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Microbial communities and their predictive functional profiles in arid soil of Saudi Arabia

Munawwar A. Khan[1], Shams T. Khan[2]

[1]Department of Life and Environmental Sciences, College of Natural and Health Sciences, Zayed University, Dubai, 19282, UAE
[2]Department of Agricultural Microbiology, Faculty of Agricultural Science, Aligarh Muslim University, Aligarh, U.P, India

Correspondence to: Shams T. Khan (shamsalig75@gmail.com)

Abstract. Saudi Arabia has world's fifth largest desert and is the biggest importer of food and agricultural products. Understanding soil microbial communities is key to improving agricultural potential of the region. Therefore, soil microbial communities of semi-arid region of Abha known for agriculture and arid regions of Hafr Al-Batin and Muzahmiyah were studied using Illumina sequencing. Microbial community composition varied remarkably from other deserts and from one place to another. Highest diversity was found in rhizospheric soil of Muzahmiyah followed by Abha. Firmicutes, Proteobacteria and Actinobacteria were three main phyla detected in all the samples. Unlike other deserts, Bacteroidetes was not a major constituent and population of Firmicutes was quite high. Soils from agricultural region of Abha were significantly different from other samples in containing only 1% Firmicutes and three to six times higher population of Actinobacteria and Bacteroidetes, respectively. Presence of photosynthetic bacteria, ammonia oxidizers, and nitrogen fixers along with bacteria capable of surviving on simple and unlikely carbon sources like DMF was indicative of their survival strategies under harsh environmental condition. Functional inference using PICRUSt show abundance of genes involved in photosynthesis and nitrogen fixation. Microbial communities show greater similarity with hot Namib desert than with cold Antarctic desert.

Keywords: Saudi Arabian Desert; soil microbiome; arid soil; Actinobacteria; PICRUSt

1 Introduction

Saudi Arabian desert also referred to as Sahara Arabian desert is the fifth largest desert of the world bordering with Yemen, Persian Gulf, and Iraq (Holm, 1960). The desert is characterized by the presence of vast barren areas of sand referred to as empty quarters or Rub-al Khali and Wadi al-Batin. Most of the area is barren with almost no vegetation and the growing population is dependent on imported agricultural products for food (Fiaz et al., 2016). According to the government of USA, Saudi Arabia imports 14.8 billion US dollar worth of agricultural products every year (https://www.export.gov/apex/article2?id=Saudi-Arabia-Market-Overview). Although the climatic conditions are not favourable, but the Saudi government has launched various programs to promote agriculture. In fact, 52.7 million ha area, which is 25% of the total country's area is currently cultivable (Fiaz et al., 2016). Especially the Asir region, with Abha as its capital is well known for agriculture and receives more rain fall than the rest of the country.

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The microbial communities of Arid regions are largely uncharacterized, to the best of our knowledge no report from Saudi-Arabia is available (Makhalanyane et al., 2015;Schulze-Makuch et al., 2018). Although the vast desert lacks vegetation and therefore are expected to be devoid of macromolecules and the microbial communities involved in the recycling of the nutrients. However, active microbial communities have been detected even in hyper arid deserts of Atacama where the rain is received only once in a decade (Schulze-Makuch et al., 2018). Such studies are crucial in improving the agricultural potential in these extreme habitats. And to design strategies for the modification of soil with microbial consortia for improving the agricultural potential of the arid soil (Fierer et al., 2012;Fierer, 2017). These microorganisms may alter soil fertility through sustaining the soil nutrient cycling, carbon sequestration and by influencing other geochemical processes. Consequently, improving soil fertility and the agricultural potential of the soil. This study therefore was aimed at comparing the soil microbial communities of Abha from Asir region and from arid regions of Mazamiyah and Hafr Al-Batin. The knowledge of the microbial communities present in these regions may provide some insights into the role of microorganisms in various geochemical processes.

2 Materials and Methods

2.1 Soil Sample Collection

The desert soil samples were collected from three regions namely Abha of Asir region (semi-arid), and from the arid regions of Muzahmiyah (near Riyadh) and Hafr Al-Batin. Samples were collected between 26 Jan and 18 Feb, 2019. Figure 1 shows locations of the sampling sites and the climatic conditions at these sites. Each sample was a mixture of three samples from the upper 5 cm of soil. From Muzahmiyah two samples namely a rhizospheric soil and a non-rhizospheric soil sample was collected. Following collection, samples were transported to the lab at room temperature and were processed immediately after arriving to the lab. Portions of soils were stored at −20 °C for DNA extractions. Biogeochemical properties and other details of sampling sites are given in table 1. The pH, of the soil was determined by using a pH meter. While total aerobic counts in the soil were determined on 1/5th diluted nutrient agar plates using dilution plating method.

