. The samples were transported to lab in cooled condition and soil samples meant for molecular analyses were stored at -20ºC until DNA isolation. Pictorial representation of workflow adapted in this study is given in Figure 1.

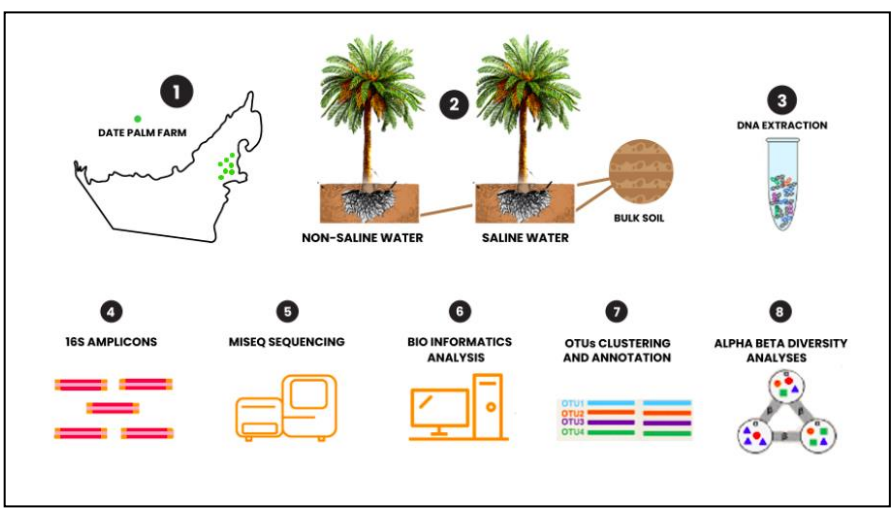

Figure 1: Pictorial representation of workflow adapted in this study.

1) Sampling Sites, 2) Treatments, 3) DNA Extraction, 4) 16s Amplicons 5) Miseq Sequencing 6) Bioinformatics Analysis 7) OTUs Clustering and Annotation 8) Alpha Beta Diversity Analysis.

2.2 Soil and water chemistry analyses

Soil samples were pulverized and passed through a 2 mm sieve in order to remove plant debris. One gram of fine soil was mixed thoroughly with 9 mL of milliQ water and homogenized for 1 hour at 200 rpm. This soil–water mixture was passed through Whatman filter paper and the filtrate was used for measuring soil chemistry (EC and pH). Soil organic matter (soil OM) of the samples were measured using loss on ignition method (Nelson, Sommers, 1996). Briefly, 5 g of air–dried soil was kept at 360°C for 4 hours and loss of mass after incubation was used for the calculation of soil OM.

2.3 Soil DNA isolation and Illumina sequencing

The DNA isolation was performed using E.Z.N.A soil DNA kit following manufacturer's protocol. In order to amplify the V3–V4 region of 16S rRNA I used 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) (Herlemann et al., 2011) primer combination. A 50 µl PCR reaction consisting of forward and reverse primers (1 µM each), 250 µM dTNPs (0.5 µM of each), 0.02 U Phusion High–Fidelity DNA Polymerase (Finnzymes OY, Espoo, Finland), 0.3 mg/mL BSA (Bovine Serum Albumin) and 5x Phusion HF buffer containing 1.5 mM $MgCl_2$ was set up. The applied PCR conditions consisted of initial denaturation at 95°C for 5 min, 25 repeating cycles of denaturation (95°C for 40 s), annealing (55°C for 30 s) and extension (72°C for 1 min), a final extension step (72°C for 7 min). DNA Normalization Kit (Charm Biotech) was used for the purification and normalization of PCR amplicons. The MiSeq (2X300 bp; paired–end) sequencing was performed at IMR lab, Halifax, Canada (https://www.imr.bio.com) following standard Illumina

protocol. The demultiplexed raw sequence data are archived at the Zenedo repository (10.5281/zenodo.6371857). Pictures of some steps of DNA extraction is given in Figure 2.

Figure 2: DNA extraction

2.4 Bioinformatics analyses

The raw sequence reads were analyzed using Divisive Amplicon Denoising Algorithm 2 (DADA2_v1.12) R package (Callahan et al., 2016). The forward and reverse primers present in the sequence data files (R1 and R2) were removed using rbind function of *SparkR* package. After primer removal, the sequences were processed for quality filtering (maxN = 0, truncQ = 2, maxEE = 2) and trimming (>275 bp for forward, >225 bp for reverse reads) using filterAndTrim function. Subsequently, the trimmed reads were processed for

error model generation (learnErrors), denoising (dada), merging (mergePairs), amplicon sequence variant (ASV) inference and chimera removal (removeBimeraDenovo) using respective functions of DADA2 package. Additional clustering of ASVs to operational taxonomic units (OTUs) at 97% sequence similarity using *vsearch* v2.15.1 (Rognes et al., 2016) were performed. After clustering, singleton and chimera screening were carried out using *vsearch*. The taxonomic assignment of OTUs was carried using Silva database v138.1 (downloaded from https://zenodo.org/record/4587955) using assignTaxonomy function (Quast et al., 2012) of DADA2, which is based on naïve Bayesian classifier, with minBoot=80. Non–bacterial OTUs belonging to archaea, eukaryotes, mitochondria, chloroplast, sequences unclassified at kingdom level and OTUs represented by less than 4 sequences were removed manually from OTU count table, which contain sample wise numerical data on the detection frequency of unique sequences (97% sequence similarity). Prior to alpha and beta diversity analyses, the OTU table was normalized to sample with lowest number of sequences (1770) using rrarefy function of R package *vegan* (Oksanen et al., 2020). The OTUs were classified as abundant (>1%), moderate (0.1–1%) and rare taxa (<0.1%) based on % occurrences according to a previous study (Dai et al., 2016).

