2018
读书报告

牛铭铭

2018.08.18

IF=4.16

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ORIGINAL RESEARCH ARTICLE

Front. Microbiol., 23 May 2018 | https://doi.org/10.3389/fmicb.2018.01068

Diversity of Bacteria and the Characteristics of Actinobacteria Community Structure in Badain Jaran Desert and Tengger Desert of China

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CONTENT

01

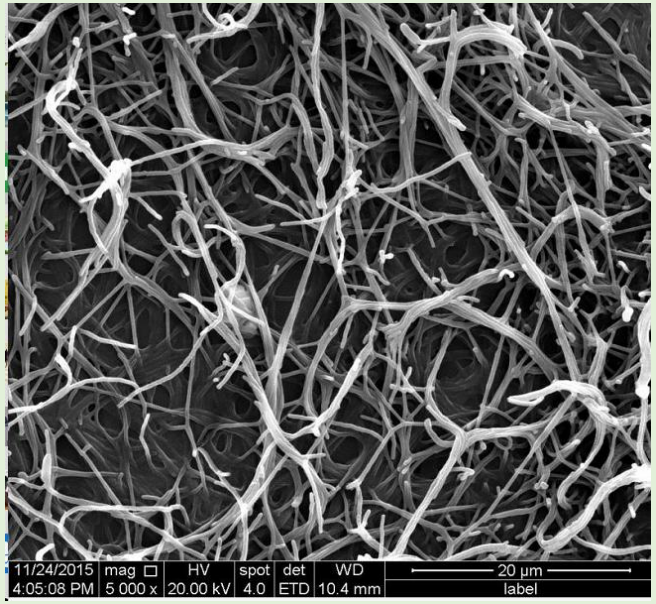
Introduction

01/ Introduction

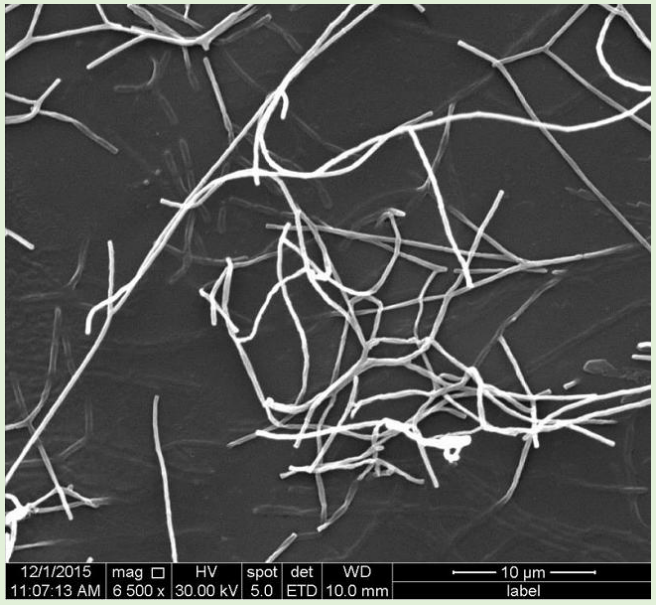
Deserts are extreme environments that are characterized by extreme aridity, intense solar UV radiation, and extreme shifts of temperature in day-night cycles. Consequently, deserts harbor numerous extremophiles (Subramani and Aalbersberg, 2013; Júlia et al., 2016).

Xerophilous microorganisms that are adapted to relatively high temperatures and radiation levels are likely to be the dominant populations in these ecosystems, including desiccation and radiation resistant phyla, such as Actinobacteria, Proteobacteria, and Bacteroidetes (Vikram et al., 2016).

01/ Introduction



Actinobacterial members have been considered as an important source of new antibiotic-producing bacteria. Currently, about 70% antibiotics in clinical use are produced by various actinomyces (Doull and Vining, 1990; Jose and Jebakumar, 2013).



Detecting various actinobacteria from extreme environments has become an important strategy for the discovery of new antibiotics (Phoebe et al., 2001; Wilson and Brimble, 2009).

01/ Introduction

FIGURE 1 | Map showing Badain Jaran desert location and Tengger desert location.

02

Materials and Methods

02/ Sample Collection

BJD

- SP : 具有零星植物（SP）的沙子
- NV： 没有任何植被的沙子
- SL： 盐湖周围的沙子

Total : 79个样品

TGD

- CC ：蓝藻主导的结壳
- MC： 苔藓主导的结壳
- LC ： 地衣主导的结壳
- BS ： 裸砂

Total : 50个样品

02/ Isolation Media

M1: 1/5 strength R2A (Difco).

M2: 5 g/L yeast extract, 2 g/L cellobiose, 2 g/L $CaCO_3$, 0.5 g/L MgSO4•7H2O, 1 g/L K_2HPO_4, and 15 g/L agar.

M3: 0.1 g/L NH_4NO_3, 2 g/L sodium propionate, 0.05 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L KCl, and 15 g/L agar.

M4: 1 g/L humic acid, 1 g/L asparagine, 0.01 g/L $FeSO_4$, 0.5 g/L Na_2HPO_4, 1.7 g/L KCl, 0.02 g/L $CaCO_3$, and 15 g/L agar.

pH=7.2-7.5

M5: 2 g/L trehalose, 5 g/L yeast extract, 2 g/L $CaCO_3$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 1 g/L K_2HPO_4, and 15 g/L agar.

M6: 0.5 g/L K_2HPO_4, 0.25 g/L yeast extract, and 15 g/L agar.

M7: 5 g/L yeast extract, 3 g/L peptone, 10 g/L glycerol, 1.25 g/L sodium pyruvate, 1.25 g/L glycine betaine, and 15 g/L agar.

将甜菜碱（0.125%w / v），丙酮酸钠（0.125%w / v），微量盐溶液（0.1%v / v）和复合维生素（0.1%w / v）加入培养基中以促进难以培养的菌株的分离。 向培养基中加入制霉菌素（25 mg / L）和重铬酸钾（50 mg / L）以抑制真菌和革兰氏阴性菌的生长。

02/ Cultivation Media

PYG medium	1L
peptone	3g
glycerol	10g
yeast extract	5g
sodium pyruvate	1.25g
glycine betaine	1.25g
agar	15g
supplement	
trace salts solution	0.1% v/v
compound vitamins	0.1% w/v

02/ Total DNA Preparation From Sand Samples and PCR Amplification

Table S3. Detail information of the 15 samples

sample number	sample type	sample resource
SPT8001BS	BS	Tengger Desert
SPT8002BS	BS	Tengger Desert
SPT8003LC	LC	Tengger Desert
SPT8004LC	LC	Tengger Desert
SPT8005CC	CC	Tengger Desert
SPT8006CC	CC	Tengger Desert
SPT8007MC	MC	Tengger Desert
SPT8008MC	MC	Tengger Desert
BD201610S1	NV	Badain Jaran Desert
BD201610S2	NV	Badain Jaran Desert
BD201610S3	NV	Badain Jaran Desert
BD201610S4	SL	Badain Jaran Desert
BD201610S5	SL	Badain Jaran Desert
BD201610S6	SP	Badain Jaran Desert
BD201610S7	SP	Badain Jaran Desert