2.2 DNA extraction and HiSeq analysis

Genomic DNA from soil the soil was prepared using direct lysis method of Robe et al (Robe et al., 2003). For obtaining enough DNA, extraction was carried out in replicates and the DNA was pooled and concentrated. The composition and diversity of bacterial communities in soil were determined by amplifying the V3-V4 regions of bacterial 16S ribosomal RNA (rRNA) genes. A set of 341F and 806R primers and the DNA extracted from the samples as template were used for amplification. PCR reactions were carried out at 50 µl scale containing ~40 ng of DNA template, 25 µl Dream Taq Green PCR Master Mix (2×), 20.5 µl H2O, 0.5 µl of 1% bovine serum albumin, and 0.2 µM of each primer. The PCR was carried out by programming the thermal regime as initial denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s; and a final extension step at 72 °C for 7 min. Amplicon sequencing was

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conducted on an Illumina HiSeq 2500 platform. Further processing of the reads and quality filtering was conducted as described earlier (Yuan et al., 2018).

2.3 Bioinformatics processing

Raw data obtained from the sequencing was processed using QIIME (Caporaso et al., 2010). Sequences were clustered into operational taxonomic units using UCLUST and an identity threshold of 97%. Reads per sample varied between 68640 to 420570. For analysis, a total of 12,790 data points were included for each sample. Sequences were assigned to their phylogenetic groups using the QIIME pipeline and the green genes database ver. 13.5 (Santamaria et al., 2012). Further processing of the sequences was carried out using Calypso (Zakrzewski et al., 2017). Rarefaction curves were calculated for number of species present in each sample. Alpha diversity was determined using both taxonomic metrics (numbers of phylotypes). Principal coordinates analyses were conducted as input the pairwise distances between bacterial communities (Bray–Curtis and UniFrac distances calculated from the 16S rRNA gene amplicon data). To test whether sample categories harboured significantly different microbial communities, we used analysis of similarities (ANOSIM). To determine whether the relative abundances of individual taxa were significantly different between sample categories pairwise t tests with P values were calculated. The abundance of various taxa in the samples as correlation charts were also calculated using Calypso. While abundance pie charts were calculated using Krona (Ondov et al., 2013). Online program Galaxy was used for functional inference using PICRUSt and STAMP (statistical analysis of taxonomic and functional profiles) software v 2.1.3 was used for statistical analyses and to detect differentially abundant OTUs between two sample groups (Parks et al., 2014).
The sequences have been submitted to SRA with the accession numbers from SAMN12651127-12651133.

3 Results and discussions

3.1 Properties of soil samples

The details of the collected soil samples are given in table 1. The pH of all the soil samples was alkaline and ranged from 7.9 to 8.4. Abha located at high elevation near Yemen is a semi-arid region. The weather of the city's is generally mild throughout the year, and is especially cooler during the “low-sun” season. The annual average temperature of Abha is only 17.5 °C and seldom rises above 35 °C. The city receives an annual rain fall of about 230 mm most of which occurs between February and April. The soil type in Abha is sand and gravel and the pH of the soil sample was slightly alkaline i.e. 7.9. The total CFU counts obtained on nutrient agar medium was $3.63 \pm 1.9 \times 10^5$ CFU/g of soil. While, Muzahmiyah is an arid region with an average annual temperature of 25.3 °C, and in summer it may cross 45 °C. The area receives an annual precipitation of only 88 mm. The soil type in Muzahmiyah is reported to be sandy loam (Siham, 2007). Two samples were collected from Muzahmiyah the pH of both samples was alkaline (8.2). The CFU counts of the rhizospheric soil of *Haloxylon persicum* was ten times higher ($5.5 \pm 1.9 \times 10^5$ CFU/g of soil) than the non-rhizospheric ($1.1 \pm 0.9 \times 10^4$ CFU/g of soil) soil at a distance of a few meters. Hafr Al-Batin is also an arid region with an average annual rainfall of only 126 mm. The soil was alkaline with a pH of 8.4 and the aerobic bacterial count on nutrient agar was an $8.1 \pm 2.7 \times 10^4$ CFU/g of soil.

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3.2 Microbial diversity of soil samples

The alpha diversity in the samples was calculated using species as OTUs, rarefaction curves and Shanon diversity index show the highest diversity is associated with the samples collected from the rhizospheric soil (M15) of *Haloxylon persicum* collected from Muzahmiyah (Figure 2). While, microbial population was least diverse in sample collected from the non-rhizospheric soil from the same region (M5). It is to be noted that the diversity of rhizospheric soil from Muzahmiyah was comparable to the diversity observed in the samples collected from Abha, a region known for agriculture. Aerobic plate counts obtained from the two samples were also comparable. The species richness of Hafr Al-Batin (HB) was also comparable to the samples collected from Abha and Muzahmiyah although the aerobic plate count was as low as found in non-rhizospheric soil from Muzahmiyah (M5). R values of 0.85 obtained in ANOISM analysis show that the studied microbial communities are significantly different from each other (Figure 3 A)