2.5 Statistical analyses

All the statistical analyses in this study were performed using R v4.0.3 from R Core Development Team. Prior to the statistical analyses, the OTU count data of samples were arcsine–transformed to increase the homogeneity of variance. The water (pH and EC) and soil (pH, EC and soil OM) chemistry values were standardized to scale of 0–1 by Z transformation. Analyses of variance (ANOVA) test followed by Tukey's HSD post–hoc test was performed using *agricolae* package to test the differences of soil chemistry

(pH, EC and OM), water chemistry (pH and EC) and bacterial diversity (bacterial richness, Shannon diversity index and evenness) between irrigation water sources (non–saline water and saline groundwater irrigation). Bacterial OTUs with 0.02% relative abundance in at least 80% occurrence (28 out of 35 samples) were defined as core taxa (Gschwend et al., 2022) using microbiomenalyst web platform (www.microbiomenalyst.ca). The indicator species analysis was performed using multiplatt function of *indicspecies* R package and indVal (>0.5) with $P<0.05$ were obtained for the prediction of indicator species in soil while non–saline water and saline groundwater irrigation. To understand the effect of environmental variables on bacterial community structuring patterns between irrigation water sources (non–saline water and saline groundwater irrigation), two dimensional non–metric multidimensional scaling (NMDS) analyses based on Bray–Curtis dissimilarities was carried out using metaMDS function of *vegan* package (Oksanen et al., 2020). NMDS analyses was performed with the following settings: dimensions (k) = 2; maximum iterations = 1000; initial configurations = 100; minimum stress improvement in each iteration cycle = 10^{-5} in order to find a stable solution with minimum stress values. The vectors respective to environmental factors ($P<0.05$) and centroids representing irrigation water sources (non–saline vs saline groundwater irrigation) were fitted to NMDS ordination plot using envfit function and 95% confidence intervals (CI) of the plots generated using ordiellipse function of *vegan* package (Oksanen et al., 2020). To test the differences between bacterial communities of irrigation water sources, permutational analysis were carried out of variance (PERMANOVA) using adonis function of *vegan* package (Oksanen et al., 2020), in which pseudo–F statistics was carried out by computing 9999 permutations of dissimilarity matrices. A forward selection procedure was used to optimise the final model for PERMANOVA analyses

(Blanchet et al., 2008). Initially, single factor models were performed and in the next step, factors were ranked based on their R^2 values in the final model.

Chapter 3

Chapter 3: Results

3.1 Soil and water chemistry analyses

Water chemistry (EC and pH) and soil OM were significantly different between irrigation water sources (non–saline vs saline groundwater irrigation) ($P<0.05$) (Figure 3a & b). Saline groundwater showed higher EC and decreased water pH while non–saline groundwater showed opposite pattern (Figure 3a & b). The irrigation water salinity ranged from 0.33–28 ds m^{-1}, while water pH differed between 6.96–7.99 (Figure 3a & b). The soil OM while saline groundwater irrigation was lower compared to non–saline groundwater irrigation ($P<0.05$) within the range of 1.17–6.28% (Figure 3c)

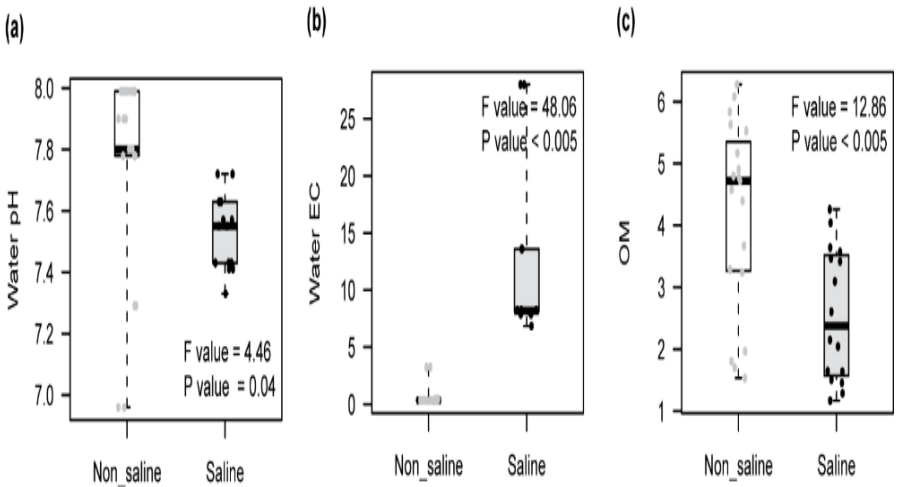

Figure 3: Soil and water chemistry of soil between irrigation water sources (non–saline water and saline groundwater irrigation).

The box plots of (a) water pH; (b) water electrical conductivity (EC in m/S); (c) soil organic matter (OM%) under different irrigation water sources (non–saline vs saline groundwater irrigation). The P values of ANOVA followed by Tukey's HSD post hoc test is given within each panel. The box spans the

interquartile range (IQR; first quartile to the third) with the median indicated by a dark horizontal line, the whiskers show the 1.5xIQR. Data for each sample is also displayed with strip chart.

3.2 Sequence data statistics

Total of 907,507 raw sequence reads were generated after sequencing and 517,217 reads passed the strict quality threshold. The high–quality reads were clustered into 7184 non–chimeric OTUs. Of total OTUs, 35 archaeal (185 reads), 7 chloroplast (3861 reads), 4 mitochondrial (346 reads), 1 non–bacterial (2 reads), 1 unclassified_kingdom OTU (5 reads) and 1980 OTUs with <5 reads (5702 reads) were removed. The final OTUs table contained 5155 OTUs (507,111 reads) from 42 samples (range 1770:47,721 reads per sample).

