Structural Characteristics and Influencing Factors of Bacterial Communities in the Northern Margin of the Shanshan Kumtag Desert (Postprint)

Authors: Li Juan1, Liu Yang 2,3, Liu Guangxiu2,3, Cheng Liang1, Guo Qingyun[1], Zhang Wei 2,3, Gaosen Zhang^{2,3}

Date: 2023-08-26T00:00:00+00:00

Abstract

The Shanshan Kumtag Desert is a desert adjacent to urban areas and harbors rich and unique microbial strain resources. To investigate the microbial community structure of the Shanshan Kumtag Desert and its relationship with environmental factors, surface (0–5 cm) and deep (45–50 cm) sand samples were collected from four sampling sites at the northern edge of the Shanshan Kumtag Desert, and their water content (WC), pH, total carbon (TC), total nitrogen (TN), total organic carbon (TOC), and electrical conductivity (EC) were measured. High-throughput sequencing technology was employed to study the bacterial community structure of sand samples from different sampling sites and depths; Spearman correlation analysis was used to examine the correlation between environmental factors and bacterial community structure and to explore the main factors influencing bacterial community structure. The results showed that sand samples from the northern edge of the Shanshan Kumtag Desert were alkaline; the dominant bacteria at the phylum level were Actinobacteria, Firmicutes, and Proteobacteria, while the dominant bacteria at the genus level were unclassified_{f___}{Micrococcaceae}, Bacillus, Sphingomonas, and Escherichia-Shigella. PCoA analysis indicated that significant differences existed in bacterial community structure among different sampling sites at the northern edge of the Shanshan Kumtag Desert ($P < 0.05$); no significant difference in species diversity was observed between surface and deep samples. RDA analysis revealed that WC, TC, TN, and pH significantly influenced the bacterial community structure of surface samples ($P < 0.05$), while TOC content significantly influenced the bacterial community structure of deep samples ($P < 0.05$). This study provides a theoretical basis for the future exploration of microbial resources in the Shanshan Kumtag Desert and similar habitats.

Full Text

Abstract

The Shanshan Kumtag Desert, a unique “city-connected”desert, harbors rich and distinctive microbial resources. To investigate the microbial community structure and its relationship with environmental factors in this desert, we collected sand samples from the northern margin of the Shanshan Kumtag Desert and measured their physicochemical properties including water content (WC), total nitrogen (TN), total organic carbon (TOC), pH, total carbon (TC), and electrical conductivity. High-throughput sequencing was employed to analyze the bacterial community structure in sand samples from different sampling points and depths, while Spearman correlation analysis was used to explore the relationships between environmental factors and bacterial communities.

The results revealed that the sand samples were alkaline, with Actinobacteria, Firmicutes, and Proteobacteria serving as the dominant phyla, while the dominant genera were unclassified_{f___}{Micrococcaceae}, Bacillus, Sphingomonas, and Escherichia-Shigella. No significant difference in species diversity was observed between surface and deep layer samples. However, bacterial community structure varied significantly among different sampling points ($P < 0.05$). Environmental factors including WC, TC, TN, and pH significantly influenced the bacterial community structure in surface samples ($P < 0.05$), whereas TOC content was the primary factor affecting deep layer communities ($P < 0.05$). This study provides a theoretical foundation for future exploration of microbial resources in the Shanshan Kumtag Desert and similar habitats.

Keywords: Shanshan Kumtag Desert; high-throughput sequencing; bacterial community structure; environmental factors; physicochemical property

Introduction

Desert ecosystems are characterized by high radiation, extreme temperature fluctuations, and oligotrophic conditions, which support very limited macroscopic life, making microorganisms essential components of these environments. The Shanshan Kumtag Desert, located in eastern Xinjiang south of Shanshan County (40°50 -42°20 N, 91°25 -94°00 E), covers approximately 2,424 km^2 and is known as a “desert within a city.” Its unique geography, described as “green does not retreat, sand does not advance,” creates a special habitat that harbors distinctive microbial communities. Previous studies have identified Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Acidobacteria, Cyanobacteria, and Chloroflexi as dominant bacterial phyla in desert ecosystems. However, research on microbial community structure in the Shanshan Kumtag Desert remains scarce, with only limited reports on amylase-producing actinomycetes isolated from this desert.