Between 69-88% of the total reads were assigned to Bacteria and majority of the reads can be assigned to phylum Actinobacteria, Firmicutes and Proteobacteria. While, remaining reads were assigned to unknown groups detailed in supplementary figure 1. Populations of Firmicutes in samples collected from Muzahmiyah M15, M5, Hafr Al-batin and Abha were 50, 0.7, 13 and 1% of the total bacteria, respectively. While the population of proteobacteria were 25, 90, 31 and 53% in soil samples from Muzahmiyah M15, M5, Hafr Al-batin and Abha, respectively. Population of Actinobacteria was highest in soil from Abha (31% of the total bacteria). While, a comparable population of Actinobacteria in soils of Hafr Al-Batin (10%) and rhizospheric soil of Muzahmiyah (9%) were observed (Figure 3 B). Earlier reports show that the major soil bacteria found in desert soil belong to Actinobacteria, Bacteroidetes and Proteobacteria (Fierer et al., 2012;Andrew et al., 2012). Soil sample from Abha show almost the same pattern where the population of Proteobacteria, Actinobacteria and Bacteroidetes was 53, 31 and 6% of the total bacteria, respectively. While, other samples vary in not having the significant populations of Bacteroidetes and an increased population of Firmicutes (Figure 3 B). The high populations of Firmicutes and Actinobacteria in desert soil may be due to their ability to produce spores under high temperature and aridity. It is to be noted that temperatures do not cross 35 ºC in Abha which has the lowest population of Firmicutes. High population of actinobacteria has been found in both cold Antarctica desert as well as hot Namib desert (Aislabie et al., 2006;Armstrong et al., 2016). Interestingly, highest population of Actinobacteria (34% of the total bacteria) was observed in Abha which is Geographically close to Namib desert. Acidobacteria were only found in the rhizospheric soil of Muzahmiyah (2% of the total), while planctomycetes were found in the soils of Abha and Hafr Al-Batin. Among other minor phyla notably present in soil samples were the members of Phylum *Gemmatimonadetes*, like Gemm 3 in some cases constituting as much as 4% (Muzahimiyah rhizospheric soil) of the total reads. These bacteria have been reported earlier also in the desert soil and recently strains from the phylum with photosynthetic capability have been cultured (Meola et al., 2015;Zeng et al., 2014). The ammonia oxidizing Archaea Candidatus *Nitrososphaera gargensis* were also found in most of the samples. Reads belonging to iii115 constituted up to 3% of the total population at least in two samples (M15 and Hafr Al-Batin), these sequences has been reported from soil in earlier studies also

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(Marasco et al., 2018). The Antarctica desert soil survey show that Actinobacteria were present prominently along with *Bacillus* spp., *Flavobacterium* spp. and Acinetobacter spp. Deinococcus–Thermus and Gemmatimonadetes clades, which have low or no representation in other surface soils, are relatively common in Dry Valley clone libraries. Members of 14 phyla have been found in the soil of Antarctica desert including Actinobacteria, *Gemmatimonas*, *Proteobacteria*, *Bacteroidetes*, *Deinococcus* and *Thermus*, *Planctomycetes*, *Chloroflexi*, *Verrucomicrobia*, *Acidobacteria*, *Cyanobacteria*, TM7 and OP11. The most dominant were *Acidobacteria*, *Actinobacteria* and *Bacteroidete*s (Cary et al., 2010). In case of the hot Namib desert 19 different phyla were observed as shown in Figure 3B. The most abundant phyla were *Bacteriodetes*, *Proteobacteria* and *Actinobacteria* (Armstrong et al., 2016).

The relative abundance of major genus found in the soil samples is shown as heatmap in figure 4B. A detailed microbial community composition generated by Calypso program is shown in Supplementary figures 1-4. The Venn diagram (Figure 4A) shows that the core genera found in all the samples were 272, while Abha and Rhizospheric soil sample from Muzahmiyah (M15) shared maximum number of genera (300). While M15 and HB also shared 295 genera and Abha and HB shared 279 genera. Some of the most abundant genera include *Pseudomonas*, *Paenibacillus*, *Bacillus*, Candidatus *Nitrososphaera*, *Devosia*, *Adhaeribacter* and others. The bacterial genera found in desert soil are expected to withstand extreme climatic conditions and to perform some vital functions. For example, genus like *Bacillus* and *Paenibacillus* or Actinobacteria like *Nocardioides*, and *Streptomyces* are spore forming bacteria and hence can survive extreme heat and arid conditions. Many of these genera have been isolated from desert already. *Ramlibacter* one of the genus found in our samples forms cyst and its genome analysis shows adaptation to desert condition (De Luca et al., 2011). *Ramlibacter* was originally isolated from the meteorite fragments buried in the sands of a desert (De Luca et al., 2011). *Modestobacter* another dominant bacteria found in our samples was isolated from Atacama desert of Chile, South America (Busarakam et al., 2016). The genera like *Pseudomonas* and *Adhaeribacter*, may produce extracellular polysaccharide to survive under arid condition and to form strong biofilms or may also contribute to water retention in soil promoting formation of soil crust.