3.3 Irrigation water influence on soil bacterial diversity and communities

Soil bacterial diversity (richness, Shannon diversity index and evenness) parameters were not significantly different while non–saline water and saline groundwater irrigation (Figure 4).

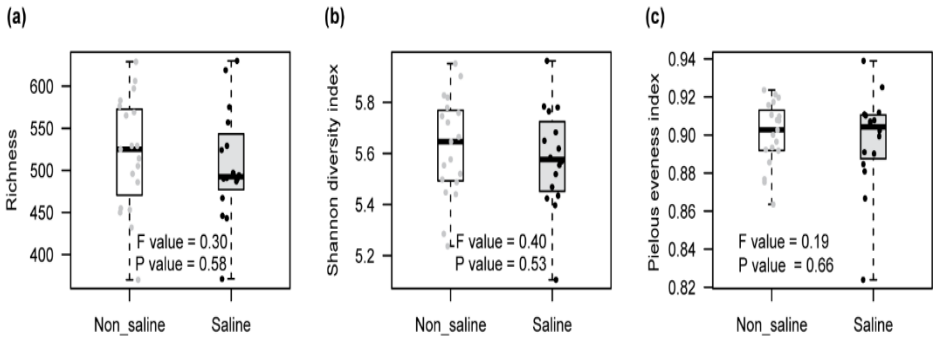

Figure 4: The bacterial diversity metrics of soil while non–saline water and saline groundwater irrigation. (a) Richness, (b) Shannon diversity index and (c) Pielous evenness index

Rarefaction curves of soil bacteria did not reach plateau for both types of soil samples while non–saline water and saline groundwater irrigation (Figure 5a). Out of 5155 OTUs, 21.3% of OTUs detected only in soil while saline groundwater compared to 31.5% OTUs while non–saline groundwater irrigation, while 47.15% of OTUs were commonly shared between both soils (Figure 5b).

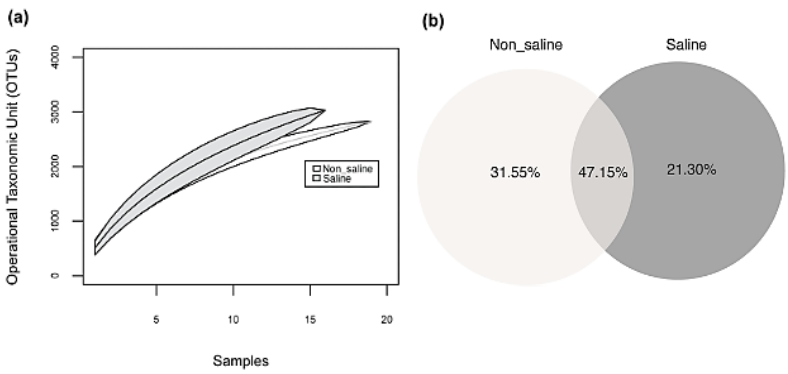

Figure 5: The species accumulation curves, unique and shared bacterial OTU analysis.

(a) Operational taxonomic unit (OTU) accumulation curves at 97% sequence similarity and (b) shared and unique OTUs of date palm-associated soil between irrigation sources (non–saline vs saline groundwater irrigation). The unique and shared OTUs are expressed as percentages of total OTUs (5155).

Soil bacterial communities were significantly different between irrigation water sources (non–saline vs saline groundwater irrigation) based on multivariate (PERMANOVA and NMDS ordination) analyses. The Bray–Curtis dissimilarities were lesser in soil while non–saline water irrigation compared to saline groundwater irrigation and distinct clusters representing irrigation water sources were observed in NMDS ordination space (R^2 = 0.1503, P = 0.013) (Figure 6a). Furthermore, the final model of PERMANOVA analyses obtained through forward selection procedure showed that out of five factors tested (soil pH, soil EC, water pH, water EC and soil OM), only irrigation water EC (R^2=0.1825, P=0.043) significantly affected bacterial community structural patterns.

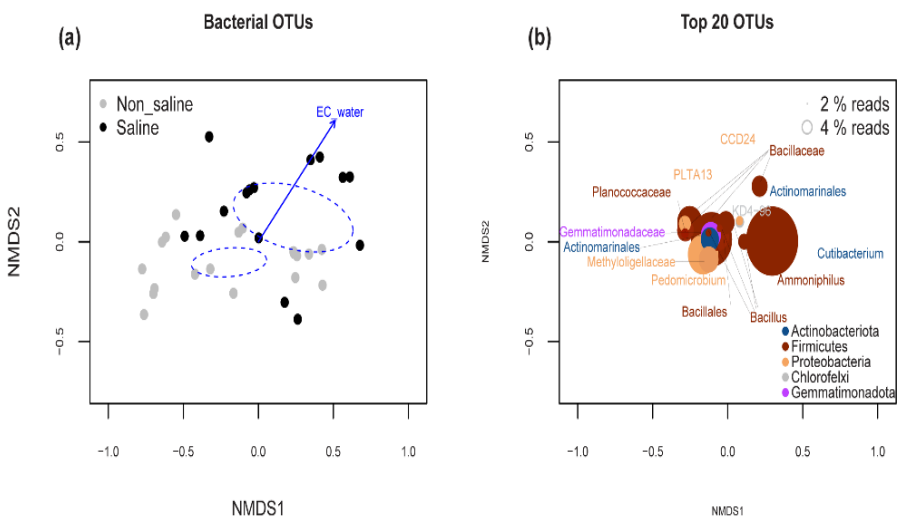

Figure 6: Non-metric multidimensional scaling (NMDS) ordination analysis of soil bacterial communities while non–saline water and saline groundwater irrigation.