通用引物

5′-ACTCCTACGGGAGGCAGCAG-3′ (338F)

5′-GGACTACHVGGGTWTCTAAT-3′ (806R)

反应体系

5×FastPfu Buffer			4 μL
2.5 mM dNTPs			2μL
primer1	5 min	1	0.8μL
primer2	94℃	30s	0.8μL
FastPfu Polymerase	55℃	45s	0.4μL
DNA	72℃	40s	10 ng
	72℃	10min	
	4℃	∞	
		35×	

02/ Illumina MiSeq Sequencing and Raw Data Preprocessing

QIIME软件包

（a）300bp读数显示任何50bp的滑动窗口，平均质量评分<20，并丢弃短于50bp的截短读数;

（b）任何条形码错配，引物中大于两个核苷酸错配，以及含有模糊字符的读数;

（c） 最后，基于它们长度超过10bp的重叠序列组装配对序列。

02/ Antimicrobial Activity Screening

通过使用含有大肠杆菌ATCC 25922，铜绿假单胞菌ATCC 27853，粪肠球菌ATCC 29212，肺炎克雷伯氏菌亚种的培养基研究分离物的抗微生物活性。肺炎衣原体ATCC 700603和白色念珠菌ATCC 10231，浓度为108菌落形成单位（CFU）/ mL。使用Kirby Bauer方法（纸片扩散法）进行这些测定，培养液浓度为1%（v / v）

采用平板倒置法，取 5.0 mL 指示菌悬液分别与相应的培养基 55 ℃以下迅速混合均匀，倒于培养皿中，制成含指示菌的平板。在培养有放线菌的平板上用无菌打孔器打孔（直径 0.5cm）制成菌圆形菌落，将有菌一面置于具指示菌凝固冷却的培养基上，并置于 28 ℃在培养 5d 后，分别测定抑菌圈直径的大小。每个重复检测三次。抗菌活性强弱评判指标以透明圈直径/菌落直径大小来表示。

03

Results and Discussion

03/ Bacterial Richness and Diversity

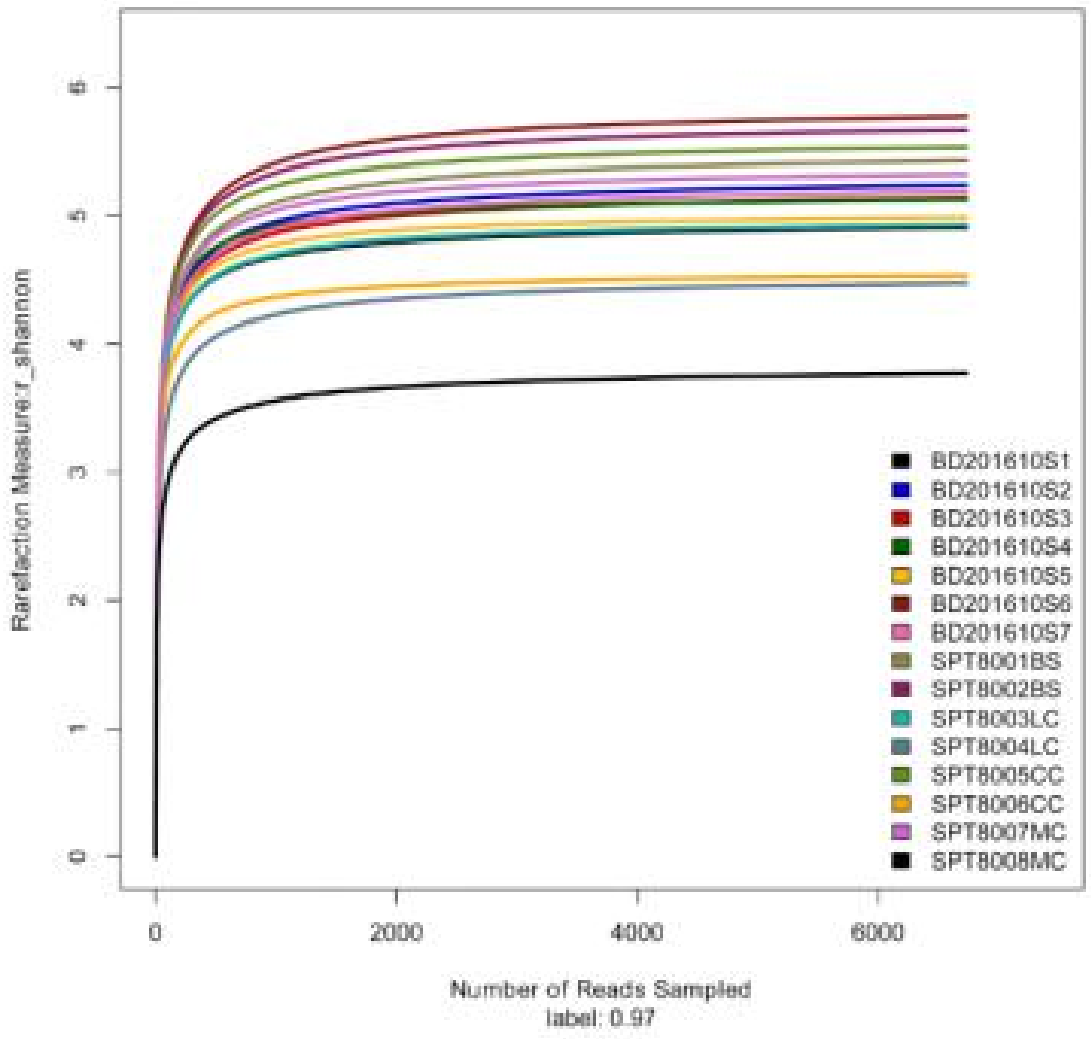


FIGURE S1 | The Shannon index curves of 15 sand samples.