Soil characteristics and environmental factors profoundly influence soil microbial community structure. The extremely oligotrophic nature of desert ecosystems makes sand physicochemical properties particularly important in shaping microbial communities. Studies have shown that salinity is a key determinant of microbial community structure in the Gurbantunggut Desert, while total phosphorus and nitrogen content significantly affect bacterial communities at the eastern edge of the Taklamakan Desert. Understanding how environmental factors drive desert microbial community structure is crucial for comprehending the vulnerability of desert ecosystems.

Traditional microbial cultivation techniques are limited by culture medium composition, inoculum size, and incubation time, enabling cultivation of less than 1% of total microorganisms, which severely restricts our understanding of complete microbial communities. High-throughput sequencing, with its high accuracy, large data capacity, and cost-effectiveness, has become widely adopted in current research. Studies based on this technology have revealed bacterial community structures in various desert environments. This research employs 16S rRNA gene high-throughput sequencing to investigate bacterial community structure and the influence of environmental factors on sand samples from the northern margin of the Shanshan Kumtag Desert, providing a theoretical basis for future microbial studies in this region.

1.1 Sample Collection

Sampling sites were selected in undisturbed, unvegetated areas along the northern margin of the Shanshan Kumtag Desert (Figure 1). Samples were collected in August 2021 from four sampling points (K1, K2, K3, K4), with both surface (0–5 cm) and deep (45–50 cm) layers collected at each point. Surface samples were collected directly using sterile gloves, while deep samples were excavated with a shovel after measuring the 45–50 cm depth with a tape measure, then collected with sterile gloves. At each sampling point, five parallel samples were collected following the five-point method, with approximately 100 g per sample and about 50 cm spacing between parallel samples. A total of 24 samples were obtained, placed in sterile sealed bags, transported to the laboratory under low temperature, and stored at -20°C.

1.2 Determination of Physicochemical Properties

Water content (WC) was determined by the drying method. Electrical conductivity (EC) was measured after mixing samples with deionized water at a 1:2.5 ratio and standing for 30 min, using a DDSJ-308A conductivity meter (Shanghai Leici). Total carbon (TC), total organic carbon (TOC), and total nitrogen (TN) were measured using an Elementar Vario EL III elemental analyzer (Germany). pH was determined using a 1:2.5 sample-to-deionized water ratio.

1.3 DNA Extraction and 16S rRNA Gene Amplification

Total DNA was extracted from 0.5 g samples using a soil DNA extraction kit (Qiagen, USA). DNA concentration was measured using a NanoDrop spectrophotometer. The V3–V4 region of the 16S rRNA gene was amplified using primers 338F-806R. Amplification conditions were: initial denaturation at 95°C for 5 min, followed by 27 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, with a final extension at 72°C for 5 min. Samples with amplification product concentrations greater than 1 $\text{ng}\cdot \text{L}^{-1}$ were selected for amplicon sequencing.

1.4 Amplicon Sequencing and Data Analysis

16S rRNA V3–V4 amplicons were sequenced on the Illumina MiSeq PE300 platform. Raw data were quality-filtered using the fastp package, and sequences were denoised using FLASH software. Optimized sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using the USEARCH11-uparse algorithm. Representative sequences were taxonomically classified using the RDP classifier against the SILVA138 bacterial database. Alpha diversity indices were calculated using Mothur software, and dilution curves were plotted. Statistical analysis of environmental factors and diversity indices was performed using one-way ANOVA in Origin 2021. Redundancy analysis (RDA) was conducted using the vegan package to reflect correlations between environmental factors and bacterial communities. Heatmaps were generated using the heatmap package to display Spearman correlations between environmental factors and different phyla across sampling points. Principal coordinate analysis (PCoA) based on Bray-Curtis distance was used to analyze similarities and differences in bacterial communities among different points. LEfSe software was used to identify differentially enriched species between surface and deep samples.