(a) The ordination plot was generated based on OTU abundances of soil while non–saline and saline groundwater samples. The colours are (a) coded according irrigation water source (non–saline vs saline groundwater irrigation) and (b) coded according to bacterial phyla. 95% ellipse represent confidence interval for the tested factor variable (i.e., irrigation water source) and direction and length point increasing influence of the significant variable ($P<0.05$) on the ordination configuration. (b) Species plots of top 20 bacterial taxa based on total OTUs composition, and the size of the circles indicate relative abundance of the OTUs.

3.4 Irrigation water effect on soil bacterial composition

Actinobacteriota (24.4%), *Firmicutes* (23.2%), *Proteobacteria* (22.8%), *Chloroflexi* (11%), *Acidobacteriota* (9.1%), *Gemmatimonadota* (3.5%), *Methylomirabilota* (1.48%) and *Planctomycetota* (1.7%) were the abundant phyla (>1% abundance) in soil while non–saline groundwater irrigation, while *Proteobacteria* (27.9%), *Actinobacteriota* (23.01%), *Firmicutes* (21.9%), *Chloroflexi* (10%), *Acidobacteriota* (9.4%), G*emmatimonadota* (3.7%), *Planctomycetota* (2%) and *Methylomirabilota* (1.3%) were abundant (>1% abundance) phyla in soil while saline groundwater irrigation (Figure 7a & Table 2). Among these phyla, the relative abundances of *Chloroflexi, Acidobacteriota, Gemmatimonadota, Methylomirabilota* and *Planctomycetota* were unchanged between irrigation water sources (Figure 7a & Table 2).

Bacilli (17.7%, 21.9%) was enriched in soil while non–saline water irrigation, while Proteobacterial classes Gamma (8%, 5.5%) and Alphaproteobacteria (17.6%, 16%) were enriched in soil while saline water irrigation. The soil samples consisted of the following orders, Bacillales, Rhizobiales, Actinomarinales, Vicinamibacterales, Paenibacillales, Tistrellales, Gaiellales, Microtrichales, Gemmatimonadales, Burkholderiales,

Thermomicrobiales and Rokubacteriales as top taxa at varied abundances while non–saline water and saline groundwater irrigation (Figure 7b & Table 2). The list of bacterial taxa at phylum (>1% of total reads) and order (>0.5% of total reads) level is given in Table 2.

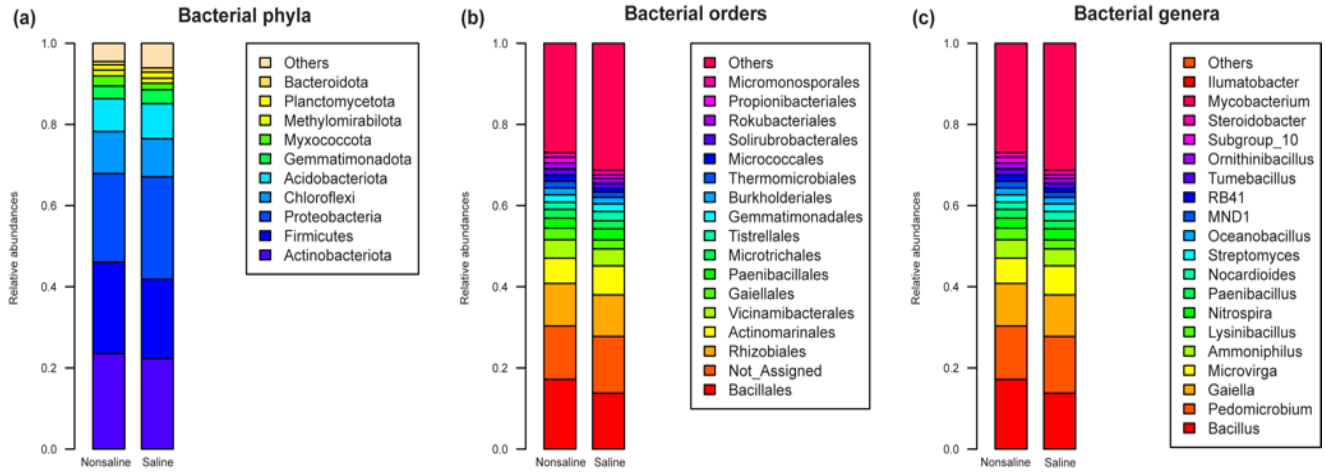

Figure 7: The relative abundance of soil bacteria at (a) phylum and (b) order and (c) genus levels. Total % reads and % occurrences from overall dataset and subset are shown here. Phylum with >0.5% reads and order with >1.0% of total reads are displayed here. *Occurrence (%) calculated from total 35 samples; #Occurrence (%) calculated from total 19 non–saline samples; $Occurrence (%) calculated from total 16 saline samples].

Table 2: Taxonomic (phylum and order level) composition of the soil bacterial community while non–saline water and saline groundwater irrigation.