Shannon指数的稀疏分析表明，本试验测序数据量渐进合理，几乎涵盖了预期在这些样品中发现的所有多样性。

03/ Bacterial Richness and Diversity

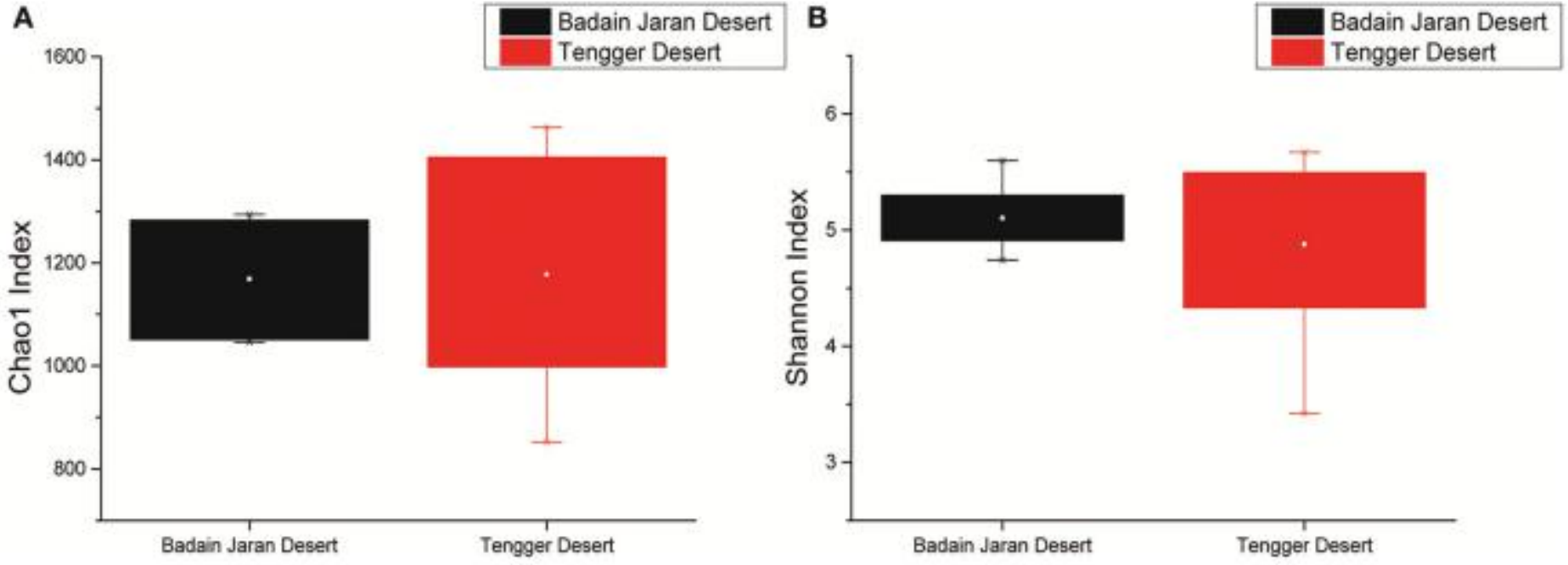


FIGURE 2 | Statistical comparisons of Chao1 and Shannon indices between two desert sand samples. **(A)** Values of Chao1 index; **(B)** values of Shannon index.

03/ Bacterial Richness and Diversity

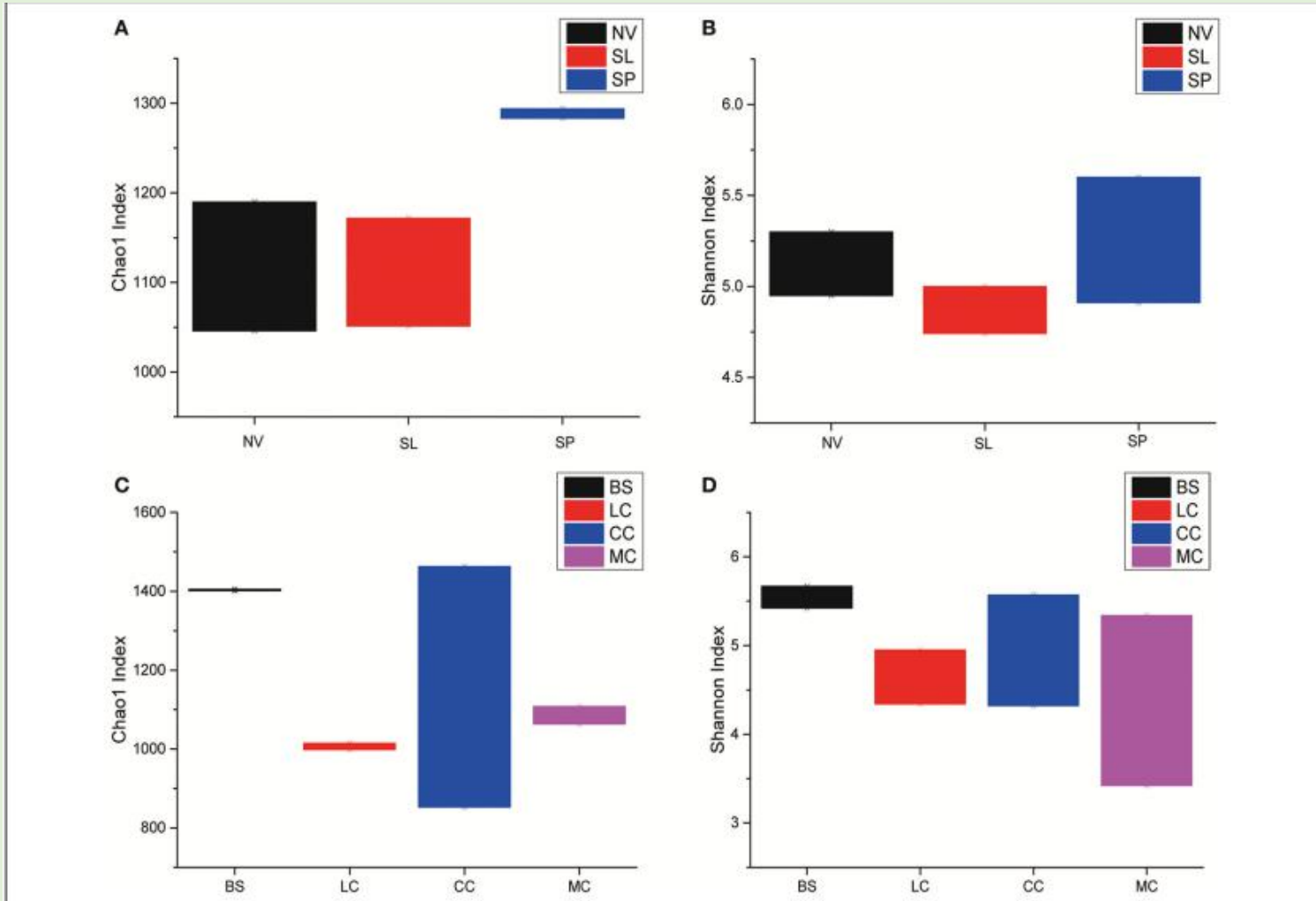


FIGURE 3 | Statistical comparisons of Chao1 and Shannon indices among three types of Badain Jaran desert sand samples (NV, SP, and SL) and four types of Tengger desert sand samples (BS, LC, MC, and CC). **(A)** Values of Chao1 in of BJD; **(B)** values of Shannon index of BJD; **(C)** values of Chao1 of TGD; **(D)** values of Shannon index of TGD.