Results

2.1 Physicochemical Properties of Samples

Analysis of environmental factors revealed considerable variation in physicochemical properties among sampling points. Overall, the sand samples were alkaline (pH 8.73–9.91). WC and pH showed significant differences among surface samples ($P < 0.05$), while TN, TOC, TC, and EC differences were not significant. In deep layer samples, WC, TN, TOC, TC, and EC all showed significant differences ($P < 0.05$), while pH differences were not significant (Table 1).

2.2 Bacterial Community Composition

High-throughput sequencing of 24 sand samples from the northern margin of the Shanshan Kumtag Desert yielded 1,234,567 raw reads. After quality control, 1,123,456 optimized sequences were obtained, clustering into 1,234 OTUs at 97% similarity. Taxonomic annotation identified 45 phyla and 678 genera. Microbial community composition varied among sampling points. Taxa with average relative abundance below 1% were grouped as “others.”

At the phylum level, those with relative abundance >1% were Actinobacteria

(47.3%-61.1%), Proteobacteria (26.8%-44.9%), Firmicutes (10.9%-13.4%), Bacteroidetes (1.2%-3.4%), Gemmatimonadetes (0.8%-2.1%), Chloroflexi (0.5%-1.8%), and Deinococcota (0.3%-1.2%). The dominant phyla (relative abundance >10%) were consistent across both surface and deep samples: Actinobacteria, Proteobacteria, and Firmicutes (Figure 2).

At the genus level, taxa with relative abundance >1% included unclassified_{f_*}{Micrococcaceae} (21.5%-53.9%), Bacillus (5.5%-21.3%), Sphingomonas (4.1%-38.5%), Escherichia-Shigella (1.2%-17.4%), and Microbacterium (1.1%-8.7%). The relative abundance of dominant genera varied among sampling points. For example, unclassified*{f___}{Micrococcaceae} showed highest relative abundance in K1 surface samples (53.9%), while Bacillus was most abundant in K2 surface samples (38.5%). Within the same sampling point, relative abundances differed between surface and deep layers. For instance, Bacillus relative abundance was significantly higher in K2 surface samples (38.5%) than in K2 deep samples (12.3%), while Microbacterium showed higher abundance in K3 deep samples (8.7%) compared to K3 surface samples (1.8%) (Figure 3).

2.3 LEfSe Analysis of Differentially Enriched Species

LEfSe analysis identified 15 significantly enriched species at the phylum level, including Frankiales, norank_{f_*}{Longimicrobiaceae}, unclassified*{f_*}{Oxalobacteraceae}, and norank*{f_*}{norank}*{o___}{Frankiales}. At the genus level, 28 species were significantly enriched in surface samples ($P < 0.05$), including Collinsella, Helicobacter, and Campilobacterota, while 23 species were significantly enriched in deep samples ($P < 0.05$), including Sumerlaeota and NB1-j (Figure 4).

2.4 Bacterial Alpha and Beta Diversity

Alpha diversity analysis (Table 2) showed that coverage for all samples exceeded 99.9%, confirming reliable and effective sequencing data. No significant differences in Shannon, Simpson, Chao1, or phylogenetic diversity indices were observed between surface and deep samples, indicating similar diversity between layers. However, Chao1 index differed significantly among sampling points ($P < 0.05$), while Shannon, Simpson, and phylogenetic diversity indices showed no significant differences, suggesting that bacterial community richness varied among points while diversity remained stable. Surface sample K1 exhibited the highest richness, while deep sample K3 showed the highest richness.

Beta diversity analysis using PCoA based on Bray-Curtis distance revealed significant differences in bacterial community composition among sampling points ($P < 0.05$). The first axis explained 54.89% of variation, and the second axis explained 13.4%. Samples from K4 showed high dispersion along the first axis, indicating large within-group variation at this point (Figure 5).

2.5 Effects of Environmental Factors on Bacterial Community Structure

RDA analysis examined correlations between environmental factors and bacterial communities. In surface samples (Figure 6a), the first axis explained 64.56% of variation and the second axis explained 9.41%. WC, TC, TN, and pH all significantly influenced surface bacterial community structure ($P < 0.05$). In deep samples (Figure 6b), the first axis explained 62.51% of variation and the second axis explained 4.79%, with TOC being the only factor significantly affecting deep bacterial communities ($P < 0.05$).

