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

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Abstract. Saudi Arabia has the world's fifth-largest desert and is the biggest importer of food and agricultural products. Understanding soil microbial communities is key to improving the agricultural potential of the region. Therefore, soil microbial communities of the semi-arid region of Abha known for agriculture and arid regions of Hafr Al-Batin and Muzahmiyah were studied using Illumina sequencing. The results show that the microbial communities of Saudi desert were characterized by the presence of high numbers of Actinobacteria, Proteobacteria, and Firmicutes. In addition to Saharan desert signature phyla like Gemmatimonas, biogeochemically important microorganisms like primary producers, nitrogen-fixers and ammonia oxidizers were also present. The composition of the microbial community varied greatly among the sites sampled. The highest diversity was found in the rhizospheric soil of Muzahmiyah followed by Abha. Firmicutes, Proteobacteria, and Actinobacteria were three main phyla detected in all the samples. Soils from the 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. The presence of photosynthetic bacteria, ammonia oxidizers, and nitrogen fixers along with bacteria capable of surviving on simple and unlikely carbon sources like dimethylformamide was indicative of their survival strategies under harsh environmental conditions in the arid soil. Functional inference using PICRUSt analysis shows an abundance of genes involved in photosynthesis and nitrogen fixation.

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

1 Introduction

Saudi Arabian desert also referred to as the Sahara Arabian desert is the fifth-largest desert of the world bordering with Yemen, the 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 the 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 favorable, 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 rainfall than the rest of the country.

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 the 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 the Asir region and 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 the arid regions of Muzahmiyah (near Riyadh) and Hafr Al-Batin. Samples were collected between 26 Jan and 18 Feb 2019. The weather of the city 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 rainfall of about 230 mm most of which occurs between February and April and is located at an elevation of about 2,270 metres (7,450 feet) above sea level. The soil type in Abha is sand and gravel and the pH of the soil sample was slightly alkaline i.e. 7.9. The region is known for agriculture and the soil sample was collected from a plot with prestine 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 annual precipitation of only 88 mm and is located at an elevation of 612 m from sea level. The soil type in Muzahmiyah is reported to be aridisol, sandy loam (Siham, 2007). Two samples were collected from Muzahmiyah the pH of both samples was alkaline (8.2). One sample was from the rhizospheric region of *Haloxylon persicum* and another sample was collected from a distance of 1m from the first sample. Hafr Al-Batin is also an arid region with an average annual rainfall of only 126 mm and is located at an elevation of 358 m above sea level. The soil is aridisol with alkaline pH of 8.4. Figure 1 and table 1 shows the locations of the sampling sites and the climatic conditions at these sites. Three cores of 1.9 cm diameter from each sampling site were collected from a distance of

1m from each other and were mixed to obtain a composite sample. Debris (2 cm) from the surface was removed at the time of sampling and a soil core from a depth of 5 cm was then obtained. 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 homogenized via sieving (<2 mm). 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 soil pH was determined in 0.01 M $CaCl_2$ (2:1 solution to solid ratio) using a pH meter. While total aerobic counts in the soil were determined on 1/5th diluted nutrient agar plates using the dilution plating method.

2.2 DNA extraction and HiSeq analysis

Genomic DNA from the soil was prepared using the 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 a 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 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 Data analysis

Raw data obtained from the sequencing was processed using QIIME (Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2010). Sequences were clustered into operational taxonomic units using UCLUST and an identity threshold of 97%. 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 the 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 harbored significantly different microbial communities, we used an 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). Predictive functional analysis of microbial communities using 16S rRNA gene sequences was carried out using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States).and STAMP (statistical analysis of taxonomic and

functional profiles) for functional inference using PICRUSt and STAMP 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. 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. The soil type in Muzahmiyah is reported to be sandy loam (Siham, 2007). The CFU counts of the rhizospheric soil of *Haloxylon persicum* was fifty 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 centimeters. 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 $8.1 \pm 2.7 \times 10^4$ CFU/g of soil.