03/ Bacterial and Actinobacterial Community Structure

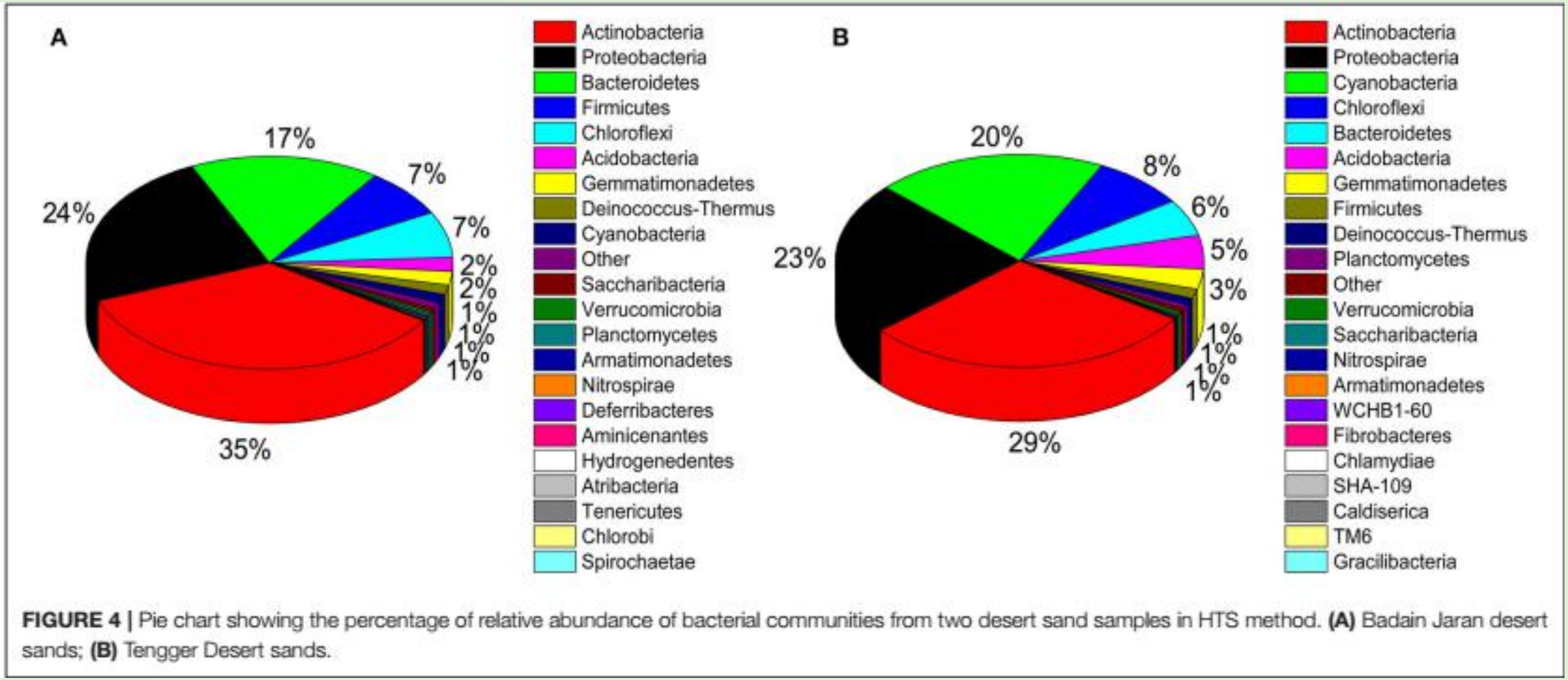


FIGURE 4 | Pie chart showing the percentage of relative abundance of bacterial communities from two desert sand samples in HTS method. **(A)** Badain Jaran desert sands; **(B)** Tengger Desert sands.