It is to be noted that some bacteria may play important role besides tolerating the extreme conditions. Candidatus *Nitrososphaera* and *Planctomyces* found in all the samples in high numbers is known to oxidize ammonia (Stieglmeier et al., 2014). It is important to note here that the desert soil is known to catalyzes ammonia formation (Schrauzer, 1978). Nitrite oxidizing bacteria *Nitrospira* were also found in all the samples but the reads were especially higher in Rhizospheric soil from Muzahimyah. The phototrophic bacterium *Rhodoplanes* present in all the samples may be one of the bacteria involved in carbon fixation in the nutrient deficient arid soil. Members of *Devosia* are known to nodulate *Neptunia* for nitrogen fixation and therefore, may serve as nitrogen source in otherwise nitrogen deficient arid soils. Members of the genus *Mesorhizobium*, *Bradyrhizobium*, and *Sinorhizobium* were also found in all the samples. While, the members of the genus Rhizobium were detected only in Rhizospheric soil from Muzahimiyah. Another genus found in significant numbers in all the soil samples and its population was especially high in Abha was *Balneimonas*. Which is the member of family *Bradyrhizobiaceae* and is known to produce extracellular material playing an important role in the formation of soil crust (Matthews et al., 2019).

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Abha soil sample was also distinct in having high populations of bacteria with the ability to survive on simple source of nutrients and extreme conditions or the ability to perform important geochemical or agricultural function. The population of genera *Adhaeribacter*, *Modestobacter*, *Ramlibacter*, radiation resistant *Geodermatophilus*, *Pseudonocardia*, and Flavobacterium was high in samples from Abha. Population of *Paracoccus* and *Phenylobacterium* capable of growing optimally on artificial compounds like dimethyl formamide, chloridazon, antipyrin, and pyramidon was also significantly higher (Eberspächer and Lingens, 2006). N2-fixing *Azospirillum*, *Agrobacterium*, and phototrophic *Rhodobacter* were also present in high numbers in soil from Abha. While, the rhizospheric soil of Muzahmiyah also show similar pattern containing high population of Candidatus *Nitrososphaera*, *Ramlibacter*, *Bradyrhizobium*, Phototrophic *Rhodoplanes*. But the sample was different in having the significantly high populations of bacteria like *Paenibacillus*, *Alicyclobacillus*, and *Sporosarcina*. Soil samples collected from Hafr Al-Batin have completely different community with high populations of *Pseudomonas*, *Propionibacterium*, *Brevundimonas*, *Staphylococcus* and *Burkholderia*. The functional inference using PICRUSt show similar results (Figure 5 and 6). Some of the most abundant genes belong to transporters, peptidases, housekeeping genes and general function. Genes involved in the prokaryotic photosynthesis, chlorophyll metabolism constitute more than 2% of the total genes (Figure 5 and 6). Furthermore, genes involved in the metabolism of simple substrates like methane, butanoate and benzoate were also predicted to have high proportion. This indicates the survival strategy of the microbial community under nutrient deficient harsh environmental conditions.

4. Conclusion

Understanding the composition of desert microbial community can help to understand the role of different microorganism in extreme environments. The analysis shows that the microbial communities of Saudi desert were characterized by the presence of high numbers of Actinobacteria, Proteobacteria and Firmicutes. These microbial communities besides showing Saharan signature desert phyla like Gemmatimonas also show biogeochemically important microorganisms exemplified by primary producers like *Rhodoplanes* and Cyanobacteria, nitrogen fixing members of the genus *Rhizobium*, *Bradyrhizobium*, and ammonia oxidizers Candidatus *Nitrososphaera*. Communities were also characterized by the presence of microbes capable of growing on simple and unlikely carbon sources such as methane, butanoate, dimethyl formamide indicating towards the survival strategies adopted by microbial communities under nutrient deficient condition.

Appendix A: Supplementary data: Figure 1-4 show the diversity of microbial community as Krona pie charts generated using Calypso for M15, M5, Abha and Hafr Al-Batin, respectively.

Code and data availability. The nucleotide sequence data is submitted to GenBank with the accession number SAMN12651127-12651133.

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Author contributions. MAK performed data analysis and prepared the manuscript. STK collected the samples, prepared genomic DNA and carried out sequencing.

Competing interests. The authors declare that they have no conflict of interest.