3.2 Microbial diversity of soil samples

Reads per sample varied between 68640 to 420570. For analysis, a total of 12,790 data points were included for each sample. 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 the microbial population was least diverse in the 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 the 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 was 25, 90, 31, and 53% in soil samples from Muzahmiyah M15, M5, Hafr Al-batin, and Abha, respectively. The 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%) was 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). Interestingly,

a recent study of Saudi desert soil samples also shows the presence of Actinobacteria, Bacteroidetes, and Proteobacteria as the major soil phylum (Eida et al., 2018). Soil sample from Abha shows almost the same pattern where the population of Proteobacteria, Actinobacteria, and Bacteroidetes was 53, 31, and 6% of the total bacteria, respectively. While samples from Muzamiyah and Hafr Al-Batin vary in not having the significant populations of Bacteroidetes and an increased population of Firmicutes (Figure 3 B). A high population of Bacteroidetes in samples other than Abha may be due to the unavailability of complex organic matter in these soils. As members of the Bacteroidetes are known to degrade various macromolecules in the soil. 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 which may not be the case with other deserts. A high population of actinobacteria has been found in both the cold Antarctica desert as well as hot Namib desert (Aislabie et al., 2006;Armstrong et al., 2016). Interestingly, the highest population of Actinobacteria (34% of the total bacteria) was observed in Abha which is Geographically close to the 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* was 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 have been reported from the soil in earlier studies also (Marasco et al., 2018). The Antarctica desert soil survey shows 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 the 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 the major genus found in the soil samples is shown as a heatmap in figure 4B. A detailed microbial community composition generated by the 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 the 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 genera found in our samples forms cyst and its genome analysis shows adaptation to arid conditions (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 the Atacama desert of Chile, South America (Busarakam et al., 2016). The genera like *Pseudomonas* and *Adhaeribacter*, may produce extracellular polysaccharide to survive under the arid condition and to form strong biofilms or may also contribute to water retention in soil promoting the formation of soil crust.

It is to be noted that some bacteria may play important roles besides tolerating the extreme conditions. Candidatus *Nitrososphaera* and *Planctomyces* found in all the samples in high numbers are known to oxidize ammonia (Stieglmeier et al., 2014). Notably, it was observed that the desert soil 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 a 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).

Abha soil sample was also distinct in having high populations of bacteria with the ability to survive on the simple source of nutrients and extreme conditions or the ability to perform an important geochemical or agricultural function. The population of genera *Adhaeribacter*, *Modestobacter*, *Ramlibacter*, radiation-resistant *Geodermatophilus*, *Pseudonocardia*, and *Flavobacterium* was high in samples from Abha. The population of *Paracoccus* and *Phenylobacterium* capable of growing optimally on artificial compounds like dimethylformamide, chloridazon, antipyrin, and pyramidon was also significantly higher (Eberspächer and Lingens, 2006). N_2-fixing *Azospirillum*, *Agrobacterium*, and phototrophic *Rhodobacter* were also present in high numbers in soil from Abha. While, the rhizospheric soil of Muzahmiyah also shows similar pattern containing a 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 a completely different community with high populations of *Pseudomonas*, *Propionibacterium*, *Brevundimonas*, *Staphylococcus,* and *Burkholderia*. The microbial community in Hafr Al-Batin soil is completely different probably due to the completely different environmental conditions in Hafr Al-Batin. This region is well known for its extreme arid condition in Saudi Arabia. The functional inference using PICRUSt analysis 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 prokaryotic photosynthesis, chlorophyll metabolism constitute more than 2% of the total genes (Figures 5 and 6). Furthermore, genes involved in the metabolism of simple substrates like methane, butanoate, and benzoate

were also predicted to have a high proportion. This indicates the survival strategy of the microbial community under nutrient-deficient harsh environmental conditions. Interestingly, it was found that the proportion of genes for prokaryotic photosynthesis was lowest (1/4th) in samples from Abha compared to other samples. Probably comparatively higher soil fertility and semiarid nature of soil do not require a high population of photosynthetic bacteria for maintaining and providing carbon to other soil organisms. Similarly, the proportion of genes involved in methane and nitrogen metabolism and peptidoglycan biosynthesis were lowest (~1/3rd) in Abha samples. Indicating that the population of Gram-positive bacteria in all the samples other than the sample from Abha is high. Production of methane is a characteristic of arid soils and the presence of these genes in high proportion in all the samples other than Abha further confirms the fact observed in previous studies.