03/ Bacterial and Actinobacterial Community Structure

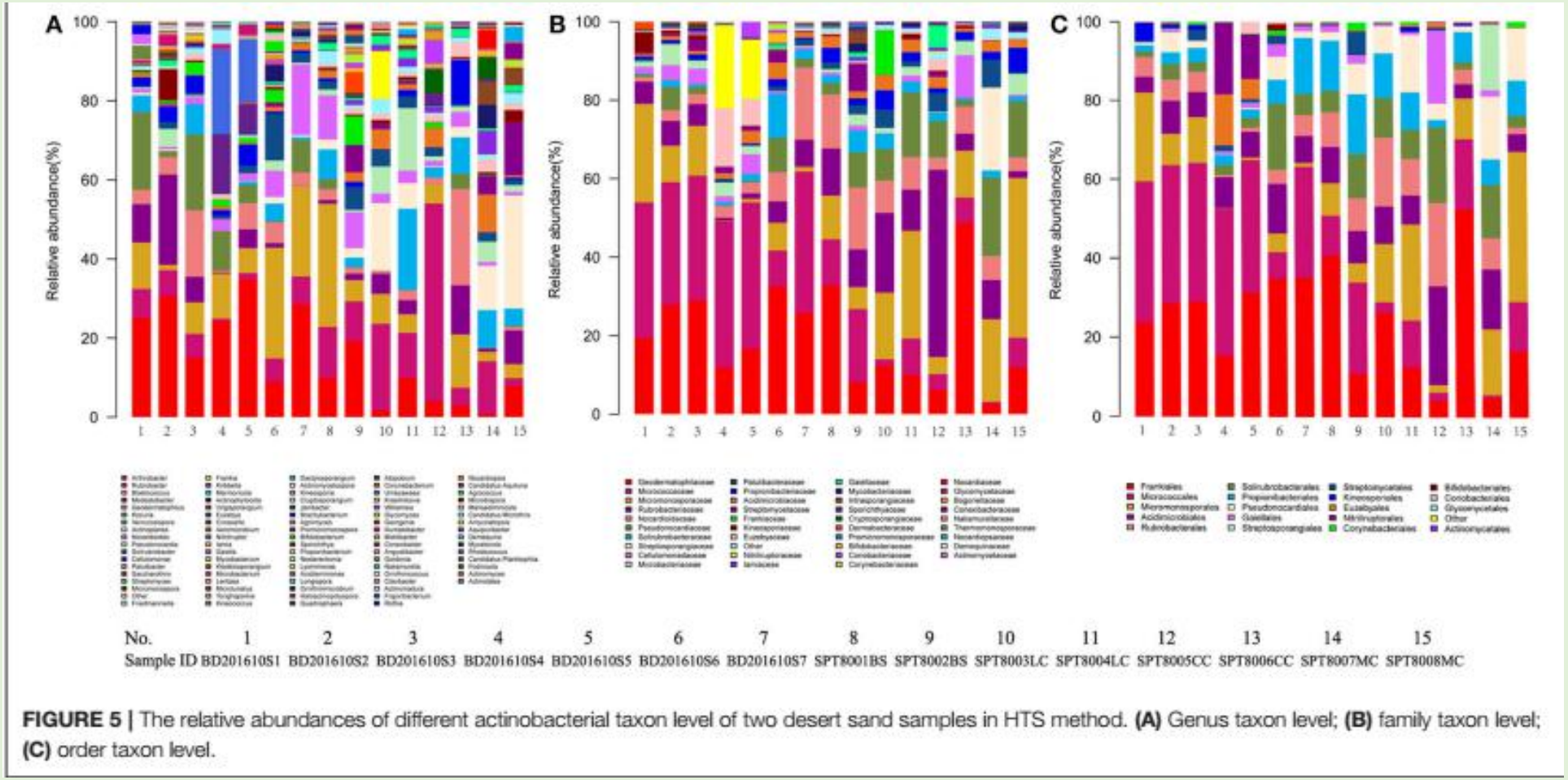


FIGURE 5 | The relative abundances of different actinobacterial taxon level of two desert sand samples in HTS method. **(A)** Genus taxon level; **(B)** family taxon level; **(C)** order taxon level.

在两个沙漠中，土壤科（*Geodermatophilaceae*），微球菌科（*Micrococcaceae*），小单孢菌科（*Micromonosporaceae*）和根瘤菌科（*Rubrobacteriaceae*）是主要的放线菌群。 其中最丰富的属是节杆菌(*Arthrobacter*)和*Rubrobacter*，其次是土壤科的芽球菌属(*Blastococcus*)，地嗜皮菌属(*Geodermatophilus*)和纯洁杆菌（*Modestobacter*）.

03/ Bacterial and Actinobacterial Community Structure

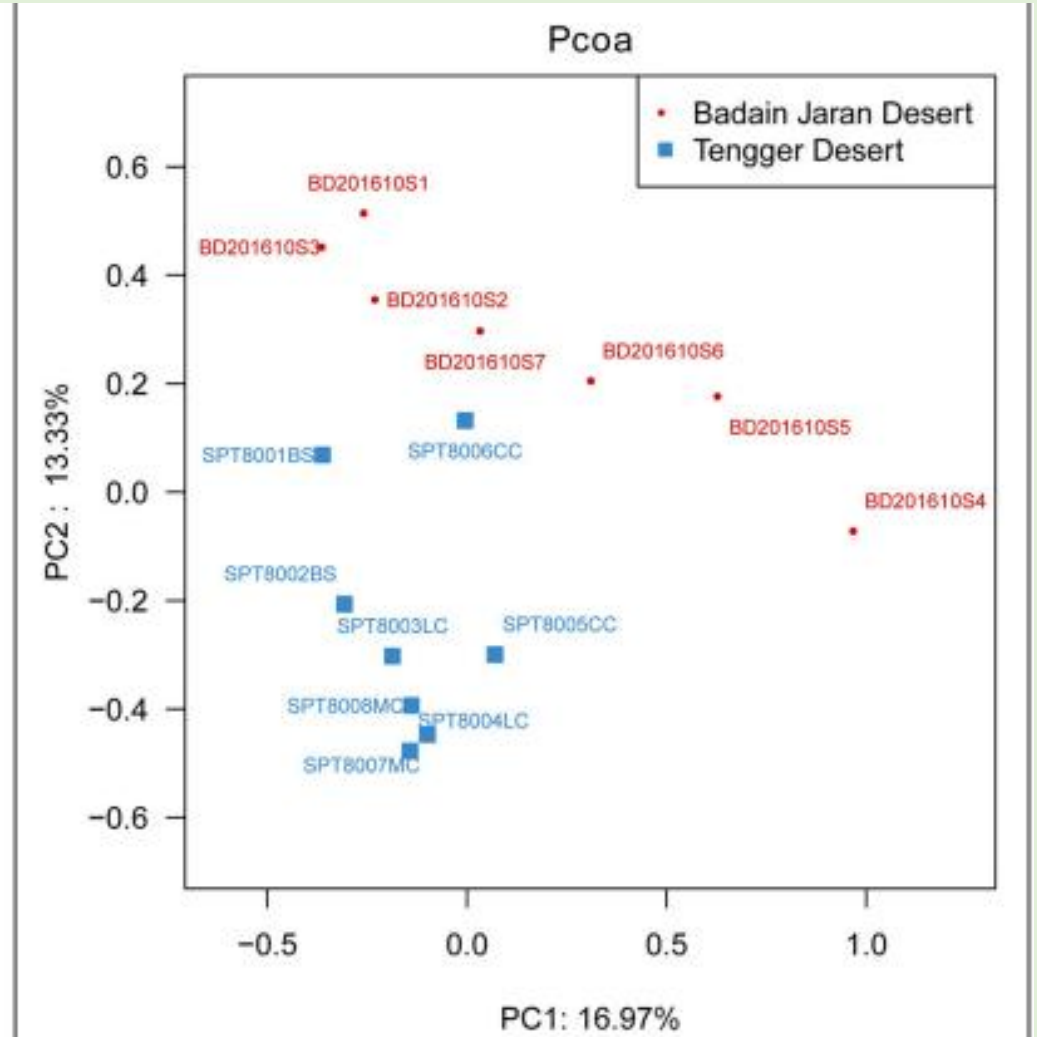


FIGURE 6 | PCoA diagram showing the significant difference in different desert samples.