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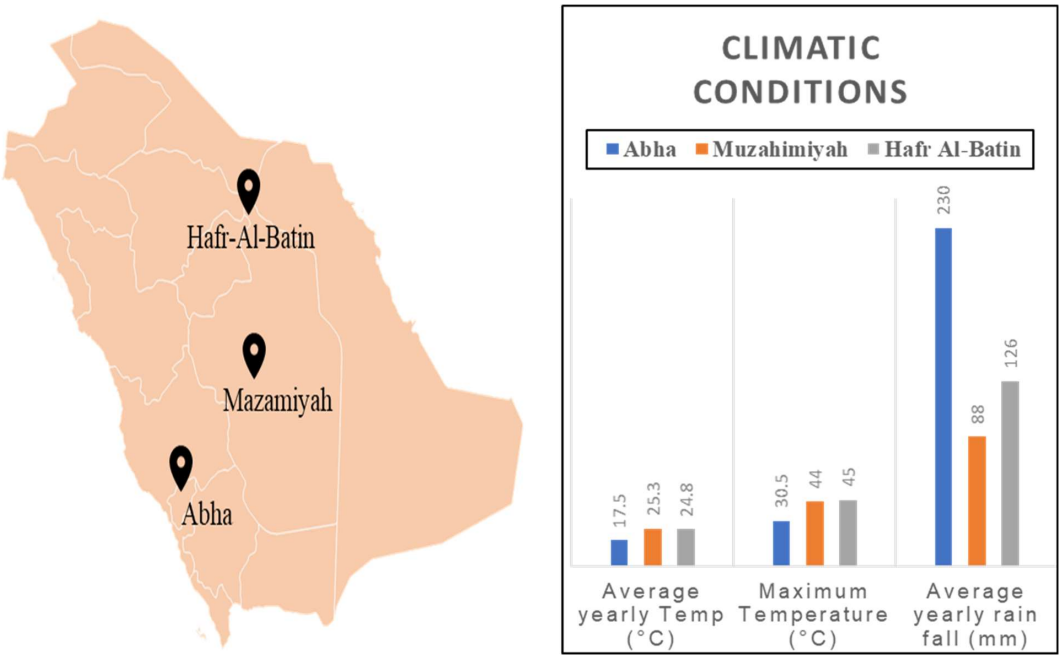

Figure 1: Location of sampling sites (A) and the climatic conditions at these sampling sites (B).

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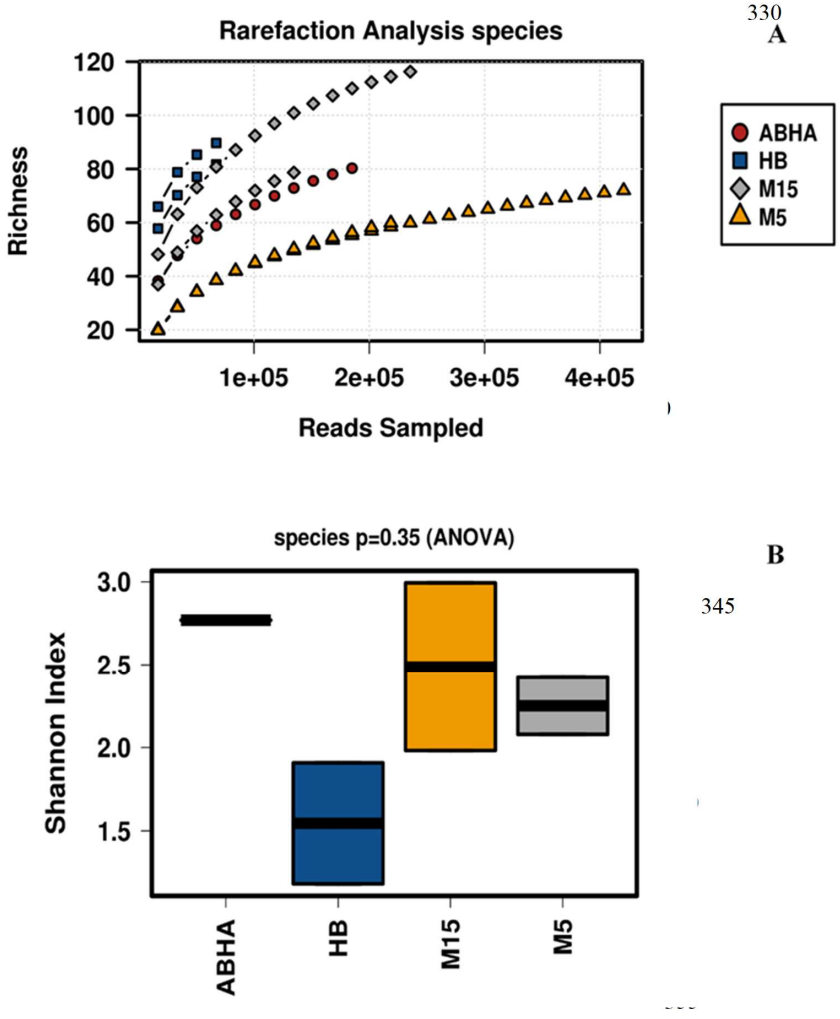

Figure 2: Estimates of Alpha diversity. Samples are rarified to a read depth of 68640. Panel A shows the rarefaction curves obtained determining the species richness. While panel B shows the Shannon diversity indices for the desert soil samples studied.