4. Conclusion

Understanding the composition of the desert microbial communities may help us in understanding the role of different microorganisms 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 desert signature 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, dimethylformamide indicating 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.

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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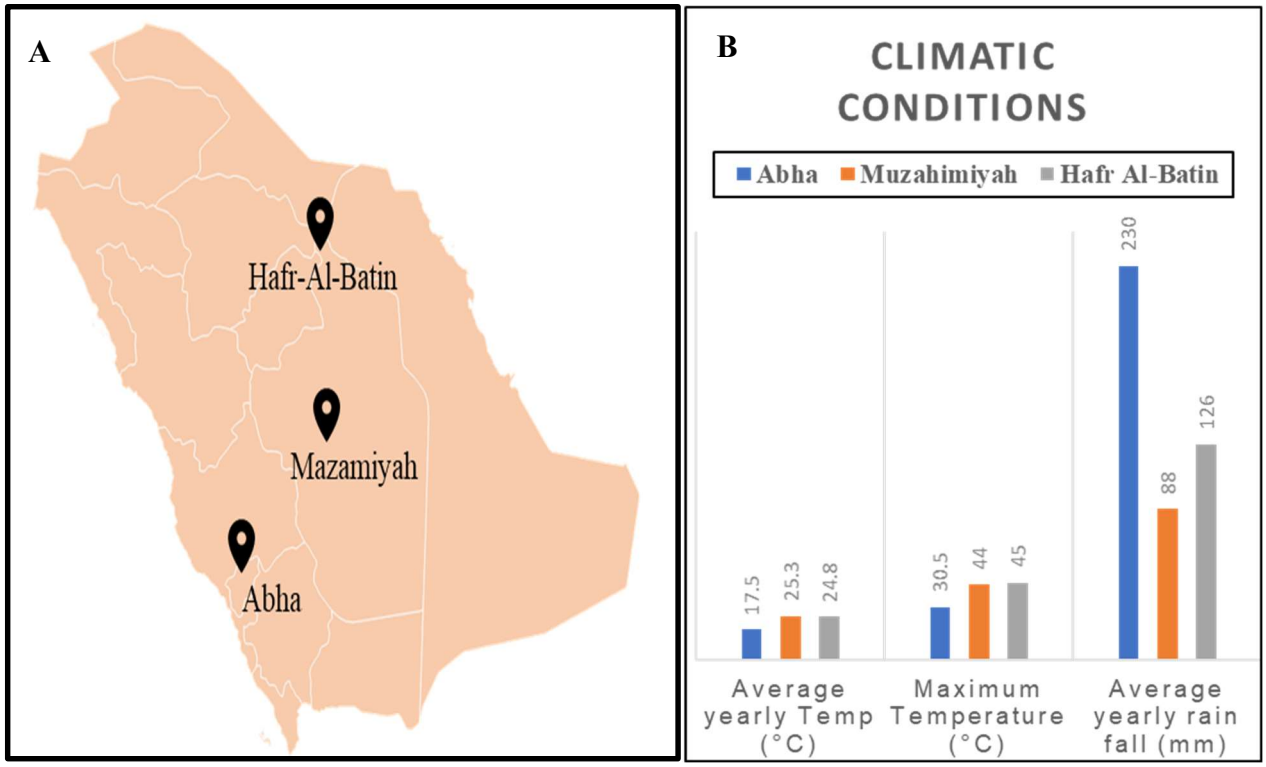


Figure 1: Location of sampling sites (A) and the climatic conditions at these sampling sites (B).