Taxonomy	Overall dataset		Non–saline		Saline	
	Reads (%)	Occurrences (%)	Reads (%)	Occurrences (%)	Reads (%)	Occurrences (%)
Acidobacteriota	9.24	100	9.10	100	9.41	100
NA	0.64	100	0.61	100	0.66	100
Pyrinomonadales	0.54	91.43	0.57	94.74	0.50	87.50
Thermoanaerobaculales	0.91	100	0.69	100	1.18	100
Vicinamibacterales	4.66	100	4.83	100	4.45	100
Actinobacteriota	23.73	100	24.34	100	23.01	100
Actinomarinales	6.56	100	6.31	100	6.86	100
Corynebacteriales	0.80	100	0.71	100	0.91	100
Gaiellales	2.79	100	3.03	100	2.51	100
IMCC26256	0.64	100	0.67	100	0.60	100
Micrococcales	1.27	100	1.51	100	0.99	100
Micromonosporales	1.27	100	1.30	100	1.24	100
Microtrichales	2.16	100	2.23	100	2.08	100
NA	2.73	100	2.66	100	2.81	100
Propionibacteriales	1.15	100	1.39	100	0.86	100
Pseudonocardiales	0.61	100	0.63	100	0.58	100
Solirubrobacterales	1.47	100	1.60	100	1.31	100
Streptomycetales	0.65	100	0.66	100	0.63	100
Chloroflexi	10.63	100	11	100	10.19	100
Ardenticatenales	0.69	100	0.48	100	0.94	100
Caldilineales	0.74	97.14	0.59	100	0.91	93.75
NA	4.49	100	4.94	100	3.96	100
S085	0.82	100	0.84	100	0.80	100
SBR1031	0.99	100	0.93	100	1.05	100
Thermomicrobiales	1.71	100	1.87	100	1.52	100
Firmicutes	21.95	100	23.25	100	20.41	100
Bacillales	15.91	100	17.41	100	14.14	100
Paenibacillales	2.64	100	2.51	100	2.81	100

Table 2: Taxonomic (phylum and order level) composition of the soil bacterial community while non–saline water and saline groundwater irrigation (Continued)

Taxonomy	Overall dataset		Non–saline		Saline	
	Reads (%)	Occurrences (%)	Reads (%)	Occurrences (%)	Reads (%)	Occurrences (%)
Peptostreptococcales-Tissierellales	0.84	97.14	1.14	100	0.49	93.75
Thermoactinomycetales	0.57	100	0.47	100	0.67	100
Gemmatimonadota	3.66	100	3.58	100	3.76	100
Gemmatimonadales	1.98	100	2.06	100	1.89	100
NA	1.61	100	1.44	100	1.82	100
Methylomirabilota	1.39	100	1.48	100	1.29	100
Rokubacteriales	1.39	100	1.48	100	1.29	100
Myxococcota	2.34	100	2.63	100	2	100
Polyangiales	0.87	100	0.97	100	0.75	100
Planctomycetota	1.87	100	1.74	100	2.02	100
Pirellulales	0.92	100	0.83	100	1.03	100
Proteobacteria	25.18	100	22.88	100	27.91	100
Burkholderiales	1.80	97.14	1.72	100	1.90	93.75
Caulobacterales	0.65	100	0.53	100	0.78	100
CCD24	0.87	97.14	0.89	100	0.84	93.75
NA	1.97	100	1.58	100	2.43	100
PLTA13	1.07	97.14	0.91	100	1.25	93.75
Pseudomonadales	0.73	97.14	0.37	100	1.17	93.75
Rhizobiales	10.88	100	10.83	100	10.93	100
Rhodobacterales	0.95	100	0.62	100	1.35	100
Sphingomonadales	0.55	100	0.50	100	0.61	100
Steroidobacterales	0.90	100	0.85	100	0.96	100
Tistrellales	2	100	1.82	100	2.21	100

Bacillus, *Pedomicrobium* and *Gaiella* were the top genera in soil samples while both types of irrigation water sources (non–saline water and saline groundwater irrigation) (Figure 7c & Table 3). *Microvirga*, *Ammoniphilus*, *Nitrospira* and *Lysinibacillus* were highly occurring in soil while non–saline groundwater irrigation (Figure 7c & Table 3). Similarly, *Subgroup_10*, *Nitrospira* and *Mycobacterium* were the top genera in soil collected from saline groundwater irrigation (Figure 7c & Table 3).

Total reads (%) and occurrences among samples were calculated for overall database, non–saline sample and saline samples subset. [**Abbreviation (A), (P), (F), (G) represent bacterial phyla Actinobacteria, Proteobacteria, Firmicutes and Gemmatimonadota respectively; † indicate core taxa with 0.02% reads across at least 28 samples (80%); *Occurrence (%) calculated from total 35 samples; $^{\#}$Occurrence (%) calculated from total 19 non–saline samples; $^{\$}$Occurrence (%) calculated from total 16 saline samples].

Table 3: Taxonomic affinity, read abundance and occurrences of the 20 most abundant operational taxonomic units (OTUs) detected in soil while non–saline water and saline groundwater irrigation.

OTU ID	Genus (Phylum) **	Overall		Non–saline		Saline	
		Reads (%)	Occurances (%)*	Reads (%)	Occurances (%)#	Reads (%)	Occurances (%)$
OTU3†	Actinomarinales_ unclassified (A)	2.54	94.29	2.67	89.47	2.42	100
OTU5	Ammoniphilus (F)	1.91	100	1.39	100	2.55	100
OTU8	Bacillales_ unclassified (F)	1.42	100	1.75	100	1.24	100
OTU1	Bacillaceae_ unclassified (F)	1.15	85.71	0.64	89.47	1.72	81.25
OTU1633†	Bacillus (F)	1.16	100	0.70	100	0.46	100
OTU14†	Pedomicrobium (P)	1.02	97.14	1	100	1.08	93.75
OTU15	Methyloligellaceae_ unclassified (P)	0.95	100	0.93	100	1.01	100
OTU37	Bacillus (F)	0.96	100	0.45	100	0.51	100
OTU6	Bacillaceae_ unclassified (F)	0.89	100	1.03	100	0.88	100
OTU66	Actinomarinales_ unclasssified (A)	0.93	97.14	0.56	100	0.37	93.75
OTU16	Bacillus (F)	0.86	100	0.83	100	0.92	100
OTU17	PLTA13 (P)	0.83	97.14	0.74	100	0.92	93.75
OTU9	KD4-96_unclassified (C)	0.78	97.14	0.84	100	0.79	93.75
OTU7	Bacillus (F)	0.78	74.29	0.10	68.42	1.38	81.25
OTU24	Gemmatimonadaceae _unclassified (G)	0.71	97.14	0.36	100	0.35	93.75
OTU27	Bacillus (F)	0.60	100	0.29	100	0.31	100

Table 3: Taxonomic affinity, read abundance and occurrences of the 20 most abundant operational taxonomic units (OTUs) detected in soil while non–saline water and saline groundwater irrigation (Continued).