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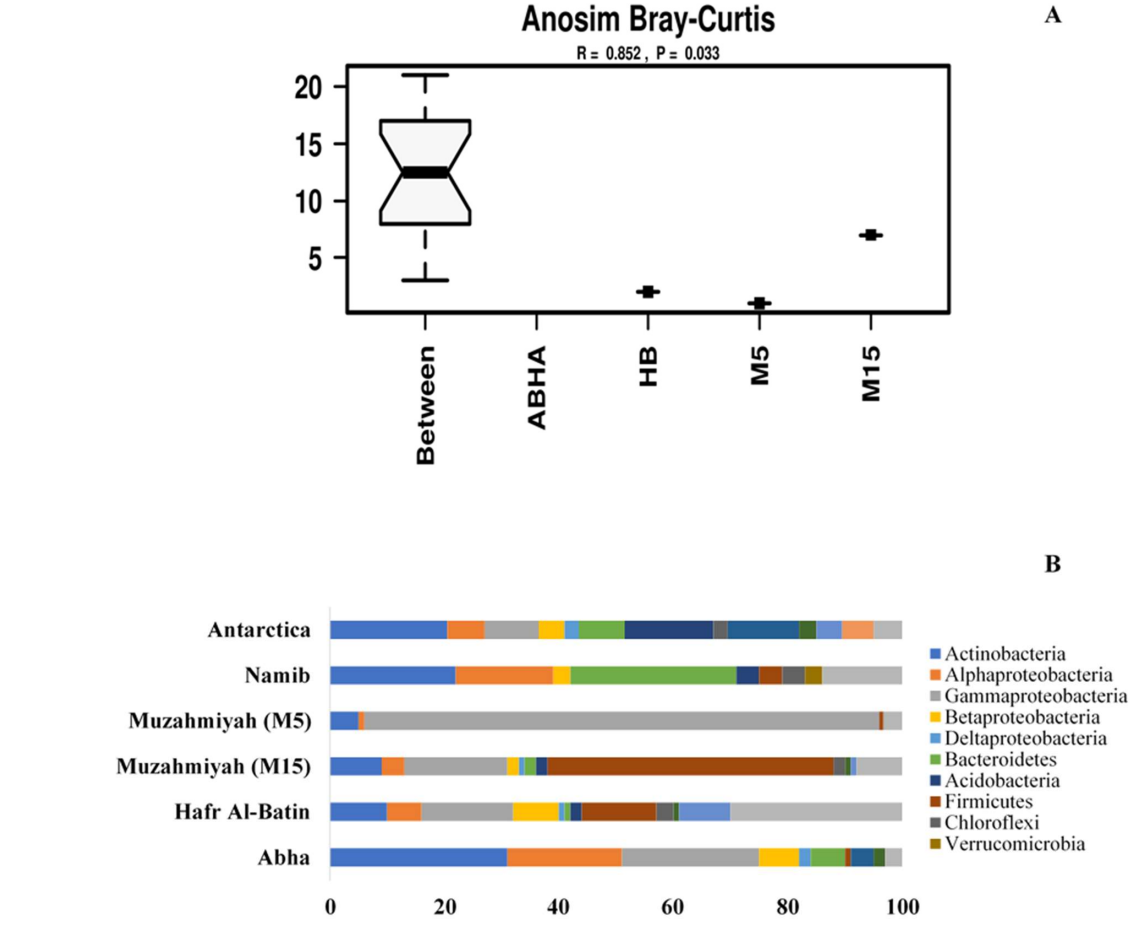

Figure 3: Analysis of similarity (ANOSIM) within and between samples (A). Abundance of different phyla in desert soil samples studied and their comparison with the hot (Namib) and cold (Antarctica) desert samples is shown in panel B. Data for Antarctic desert and Namib desert are taken from Armstrong et al., 2016 and Cary et al., 2010, respectively.