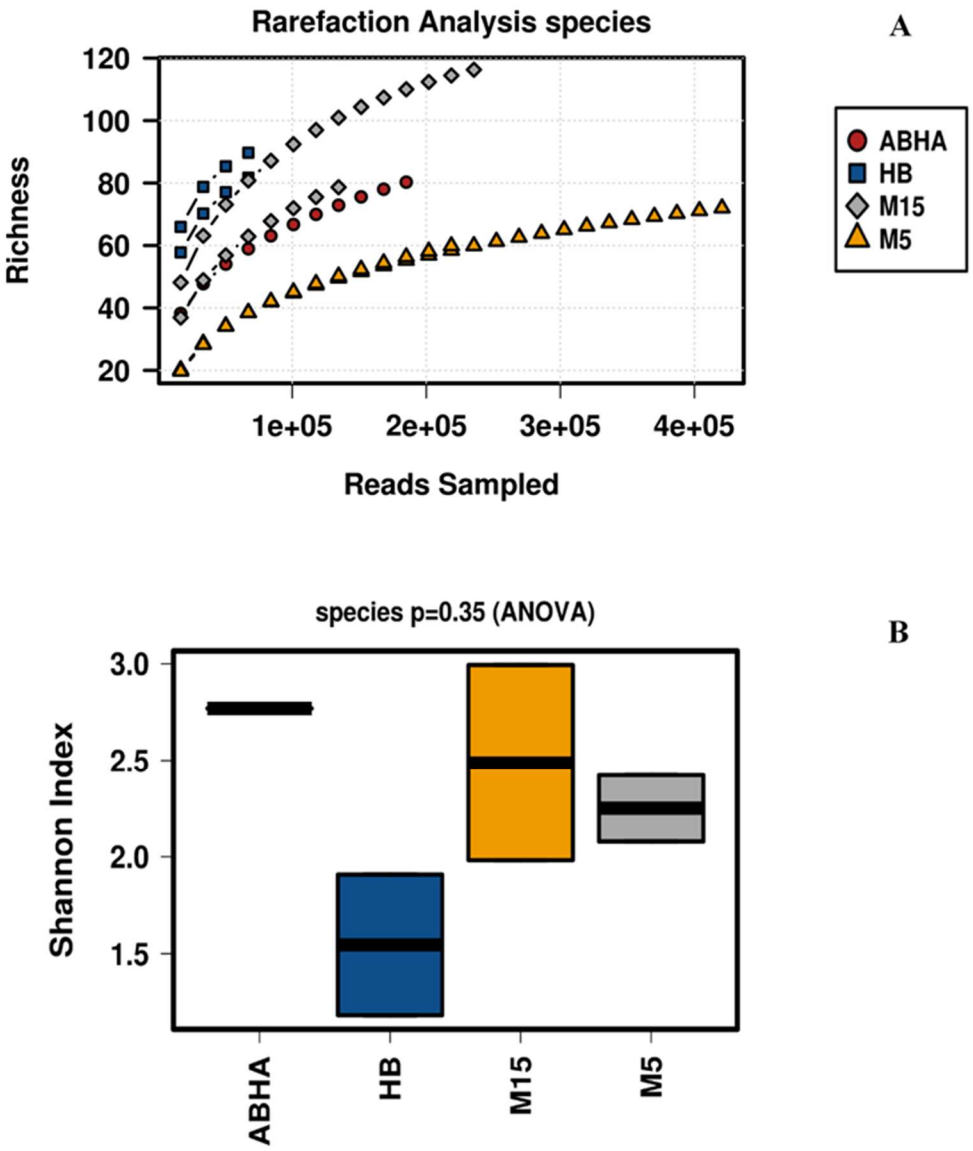