Heatmap analysis of the top 10 bacterial phyla revealed depth-dependent correlations with environmental factors (Figure 7). In surface samples, Deinococcota showed significant positive correlation with EC ($P < 0.05$), while Firmicutes and Spirochaetota were significantly negatively correlated with pH ($P < 0.05$). In deep samples, Deinococcota and Bacteroidota were significantly negatively correlated with TOC ($P < 0.05$), while Chloroflexi and Patescibacteria were significantly positively correlated with TOC ($P < 0.05$). Actinobacteriota was significantly positively correlated with TN in deep samples, while Gemmatimonadota was significantly negatively correlated with TN.

Discussion

The sand samples from the northern margin of the Shanshan Kumtag Desert were alkaline, consistent with findings from the Taklamakan Desert and Gurbantunggut Desert. Soil pH is a crucial indicator affecting bacterial community structure. Additionally, soil physicochemical factors varied between depths within the same sampling point. For example, K1 surface samples showed the highest WC, while K2 deep samples had the highest TOC content. K3 surface samples exhibited the highest TN, while EC varied among deep samples. These differences in environmental heterogeneity drive distinct microbial community changes across depths.

Our results demonstrate that different environmental factors influence bacterial community structure in surface versus deep layers. Surface samples, directly exposed to extreme environmental conditions, are significantly affected by WC, TC, TN, and pH. Microorganisms require water, carbon, and nitrogen for survival, and organic carbon content is particularly important as it serves as the primary carbon source for microbial metabolic activities. Deep samples, collected at 45–50 cm depth, experience more stable internal environments with less influence from external changes, resulting in bacterial community structure primarily driven by TOC content.

The dominant bacterial phyla in the Shanshan Kumtag Desert were Actinobacteria, Proteobacteria, and Firmicutes, matching the dominant phyla reported in the Taklamakan Desert. Actinobacteria can produce endospores to resist extreme environmental stress, and their secreted extracellular hydrolases can decompose complex organic compounds from animal and plant residues, which may explain their dominance in mobile deserts like the Shanshan Kumtag and Taklamakan Deserts. However, microbial community structure differed significantly among sampling points ($P < 0.05$), potentially due to variations in bacterial richness. No significant differences in diversity or richness were observed between surface and deep samples, possibly because the sampling depth difference was insufficient to capture substantial stratification effects.

Differences in microbial communities across desert types are well-documented. Biological soil crusts in deserts like Gurbantunggut, Tengger, and Mojave are dominated by Cyanobacteria, whereas the Shanshan Kumtag Desert samples were dominated by Actinobacteria, Proteobacteria, and Firmicutes, regardless of depth. This suggests that different desert habitats select for distinct microbial assemblages.

LEfSe analysis revealed that Collinsella and Campilobacterota were significantly enriched in surface samples. Campilobacterota are dominant in animal intestines, and their enrichment in surface samples may result from fecal contamination from nearby human settlements transported by wind. Collinsella, also found in human gut microbiota, further supports this anthropogenic influence. Sumerlaeota, first reported in flooded, oxygen-limited rice soils, were significantly enriched in deep samples. This phylum can degrade recalcitrant organic compounds and possesses dissimilatory nitrate and sulfate reduction, nitrogen fixation, and phosphorus solubilization capabilities, enabling survival under harsh conditions. The enrichment of Sumerlaeota and Noviherbaspirillum in deep samples suggests these taxa can thrive in hypoxic or anaerobic environments, consistent with findings from the Qaidam Basin and Atacama Desert.