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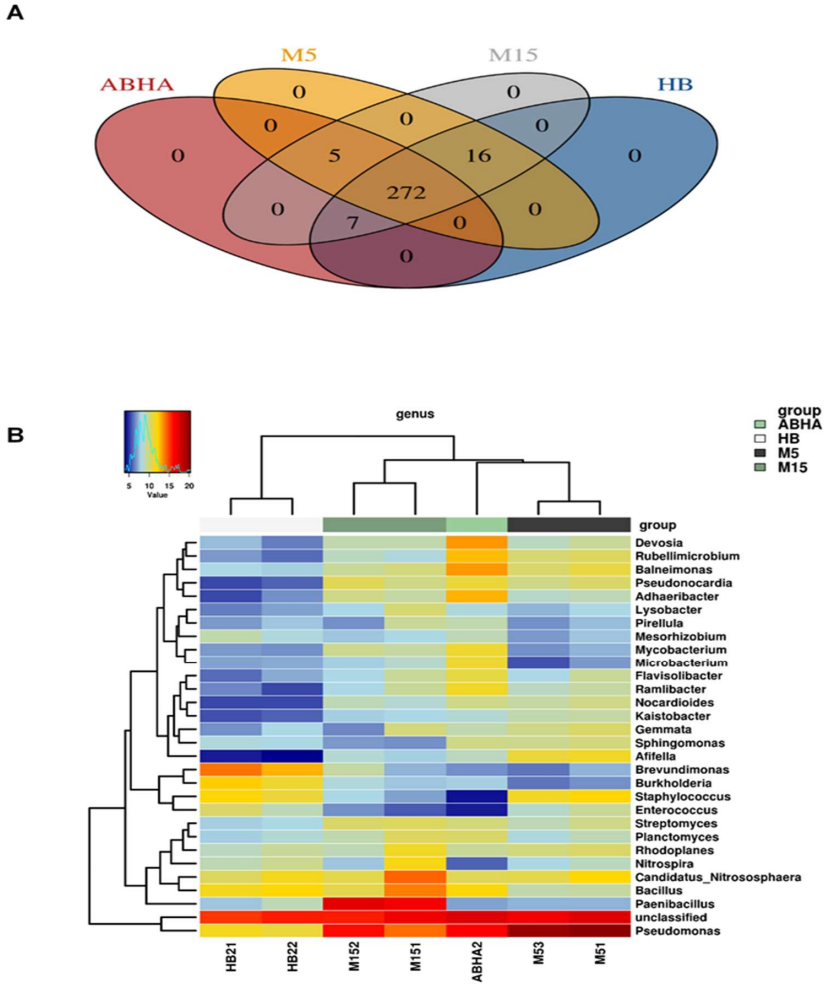

Figure 4: Venn diagram showing the number of genera present in different desert soil samples (A). Heat map showing 20 most dominant genera present in the desert soil samples.

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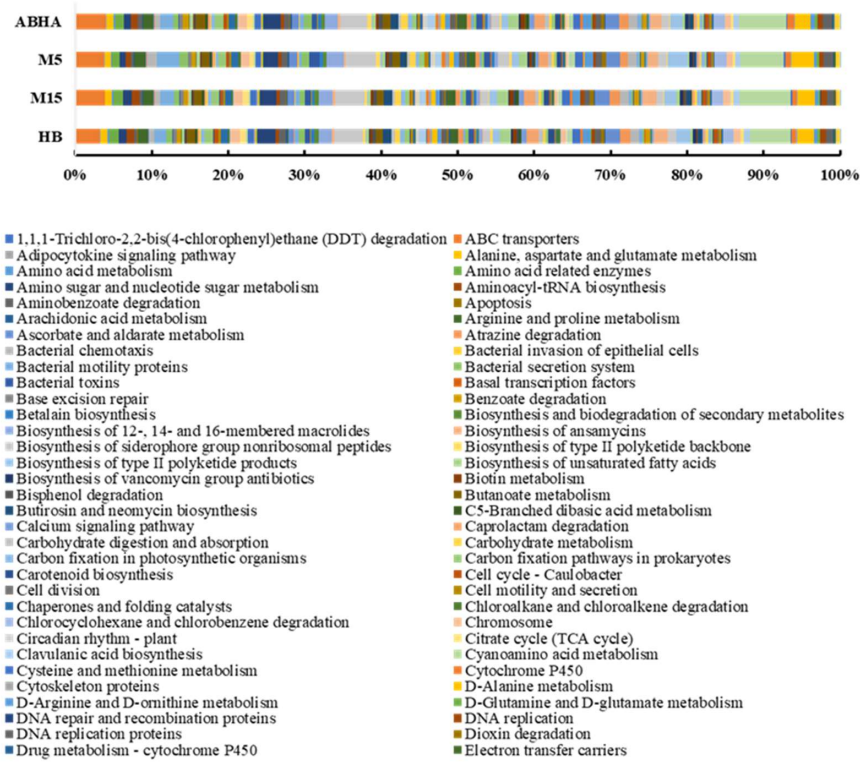

380 **Figure 5: Predicted functional gene abundance in four different samples obtained through PICRUSt analysis.**