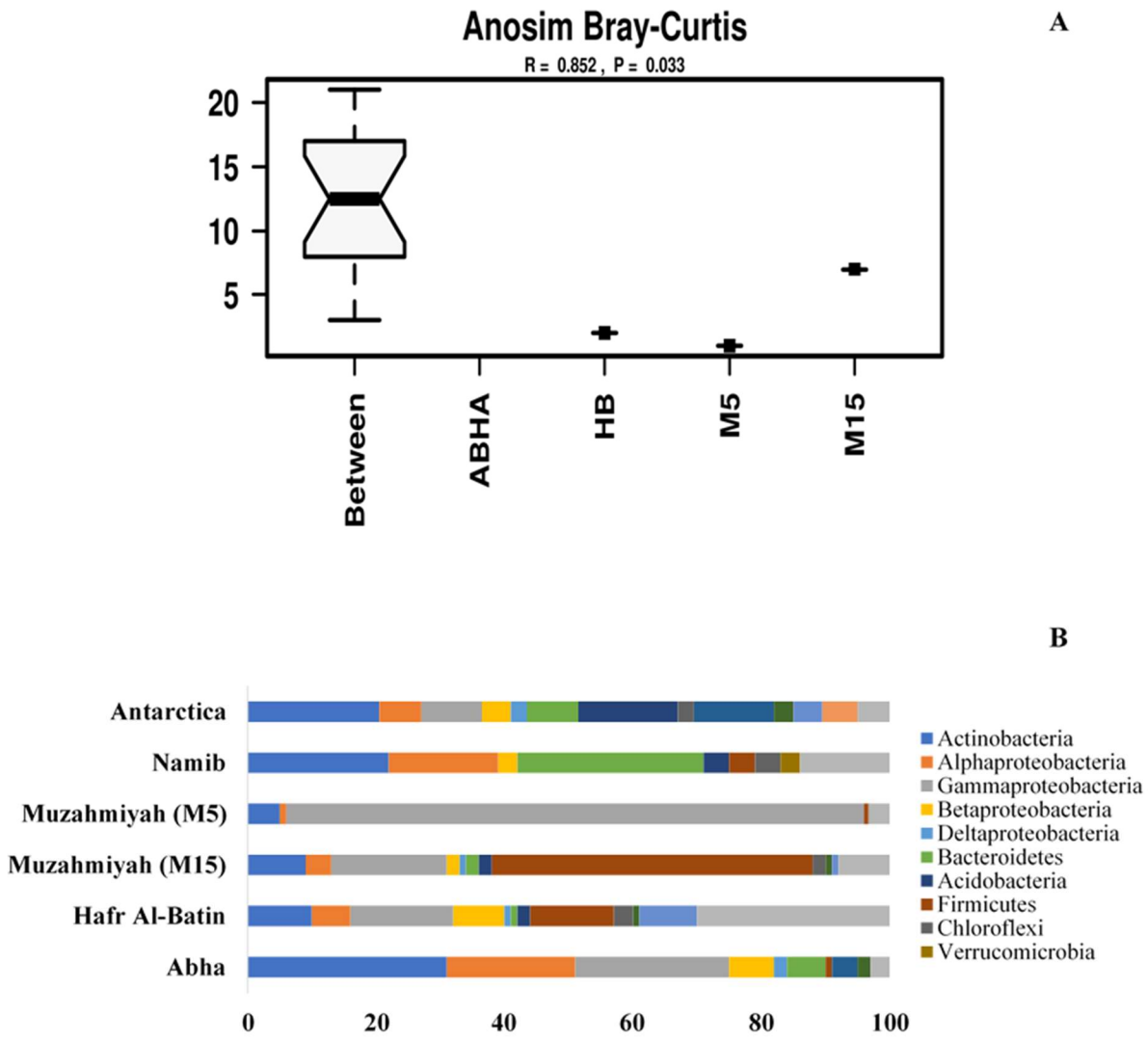