不同的沙子类型代表了不同的生态系统，通常，细菌群落组成与采样点而非微生态系统类型的相关性更强。

03/ Bacterial and Actinobacterial Community Structure

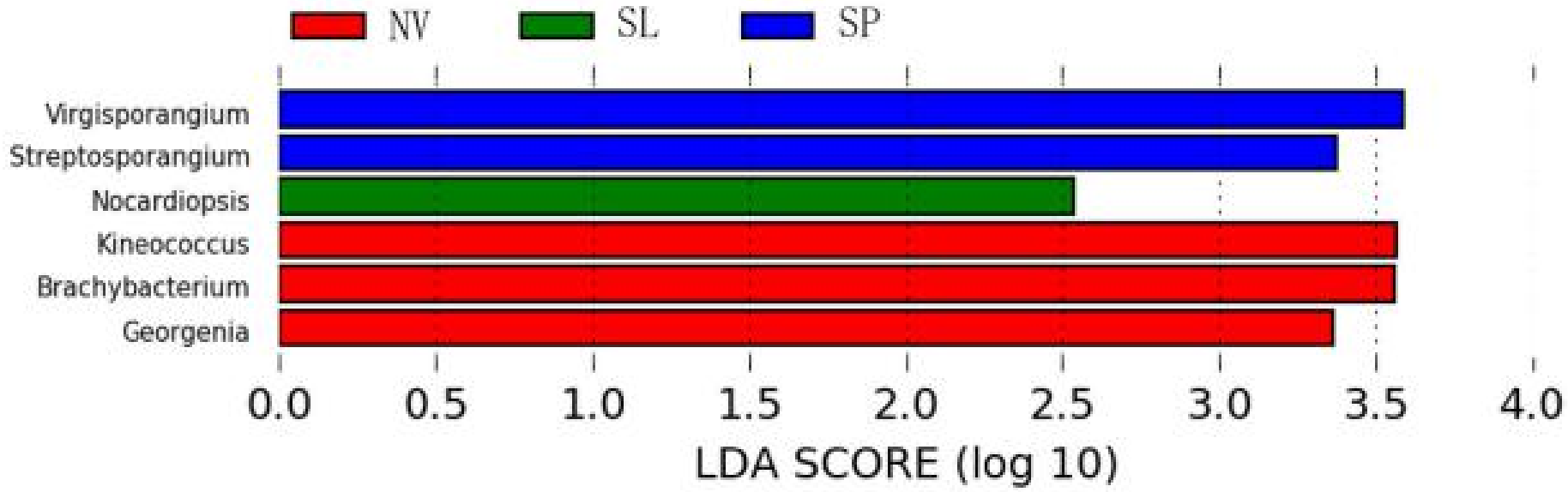
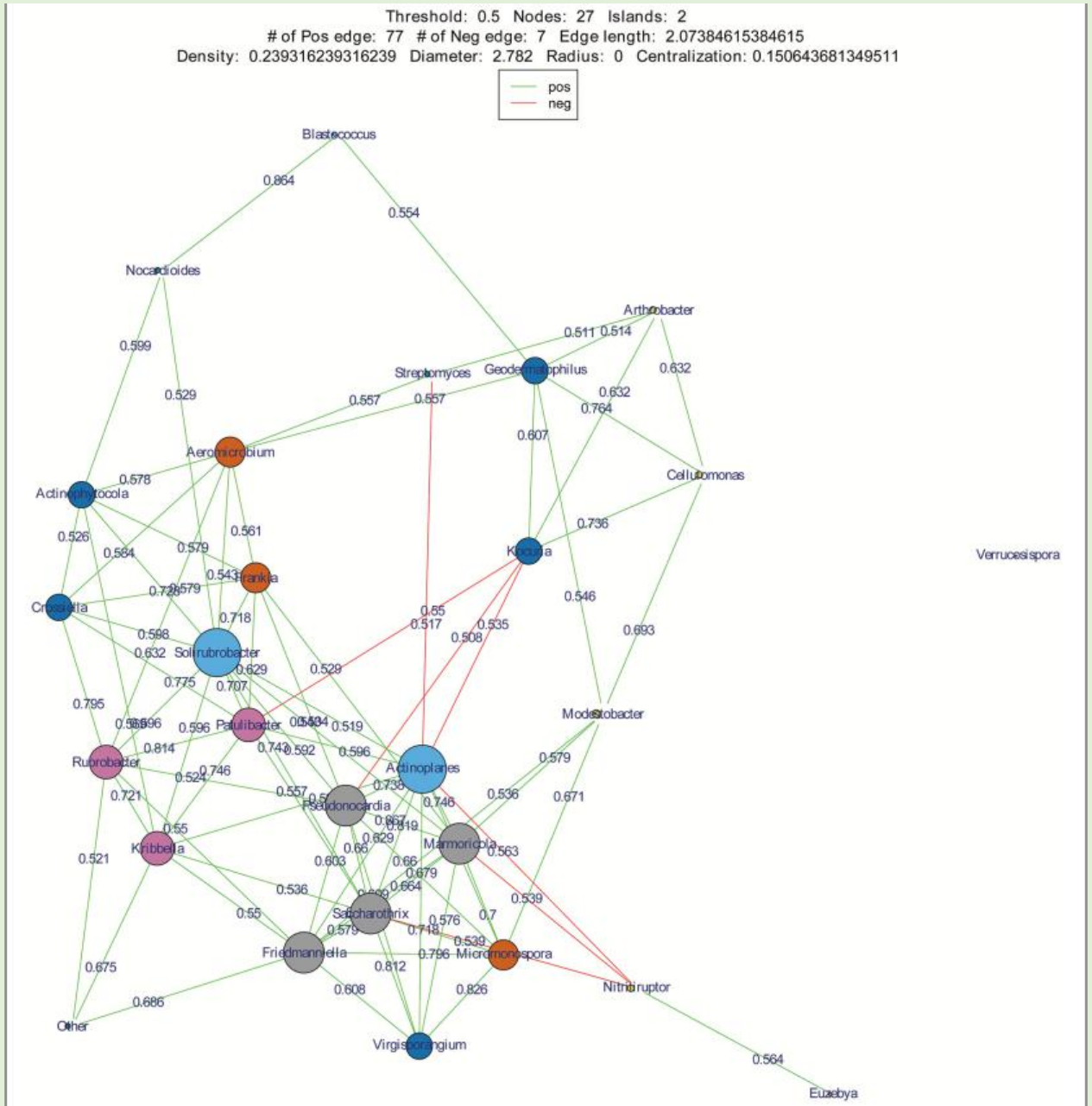
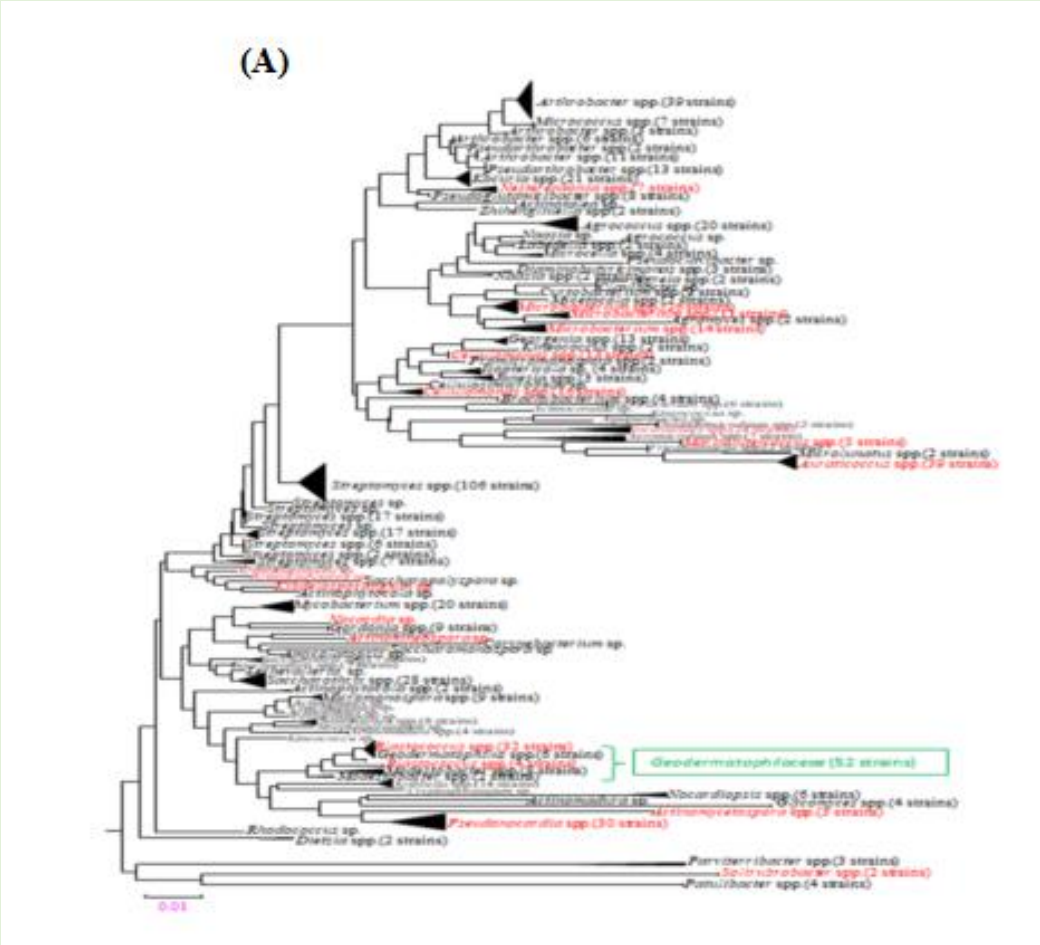