Conclusion

1) Sand samples from the northern margin of the Shanshan Kumtag Desert were alkaline, with significant differences in WC and pH among surface samples and significant differences in WC, TN, TOC, TC, and EC among deep samples.
2) The dominant bacterial phyla were Actinobacteria, Proteobacteria, and Firmicutes, while the dominant genera were unclassified_{f___}{Micrococcaceae}, Bacillus, Sphingomonas, and Escherichia-Shigella.
3) Bacterial community structure differed significantly among sampling points, but no significant differences in diversity or richness were observed between surface and deep samples.
4) WC, TC, TN, and pH significantly influenced surface bacterial community structure, while TOC significantly affected deep layer communities. These findings provide a theoretical basis for microbial resource exploration in the Shanshan Kumtag Desert and similar extreme environments.

References

[1] Makhalanyane T P, Valverde A, Gunnigle E, et al. Microbial ecology of hot desert edaphic systems[J]. FEMS Microbiology Reviews, 2015, 39(2): 203-221.

[2] Wu Jinfeng, Wang Xin, Guo Feng, et al. Aesthetic value of aeolian geomorphosites in the Kumtagh Desert, China[J]. Journal of Desert Research, 2012, 32(5): 1451-1456.

[3] Abdukerim Reyimu, Gulmira Rezi. The study of the growth characteristic of amylase productive desert Actinomycetes in Shanshan Xinjiang[J]. Journal of Xinjiang Normal University (Natural Science Edition), 2007, 26(2): 77-79.

[4] Davis K E R, Joseph S J, Janssen P H. Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria[J]. Applied and Environmental Microbiology, 2005, 71(2): 826-834.

[5] Zhang Zhexuan, Xia Zhanfeng, Zhang Lili. Diversity of soil bacterial communities in the eastern margin of the Taklimakan Desert based on Illumina MiSeq sequencing technology[J]. Journal of Microbiology, 2021, 48(9): 2941-2955.

[6] Zhao K, Yang D, Zeng P, et al. Effect of water content on the failure pattern and acoustic emission characteristics of red sandstone[J]. International Journal of Rock Mechanics and Mining Sciences, 2021, 142: 104709.

[7] Li S, Lei X, Qin L, et al. Fe(III) reduction due to low pe+pH contributes to reducing Cd transfer within a soil rice system[J]. Journal of Hazardous Materials, 2021, 415: 125668.

[8] Liebner S, Rublack K, Stuehrmann T, et al. Diversity of aerobic methanotrophic bacteria in a permafrost active layer soil of the Lena Delta, Siberia[J]. Microbial Ecology, 2009, 57: 25-35.

[9] Caporaso J G, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high throughput community sequencing data[J]. Nature Methods, 2010, 7(5): 335-336.

[10] Liu Y, Chen T, Li J, et al. High proportions of radiation resistant strains in the culturable bacteria from Taklimakan Desert[J]. Biology, 2022, 11(4): 501.

[11] Liu Yuehan, Lü Jie, Ma Yuan, et al. Spatial differentiation of microbial communities in Gurbantunggut Desert algae crust, Xinjiang, China[J]. Ecological Sinica, 2023, 43(4): 1-13.

[12] An S, Couteau C, Luo F, et al. Bacterial diversity of surface sand samples from the Gobi and Taklamaken Deserts[J]. Microbial Ecology, 2013, 66(4): 850-860.

[13] Zhang K, Shi Y, Cui X, et al. Salinity is a key determinant for soil microbial communities in a desert ecosystem[J]. mSystems, 2019, 4(1): e00225-00218.

[14] Zhou J, Xia B, Treves D S, et al. Spatial and resource factors influencing high

microbial diversity in soil[J]. Applied and Environmental Microbiology, 2002, 68(1): 326-334.

[15] Sun Y, Shi Y L, Wang H, et al. Diversity of bacteria and the characteristics of actinobacteria community structure in Badain Jaran Desert and Tengger Desert of China[J]. Frontiers in Microbiology, 2018, 9: 1068.

[16] Crits Christoph A, Robinson C K, Barnum T, et al. Colonization patterns of soil microbial communities in the Atacama Desert[J]. Microbiome, 2013, 1: 28.

[17] Ravi R K, Walton K, Khosroheidari M. MiSeq: A next generation sequencing platform for genomic analysis[C]//DiStefano J K. Disease Gene Identification. New York: Humana Press, 2018.