Figure 2: Estimates of Alpha diversity. Samples are rarified to read depth of 68640. Panel A shows the rarefaction curves obtained determining the species richness. While panel B shows the Shannon diversity indices for the desert soil samples studied.



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

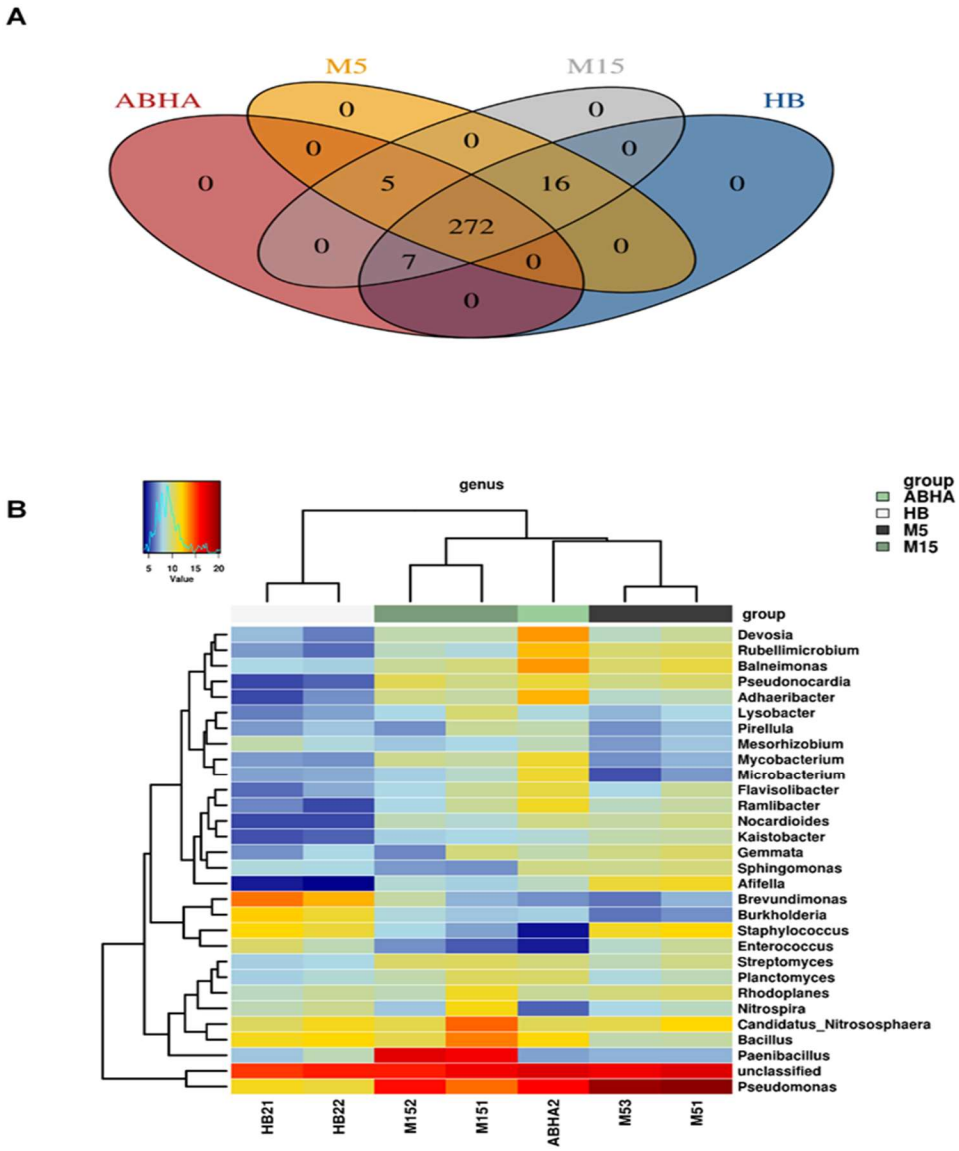


Figure 4: Venn diagram showing the number of genera present in different desert soil samples (A). Heat map showing 20 most dominant genera present in the desert soil samples. M5 & M15; Samples collected from Muzamiyah, HB; sample collected from Hafr Al-Batin.

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Figure 5: Predicted functional gene abundance in four different samples obtained through PICRUSt analysis. M5 & M15; Samples collected from Muzamiyah, HB; sample collected from Hafr Al-Batin.