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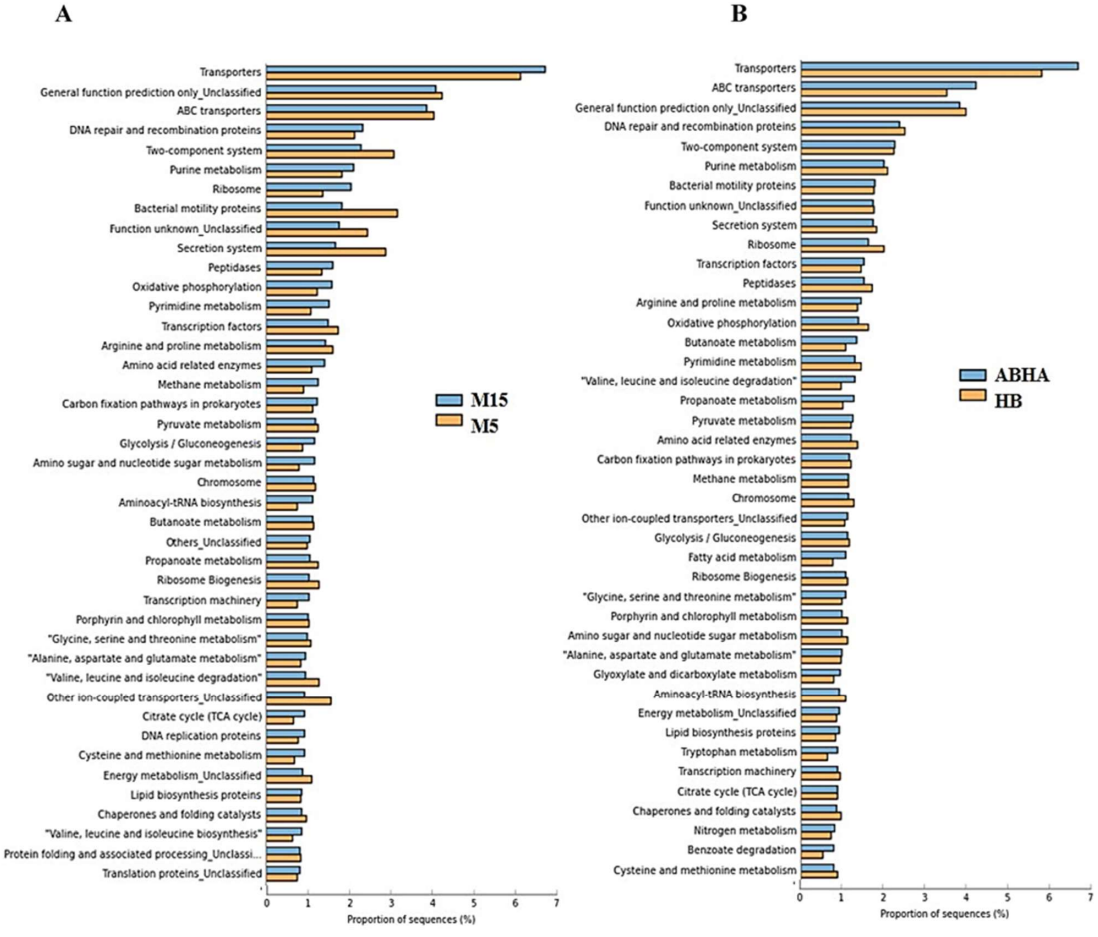

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Figure 6. Statistical comparison (Welch's t-test) of the predicted function gene abundance between M5 and M15 samples obtained from Mazamiyah (A) and between ABHA and Hafral Batin (B). P-value correction; $P < 0.05$.

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Table 1. The properties of soil samples collected for the study

Properties	Abha	Muzahimiyah		Hafr Al-Batin
		M15	M5	
Coordinates	18°15'24.4"N 42°32'45.4"E	24°24'43.6"N 46°15'31.4"E		28°02'05.3"N 45°44'03.3"E
Date of Collection	28 January 2017	6 February 2017		13 February 2017
pH	7.9	8.2		8.4
Total Aerobic Count on NA (CFU/g of soil)	$3.63 \pm 1.9 \times 10^5$	$1.1 \pm 0.9 \times 10^4$	$5.5 \pm 1.9 \times 10^5$	$8.1 \pm 2.7 \times 10^4$
*Average yearly Temp (°C)	17.5	25.3		24.8
Maximum Temperature (°C)	30.5	44		45
*Average yearly rain fall (mm)	230	88		126
*Soil type	Sand and silty	Sandy loam		Sand and Gravel
†*Other deserts*	**pH**	**Temperature (°C)**		**precipitation (mm/yr)**
Namib desert	7.9-8.5	5 to 45		5-100
Antarctica desert	-	-15 to -30		Less than 100

*Al-Zahrani 2017; Tarawneh other literature, †Makhalanyane et al., 2015; Cary et al., 2010