OTU ID	Genus (Phylum) **	Overall		Non–saline		Saline	
		Reads (%)	Occurances (%)*	Reads (%)	Occurances (%)#	Reads (%)	Occurances (%)$
OTU 25	MB-A2-108_ unclassified (A)	0.45	97.14	0.24	100	0.21	93.75
OTU 10	Planococcaceae_ unclassified (F)	0.53	80	0.24	73.68	0.89	87.50
OTU 11	Bacillaceae_ unclassified (F)	0.57	100	0.46	100	0.69	100
OTU 51	CCD24_ unclassified (P)	0.54	97.14	0.28	100	0.26	93.75

Statistical analysis using ANOVA showed significant enhancement ($P<0.05$) of *Microvirga*, *Marmoricola*, *Domibacillus*, *Oceanobacillus*, *Bhargavaea* and *Solirubrobacter* in soil while non–saline groundwater irrigation, whereas *Novibacillus* and *Bauldea* abundance was significantly increased ($P<0.05$) while saline groundwater irrigation (Figure 8). The proportions of these significantly differing taxa detected in soil while both types of irrigation water sources were at moderate level (<0.1 to 1% abundance).

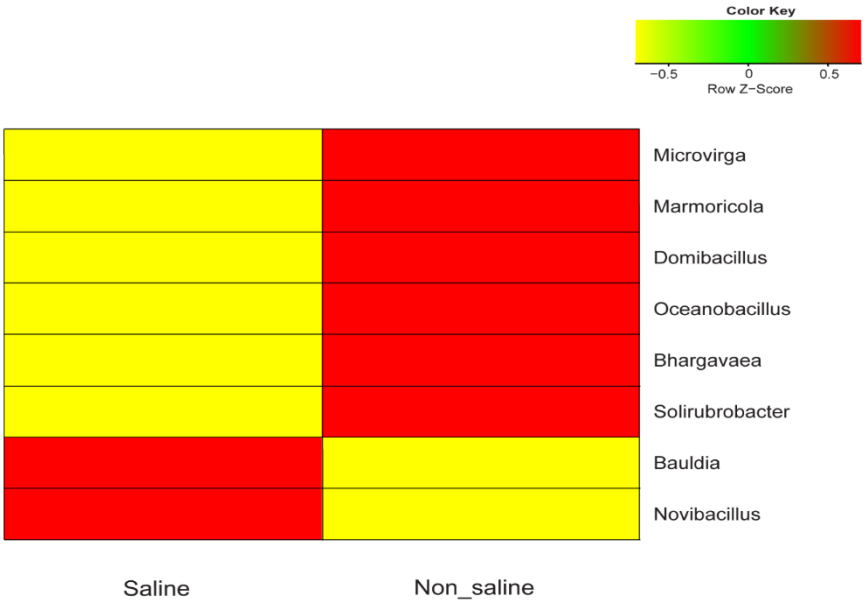

Figure 8: Heat-plot of bacterial proportional abundances in soil while non–saline water and saline groundwater irrigation.

The figure shows hierarchical clustering of significant OTUs ($P<0.05$) of soil while saline vs non–saline groundwater irrigation. The color key of the legend indicates the median-centered Z-scores values, which were calculated after normalizing relative abundance values of selected genera.

The core taxa detected in the study were Actinomarinales_unclassified, *Bacillus* and *Pedomicrobium* in soil while both types of irrigation water sources (Table 3). Six indicator taxa specific for soil irrigated with non–saline water and two saline groundwater irrigation were detected in this study. The top most indicator taxa with highest indVal in soil irrigated with non–saline groundwater irrigation were *Solirubrobacter* and *Sorangium*, whereas for the soil irrigated with non–saline water irrigation was *Mycobacterium* and *Steroidobacter* (Table 4).

Table 4: Indicator species analyses representing indicator Operational Taxonomic Unit (OTUs).

Non–saline	Genus	IndVal	P value
OTU_645	Solirubrobacter	0.668	0.0001
OTU_1061	Sorangium	0.608	0.0003
OTU_346	Geminicoccus	0.589	0.0001
OTU_145	AKYG1722_unclassified	0.576	0.0002
OTU_637	Lysobacter	0.522	0.0005
OTU_576	67-14_unclassified	0.51	0.0018
Saline	Genus	IndVal	P value
OTU_1282	Mycobacterium	0.536	0.0002
OTU_33	Steroidobacter	0.506	0.0003

Chapter 4

Chapter 4: Discussion

In this study, soil samples were examined from arid agroecosystems receiving two different irrigation water sources (non–saline water and saline groundwater irrigation) to examine the relationship of soil bacterial diversity and communities due to salinity effect. The soil bacterial diversity did not change, but the percentage of OTUs colonizing soil irrigated with saline water irrigation showed decreased percentage of unique OTUs compared to non–saline groundwater irrigation. Our results have shown occurrence of distinct soil bacterial communities while under non–saline water and saline groundwater irrigation, which was affected significantly by irrigation water salinity. In addition, saline groundwater irrigation selectively enriched specific soil bacterial taxa (Subgroup_10 genus, *Mycobacterium* and *Steroidobacter*), which are potentially involved in fatty acid and starch biosynthesis indicating their possible salinity tolerant mechanisms in arid agroecosystems.