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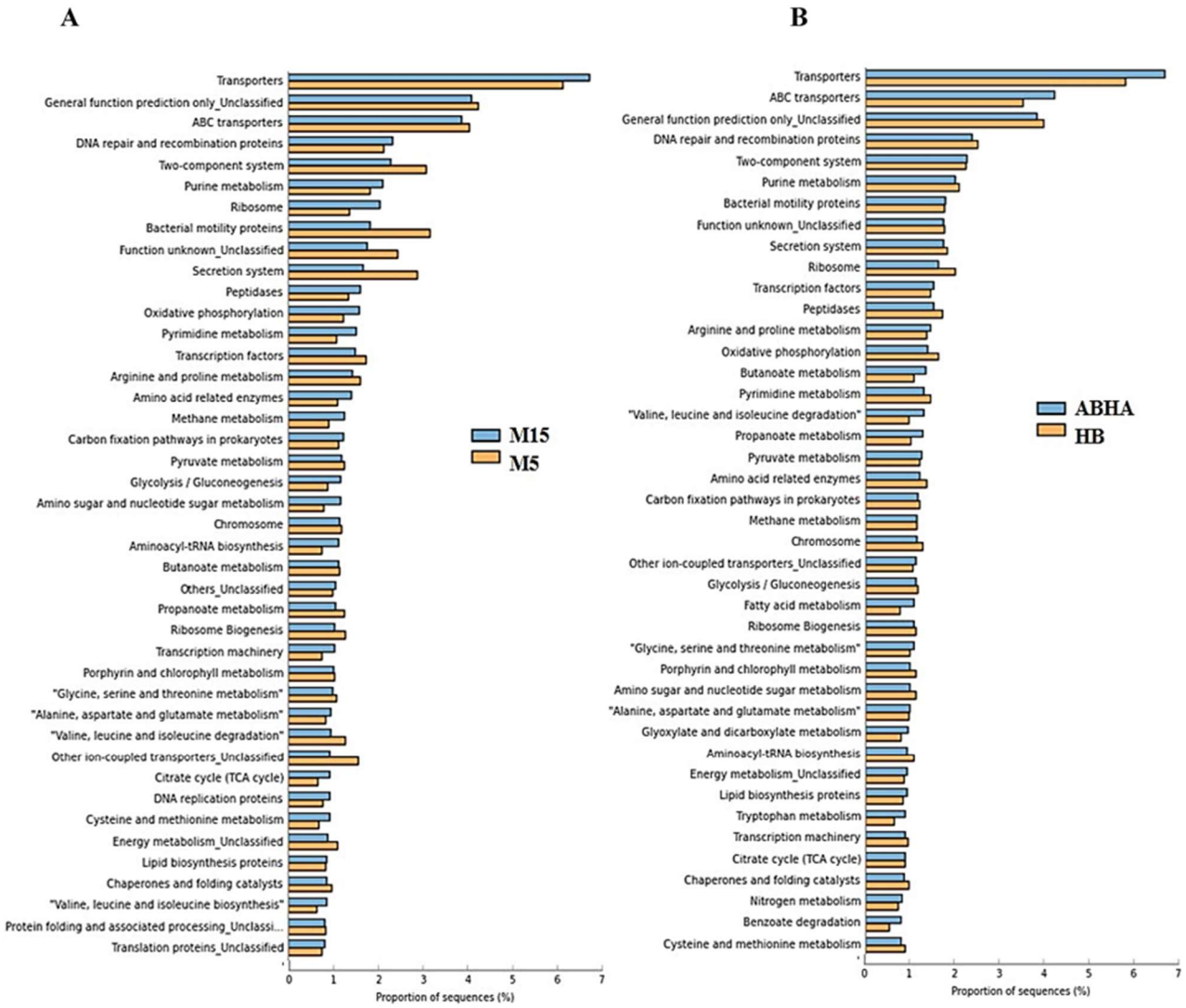

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Figure 6. Statistical comparison (Welch's *t*-test) of the predicted function gene abundance between M5 and M15 samples obtained from Mazamiyah (A) and between ABHA and Hafral Batin (B). P-value correction; $P < 0.05$.

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

Properties	Abha	Muzamiyah		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

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