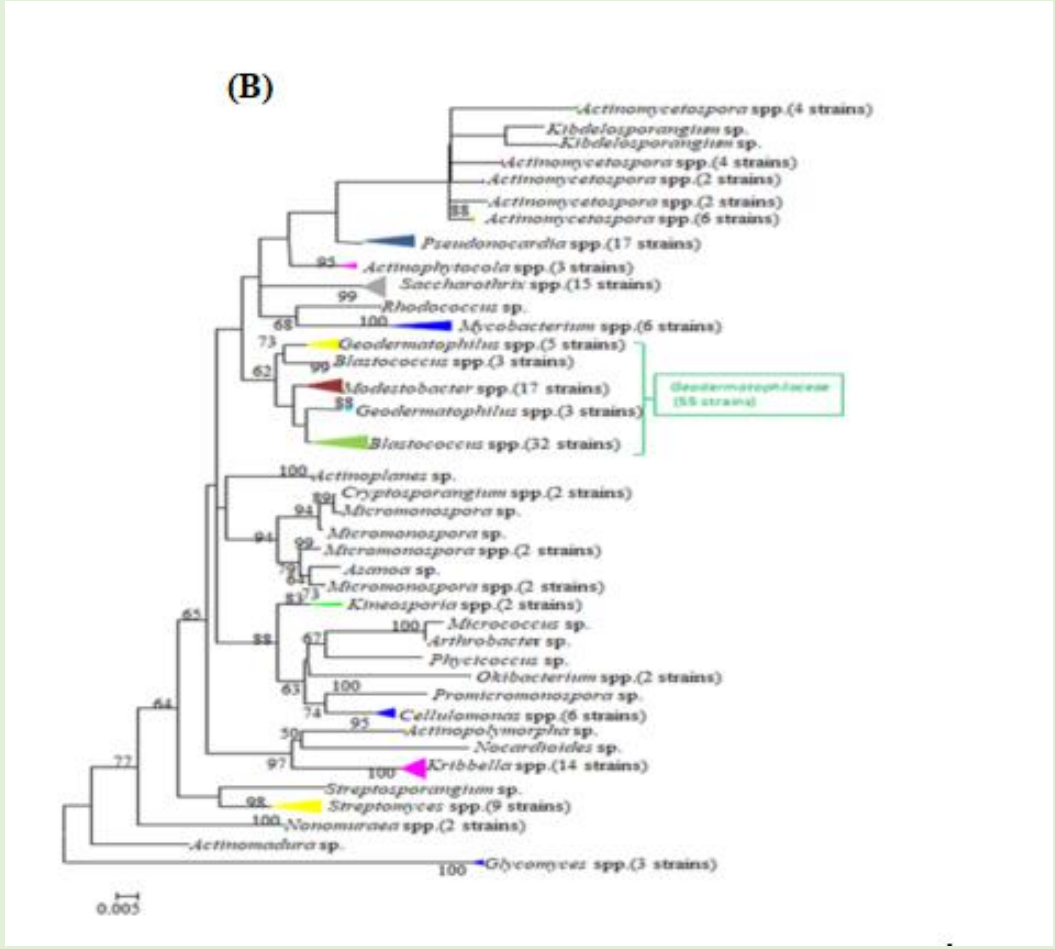
FIGURE 7 | The LEfSe analysis indicating the significant difference among three types of Badain Jaran desert sand samples.