4.1 Soil and water chemistry changes between irrigation sources

The water chemistry (EC and pH) and soil organic matter (SOM) were significantly different between irrigation water sources. Higher EC of saline groundwater may be attributed to increased sodium and chloride concentrations of groundwater used for irrigation, since previous studies reported sodium and chloride as major ions of saline groundwater (Egamberdieva et al., 2010; Khan et al., 2019). While the pH values of irrigation water samples used in this study were neutral to slightly alkaline, but a previous study from nearby region reported acidic to neutral pH for saline groundwater (Khan et al., 2019). The reason for the slightly alkaline characteristic of irrigation water samples could be due to interaction of water with soil and possible release of calcium by lime dissolution in irrigation

channels, since alkaline water pH is linked to calcium levels of irrigation water (Zaman et al., 2018). Soil OM in agricultural soil is composed of partial as well as fully decomposed organic matter derived from litter (leaf and root). In this study, soil OM was significantly lower while saline water irrigation compared to non–saline water irrigation. This finding is similar to a previous study, which showed a decrease in soil organic carbon under increasing soil salinities (Wong et al., 2008). Saline water percolation through soil layers reported to decrease soil aggregate formation resulting in loss of soil organic carbon (Ju et al., 2019; Trivedi et al., 2017; Yu et al., 2021).

4.2 Soil does not alter bacterial diversity but unique OTUs between irrigation sources

The bacterial diversity (richness, Shannon diversity and evenness) of the soil was not significantly different while non–saline water and saline groundwater irrigation, while the bacterial OTU numbers change depending on irrigation water source. The decreased proportions of unique OTUs while saline groundwater irrigation indicate only a smaller number of bacteria that are salinity–tolerant are recruited in soil compared to non–saline water irrigation. Similar decrease in bacterial OTUs in saline soil compared to non–saline soil was observed by a previous study as well (Li et al., 2021). Apart from salinity effect, I cannot rule out the selection pressure exerted by roots (i.e, root exudates and rhizodeposits) as well since reports suggest that root influence possibly extend beyond rhizosphere, therefore allowing colonization of certain bacteria in soil (Bakker et al., 2015; Moroenyane et al., 2018).

4.3 Soil bacterial communities structured by irrigation water salinity

Higher salinities of irrigation water may cause osmotic imbalance in soil, which makes soil bacteria to become dormant or lysed due to plasmolysis, depending on salinity tolerance levels, possibly affecting structure of soil bacterial communities. The structuring of bacterial communities was reported

to be dependent on salt concentrations (0–22 mg NaCl g^{-1} of soil) (Rath et al., 2017). Our results shown that soil bacterial communities were indeed structured according to irrigation water sources (non–saline water and saline groundwater irrigation) into distinct clusters and irrigation water EC (salinity) was the major structuring factor. Our results are in line with the previous studies on naturally saline soils (Guo et al., 2021; Li et al., 2021; Nan et al., 2022) and soil while saline groundwater irrigation (Chen et al., 2017; Chen et al., 2019). However, studies focusing on irrigation water source (secondary salinization) covered only a narrow range of salinities (1.09–8.41 dS m^{-1}) from a single location. Surprisingly, a study on soil from spinach field did not show any effect on bacterial communities at the salinity range of 0.85–15 dS m^{-1} (Mark et al., 2017).

Consistent bacterial community structuring depending on irrigation water sources (non–saline water and saline groundwater) as observed despite covering multiple geographical locations and wide range of salinities (0.33–28 ds m^{-1}), therefore accounting for patchiness in bacterial communities due to spatial variability. It is suggested that soil transiently select bacterial communities through deterministic 'salinity filtering' process, wherein salt–tolerant species possibly replaced less salt–tolerant members via species sorting while in contact with saline groundwater. In addition, the soil samples while saline groundwater irrigation were heterogeneously clustered compared to soil while non–saline water irrigation indicating higher within sample variation of bacterial communities while soil saline groundwater irrigation than non–saline water irrigation, which perhaps select subset of bacterial communities among soil while saline groundwater irrigation.

4.4 Taxa compositional variations according to irrigation sources

Proteobacteria and its classes Gamma– and Alphaproteobacteria were the most abundant phylum in this study while saline groundwater irrigation,

agreeing with previous studies in desert soil (Chen et al., 2017; Chen et al., 2019; Nan et al., 2022). Proteobacteria consist of rapidly multiplying bacteria capable of growing under extreme temperature and nutrient limited conditions (Chen et al., 2019; Zhang et al., 2019). At genera level, saline groundwater irrigation enriched *Microvirga*, a free–living nitrogen–fixer belonging to Alphaproteobacteria isolated earlier from desert soil (Amin et al., 2016) pointing their role in nitrogen cycle. Firmicutes (*Bacillus*, *Lysinibacillus, Domibacillus* and *Oceanibacillus*) and Actinobacteria (*Gaiella*, *Ammoniphilus* and *Mycobacterium*) members were also enriched while saline groundwater irrigation, which are known to withstand saline and dry conditions of desert environment by producing endospores, extracellular polysaccharides and also involved in organic matter degradation. *Mycobacterium,* an indicator taxon for saline water irrigation in this study was earlier isolated from rhizosphere of plants are known to be actively involved in plant growth promotion (Karmakar et al., 2021) and also capable of withstanding salinity–stress (Asmar et al., 2016).

