

We thank the handling topical editor for giving us a chance to publish in soil. The manuscript has been revised as per the comments of the referees and a point by point reply to their comments and suggestions is given below

Thanking you again

### **Anonymous Referee #1**

The manuscript entitled “Microbial communities and their predictive functional profiles in arid soil of Saudi Arabia” presents enough evidence of the existence of a microbial community with many genera well suited to their survival under extremes of arid region climate soils poor in nutrient content. But I have some minor concerns that should be addressed before the manuscript can be finally accepted for publication.

**Response:** Authors thank reviewer for their comments and suggestions, and all the suggestions given below are included in the revised manuscript.

Line 18, environmental conditions.; shows an abundance of genes

**Response:** Changed as suggested.

Line 31, rainfall

**Response:** Changed as suggested.

Line 40, Delete the sentence “consequently, improving. . .of the soil.

**Response:** Deleted.

Line 80: The sentence Online program Galaxy, should be modified as “Predictive functional analysis of microbial communities using 16S rRNA gene sequences was carried out using online PICRUSt program available with Galaxy and STAMP program.

**Response:** Modified as suggested.

Line 86, the weather of the city

**Response:** Changed as suggested.

Line 95: Please correctly calculate how many times  $5.5 \pm 1.9 \times 10^5$  is higher than  $1.1 \pm 0.9 \times 10^4$ . I think it is 50 times?

**Response:** Changed as suggested.

Line 96, Delete “an” before 8.1

**Response:** Deleted.

Line 115 was observed

**Response:** Modified as suggested.

Line 130, these sequences have been

**Response:** Modified as suggested.

Line 133, Scientific names should be italicized throughout the manuscript, examples, *Acinetobacter* in line, *Rhizobium* in line 163 and *Flavobacterium* in line 169.

**Response:** Scientific names are italicized in revised manuscript.

Line 155, Change “is known to oxidize” to are known to oxidize

**Response:** Changed as suggested.

Line 176, PICRUSt should be changed to PICRUSt analysis

**Response:** Changed as suggested.

Line 184 Correct this line use “us” after help.

**Response:** Corrected.

Line 187, delete but after *Gemmatimonas* and italicize *Gemmatimonas*.

**Response:** Italicized.

Improve the map of Saudi Arabia, clearly label A and B in figure 1.

**Response:** Figures are labelled properly in revised manuscript.

## **Anonymous Referee #2**

From reviewer's point of view the work presented in this study is an important piece of work filling in the Knowledge gap about the microbial community in the soil of arid regions in Saudi Arabia. This study also provides substantial ground to carry out many future projects related to improving soil fertility in these regions. It will be interesting to confirm in future studies that the addition of carbon sources with single carbon like butanoate and dimethylformamide can enrich certain groups of bacteria mentioned in this study. And to monitor the survival and role of these microorganisms in these arid soils.

Authors are also requested to ask add some discussion as to why the microbial community in the soil from Hafra-batin was so different.

**Response:** Information added.

What are the possible reasons for the high population of Bacteroidetes bacteria in Abha region.

**Response:** Information added to the revised manuscript.

Very recently published study "Desert plant bacteria reveal host influence and beneficial plant growth properties" by Eida shows microbial communities in the rhizospheric soil in Jizan region. Authors should consider including this report for comparing microbial community.

**Response:** Although the study is interesting but not directly related to this paper .

Please correctly calculate how many times  $5.5 \pm 1.9 \times 10^5$  is higher than  $1.1 \pm 0.9 \times 10^4$ . I think it is 50 times?

**Response:** Corrected.

Manuscripts also needs some minor corrections listed below In title better to use "in the arid"

**Response:** Corrected as suggested.

Line 15, delete "in" from the sentence, change samples in containing

**Response:** Deleted.

Line 16, The presence of "in the arid soil".

**Response:** Corrected.

Line 18, Add analysis after PICRUSt.

**Response:** Corrected.

Line 18, environmental conditions.; shows an abundance of genes

**Response:** Modified as suggested.

Line 41, Asir reion and arid regions same change in line 47

**Response:** Changed as suggested.

Line 77, use hyphen between t and test

**Response:** Corrected.

Line 86, the weather of the city

**Response:** Changed as suggested.

Line 96, remove an before 8.1

**Response:** Deleted.

Line 112, the population of proteobacteria was

**Response:** Changed as suggested.

Line 115 was observed

**Response:** Changed as suggested.

Line 130, these sequences have been

**Response:** Corrected.

Line 151, the arid conditions

**Response:** Changed as suggested.

Line 155, are known to oxidize

**Response:** Changed as suggested.

Line 169, dimethylformamide

**Response:** Corrected.

Line 178, Figures 5 and 6

Line 190, consider “indicating the” Mark A and B clearly in figure 1.

**Response:** Marked clearly.

### **Anonymous Referee #3**

Authors thank reviewer for critical assessment of the manuscript and for expert comments. We agree with the reviewer on many points and corrections according to his expert comments will be made in the revised manuscript. English and grammatical mistakes can be corrected by a native speaker. “Inappropriate writing” can also be improved by including the comments made by the learned reviewer or any other correction proposed by reviewers and editor. Please find below point wise reply to the posted comments.