03/ Bacterial and Actinobacterial Community Structure



03/ Isolation of Actinobacterial Strains





BJD: 786株（30科73属）

TGD: 376株（18科29属）

03/ Comparative Analysis of HTS and CULD Results

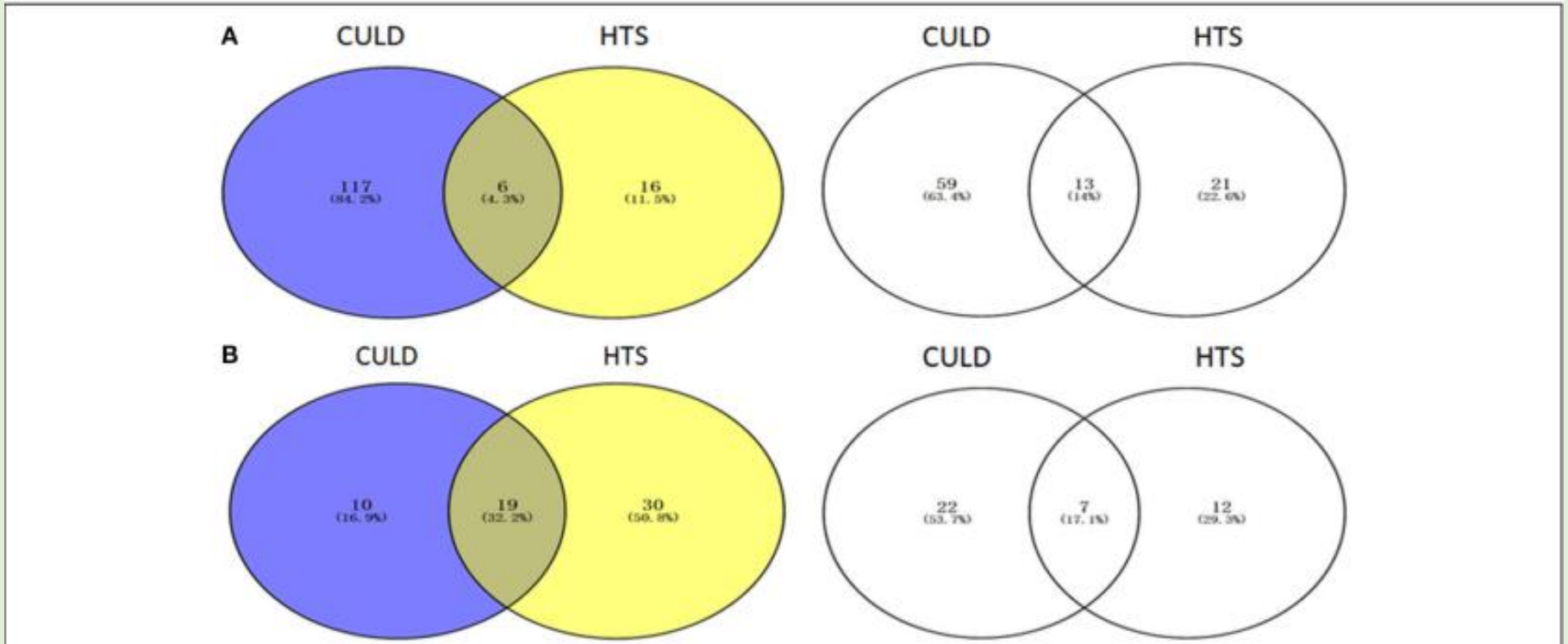


FIGURE 10 | Venn diagrams illustrating the number of genera between High-throughput sequencing analysis and Cultivation-dependent method. **(A)** Badain Jaran desert sands; **(B)** Tengger desert sands. The Venn diagrams were compared by dominant genera (1/3) and rare genera (2/4).

这些结果表明，天然微生物群落的组成不仅受到优势群体的驱动，罕见的分类群也是群落结构积极和不可分割的成员。

03/ Actinobacterial Members in Desert Sands

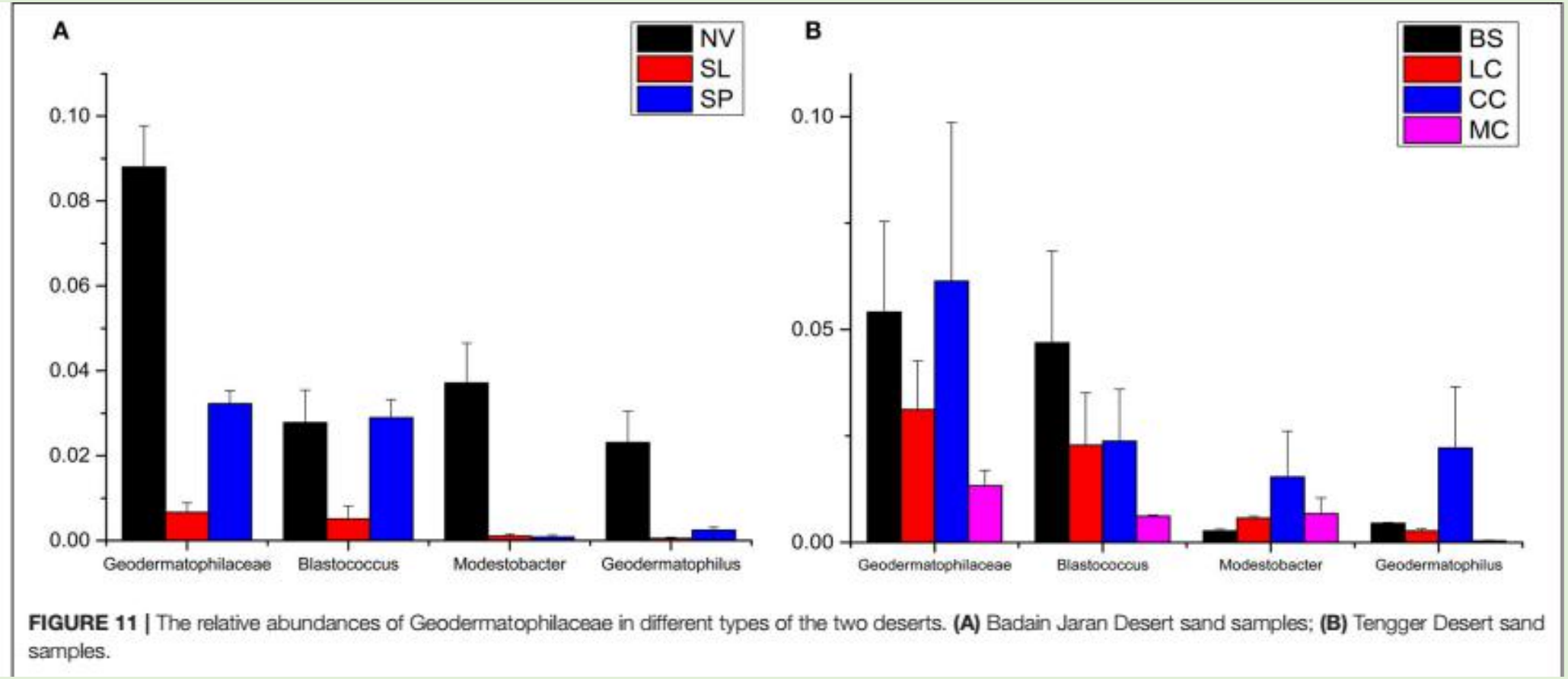


FIGURE 11 | The relative abundances of Geodermatophilaceae in different types of the two deserts. **(A)** Badain Jaran Desert sand samples; **(B)** Tengger Desert sand samples.

04

Conclusion

04/ Discussion

地理屏障在塑造细菌群落方面比微生态类型贡献得更多；另外，即使在同一地形上，土壤微生物的生态服务功能也导致了微观生态系统的区分。

放线菌是沙漠微生物群落中的主要微生物，其中土壤纲普遍存在,其在这种恶劣环境中塑造细菌群落结构中起关键作用。

链霉菌属, 考克氏菌属 和游动放线菌属 之间潜在的负相互作用可能解释了获得的游动放线菌属分离株的缺乏。

请各位老师同学批评指导

THANK YOU