Similarly, *Ammoniphilus,* an ammonia oxidising bacterium, whose abundance was increased in soil while saline groundwater irrigation points similar role in this study. Subgroup_10 genus (Acidobacteriota) abundance was increased in soil while saline water irrigation, whose members reported to accumulate starch indicating its possible role as osmoprotectant against salinity stress (Kristensen et al., 2021). Non–saline water irrigation increased Actinobacteria phylum, which has shown sensitivity to salinities in a previous study (Li et al., 2021). *Solirubrobacter,* an indicator taxon recorded for non–saline water irrigation in this study is in line with a previous study, which identified it as ‘keystone species’ for agricultural soils (Banerjee et al., 2018).

Core taxa (*Bacillus* and *Pedomicrobium*) detected in this study indicated their common role across samples irrespective of irrigation water source indicating

versatile nature of the bacteria. *Bacillus* is known to produce endospores under hot and saline conditions, secrete EPS, form biofilm and enhance soil aggregation (Marvasi et al., 2010). The other Bacillales members (*Bacillus*, *Lysinibacillus*, *Domibacillus*, *Oceanibacillus*) detected in this study also known to perform similar role (Marvasi et al., 2010). Plant growth promoting rhizobacteria (PGPR) members detected while non–saline water (*Bacillus*, *Lysinibacillus*, *Domibacillus*, *Oceanibacillus* and *Marmoricola*) and saline groundwater (*Novibacillus*) (Mandic-Mulec et al., 2015; Martínez et al., 2018; Mukhtar et al., 2021) irrigation indicate that soil may be serving as a base for bacterial recruitment in the rhizosphere of date palms. Another core taxa *Pedomicrobium*, a biofilm dwelling iron-oxidizing bacterium (Cox & Sly, 1997), whose enrichment indicate its role in iron oxidation across samples.

Chapter 5

Chapter 5: Conclusion

I showed that soil selectively allow colonization of specific set of bacterial communities between irrigation water sources (non–saline water vs saline irrigation water) at wider geographical distribution and salinity ranges due to 'salinity filtering'. Saline groundwater did not alter soil bacterial diversity but decreased the number of unique OTUs possibly due to osmotic stress. In addition, saline groundwater irrigation selected specific soil bacterial taxa (*Mycobacterium*, Subgroup_10 and *Ammoniphilus*).

Presence of several PGPRs in soil under both irrigation sources indicated that soil may serve as a selection source for rhizosphere recruitment in the neighbouring date palm. In summary, the findings of this study show that soil select specific bacterial taxa and communities under different irrigation water sources (non–saline water and saline irrigation water) in soil, which is vital for the sustainable land use, crop production and rehabilitation.

However, one of the limitations we had in this study, was lack of previous studies in the metabarcoding area related to high salinity where the EC was 6.85 - 28 ds m-1. Second, limited accuracy for the samples, 42 samples were collected but only 35 samples were high quality after filtering and bioinformatic process. If sample size is more, statistical tests would be able to identify significant interactions within data set.

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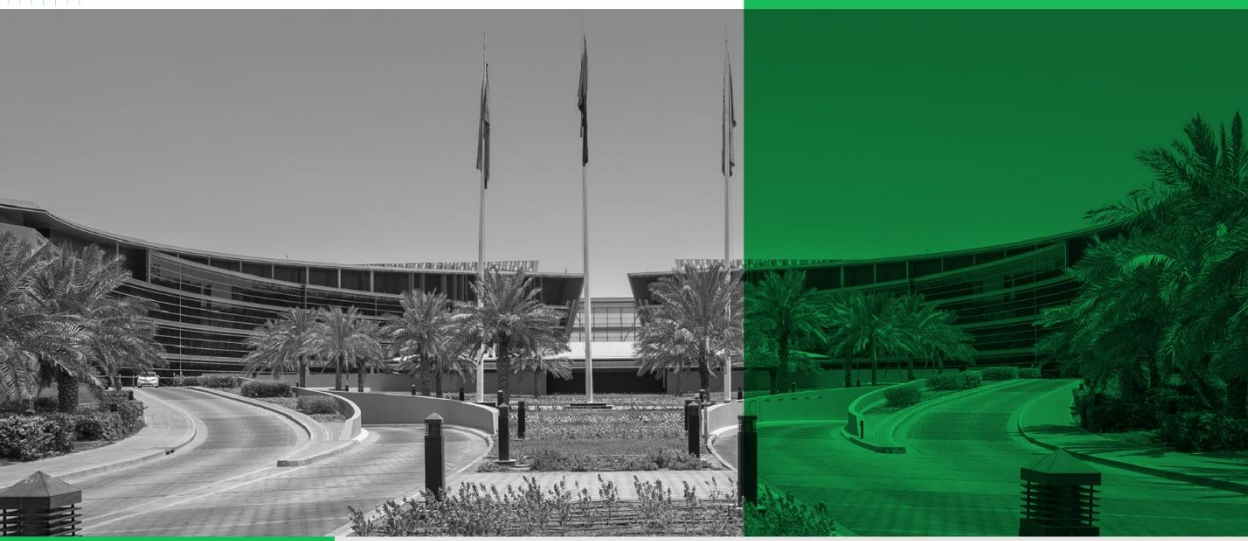

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Belowground living beings play basic parts in keeping up numerous biological system forms, counting plant efficiency. The overarching goal of the project is to investigate the role of date palm associated bacterial community composition and their functions in alleviating salinity stress for their host plant for successful establishment and fitness success. In this study, I am spotting the light on the palm tree associated microbiome that makes the tree tolerate and resist under saline stress. Plan it to employ high-throughput sequencing based 16S rRNA gene amplicon sequencing to investigate microbial communities.

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