**Reviewer comments:** Although the author wants to explore the arable potential in this region through the presented experiment, the improper experimental design, unclear sampling process, inappropriate writing, and numerous grammar errors made the text should be reconsidered to publication.

**Response:** As discussed above the experimental design and sampling process can be explained to address the concerns raised by the referee. Writing part can be improved taking into consideration technical flaws, editing and grammar issues.

**Reviewer comments:** The content of the text only involved the changes in the microbial community structure, and did not involve the functions related to microbes, such as enzyme activities related to C mineralization and changes in NO<sub>x</sub> or soil N levels related to N mineralization, therefore it cannot reflect the meaning of “predictive functional profiles” in the title.

**Response:** The data presented in the manuscript is 16S rRNA gene sequencing and functional prediction using PICRUSt analysis which has already been used in multiple studies as a powerful tool for predicting function. Keeping in view these factors we chose the title, but if the reviewer disagrees, we can remove “predictive functional profiles” from the title. Discussion can be elaborated to include changes in genes responsible for mineralization, and in nitrogen cycle etc in different samples.

**Reviewers comments:** The sampling points in each site were not enough to contrast the differences in soil community from semi-arid to arid regions and the sampling method is incorrect according to the current description.

**Response:** Details of the sampling site is provided in table 1 as mentioned in the manuscript. We agree with the reviewer that a more exhaustive sampling could have been included in the study. Nevertheless, samples were collected in triplicates from each sampling site which is a widely reported in many papers published earlier also to characterize microbial community. And the fact is mentioned clearly in the manuscript.

**Reviewers comments:** The author should set the sampling plots for collecting soils because it is impossible to set true repetitions at each site. Furthermore, the author did not detail describe the sampling method. What is the basis of sampling? How about the aboveground vegetation? What is the size of each sampling plots at each site? What points were collected in each sampling plot? How far apart between the sampling plots?

**Response:** Authors will add these details in the revised manuscript. At all the sites top 1-2 cm sand was removed before sample collection to avoid any debris and sand.

**Reviewers comments:** In the Lines 49-51, why did soil samples distinguish between rhizosphere and non-rhizosphere soils only at Muzahmiyah?

**Response:** If reviewer want, we can remove the data of rhizospheric soil sample from Muzahmiyah this was included to compare the Rhizosphere soil microbial community with that of relatively fertile soil from Abha region. Furthermore, Hafralbatin is a highly arid region and we did not find any vegetation at that sampling site.

**Reviewers comments:** Line 49, why soil samples only collected from the upper soil of 0-5cm. Due to the contrasting rainfall and temperature among these sites, soil community in the deeper soil that greatly influenced by soil moisture should include in this study.

**Response:** We checked in some preliminary experiments (not included in the manuscript) that higher plate count was observed in 0-5 cm region after removing the top layer which we considered as debris or surface sand (~1-2 cm). The CFU counts decreased to an order of 10 in samples collected from a depth of 15 cm.

**Reviewers comments:** The last and most important drawback of this experiment is why the chemical or physical properties of the soil, such as soil temperature, humidity, soil total carbon and nitrogen, are not measured when collecting soil samples. These parameters are more useful than the currently used parameters (annual average) to explain changes in the soil communities.

**Response:** We agree with the reviewer, but we have included some climatic conditions, average soil temperature, soil texture, CFU counts and average rainfall in table 1. As correctly pointed out by reviewers these parameters also influence microbial community and some discussion could have been added to the manuscript. Which can be improved in the revised manuscript. Temperature on the day of sampling, range of carbon and nitrogen content of soil in the region can be searched in the literature at this point and can be included in the discussion if available.

**Reviewers comments:** Writing skills: Data analysis should write in another subtitle different from the others in the part of 2 Materials and Methods.

**Response:** This correction will be included in the revised manuscript.

**Reviewers comments:** Many abbreviations, such as Line 17-18 DMF and PICRUSt, should give their full name when they first present in the text. Many descriptions, such as Line 85-87.

**Response:** Suggested correction will be included in the revised manuscript.

**Reviewers comments:** Many descriptions, such as Line 85-87, in the part of 3 Results and discussions should move into the part 2 acted as the background of sampling sites.

**Response:** This part will be moved to part 2.

Line 138-141, the results should be described in the order of

**Reviewers comments:** The figures, first in Figure 4A and then in Figure 4B. Table 1, no note for the indication of M5 and M15.

**Response:** We will add the note to indicate M5 and M15.

**Reviewers comment:** English grammar errors: grammatical errors existed throughout the whole text, need to rewrite. For example, in the Abstract, Line 11, 'Microbial community composition varied remarkably from other deserts and from one place to another. ' do you want to express 'the composition of microbial community varied greatly from site to site'?

**Response:** This sentence should come in the later in the abstract as by other deserts we mean Antarctic, Namib" and place to place means Abha, Muzahmiya and Hafr Al-batin.

**Reviewers comment:** Line 13, 'Unlike other deserts', what do you mean?

**Response:** Here also it means deserts in other parts of the world. But as suggested by reviewer the abstract will be restructured.

**Reviewers comment:** '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 do you want to express that 'Soil microbial community in the region of Abha contained only 1% Firmicutes, but the populations of Actinobacteria and Bacteroidetes were three to six times higher than the other desert regions.

**Response:** Yes, the sentence will be modified to increase the clarity.