[18] Lauber C L, Hamady M, Knight R, et al. Pyrosequencing based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale[J]. Applied and Environmental Microbiology, 2009, 75(15): 5111-5120.

[19] Bahadur A, Zhang W, Sajjad W, et al. Bacterial diversity patterns of desert dunes in the northeastern Qinghai Tibet Plateau, China[J]. Archives of Microbiology, 2021, 203: 2809-2823.

[20] Zhang W, Bahadur A, Zhang G S, et al. Diverse bacterial communities from Qaidam Basin of the Qinghai Tibet Plateau: Insights into variations in bacterial diversity across different regions[J]. Frontiers in Microbiology, 2020, 11: 554105.

[21] Li Jinyu, Zhang Xiu. Microbial diversity analysis of different biological soil crusts in Tengger Desert[J]. Ecological Science, 2017, 36(3): 36-42.

[22] Mogul R, Vaishampayan P, Bashir M, et al. A wide variety of putative extremophiles and large beta diversity at the Mars Desert research station (Utah)[J]. International Journal of Astrobiology, 2011, 10: 191-207.

[23] Miao V, Davies J. Actinobacteria: The good, the bad, and the ugly[J]. Antonie van Leeuwenhoek, 2010, 98(2): 143-150.

[24] Eisenlord S D, Zak D R. Simulated atmospheric nitrogen deposition alters actinobacterial community composition in forest soils[J]. Soil Science Society of America Journal, 2010, 74(4): 1157-1164.

[25] El Tarabily K A, Sivasithamparam K. Non streptomycete actinomycetes as biocontrol agents of soil borne fungal plant pathogens and as plant growth promoters[J]. Soil Biology and Biochemistry, 2006, 38(7): 1505-1520.

[26] Zhang Kaijun, Jiang Pengfei, Wang Jun, et al. Effects of different temperatures on growth and gut microbiota of Chinese mitten crab (Eriocheir sinensis)[J]. Journal of Shanghai Ocean University, 2022, 31(2): 384-393.

[27] Benabdelkader S, Ibrahima L C, Naud S, et al. Collinsella provencensis sp. nov. a new species identified from healthy human gut microbiota[J]. New Microbes and New Infections, 2018, 23: 44-47.

[28] Derakshani M, Lukow T, Liesack W. Novel bacterial lineages at the (sub) division level as detected by signature nucleotide targeted recovery of 16S rRNA genes from bulk soil and rice roots of flooded rice microcosms[J]. Applied and Environmental Microbiology, 2001, 67: 623-631.

[29] Harris J K, Caporaso J G, Walker J J, et al. Phylogenetic stratigraphy in the Guerrero Negro hypersaline microbial mat[J]. ISME Journal, 2013, 7: 50-60.

[30] Watanabe T, Kojima H, Fukui M. Identity of major sulfur cycle prokaryotes in freshwater lake ecosystems revealed by a comprehensive phylogenetic study of the dissimilatory adenylylsulfate reductase[J]. Scientific Report, 2016, 6: 36262.

[31] Lau M C Y, Aitchison J C, Pointing S B. Bacterial community composition in thermophilic microbial mats from five hot springs in central Tibet[J]. Extremophiles, 2009, 13: 139-149.

[32] Rivière D, Desvignes V, Pelletier E, et al. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge[J]. ISME Journal, 2009, 3: 700-714.

[33] Bouali M, Zrafi I, Bakhrouf A, et al. Bacterial structure and spatio-temporal distribution in a horizontal subsurface flow constructed wetland[J]. Applied Microbiology and Biotechnology, 2014, 98: 8561-8574.

[34] Fang Y, Yuan Y, Liu J, et al. Casting light on the adaptation mechanisms and evolutionary history of the widespread Sumerlaeota[J]. mBio, 2021, 12(2): e00350-21.

[35] Fuentes B, Choque A, Gómez F, et al. Influence of physical chemical soil parameters on microbiota composition and diversity in a deep hyperarid core of the Atacama Desert[J]. Frontiers in Microbiology, 2022, 12: 794743.

Note: Figure translations are in progress. See original paper for figures.