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Relationship between microbiota classification and working conditions of sequencing biofilm batch reactor process in Tibetan habitat

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ABSTRACT

Although sequencing biofilm batch reactor (SBBR) treatment has been extensively studied, there is a lack of research relating to the variation of the process microbiota's operating conditions in plateau habitat. The purpose of this study is to construct an SBBR process reaction device in the laboratory, and use high-throughput sequencing to characterize the microbiota structure and to predict its functional characteristics. The results suggested that the diversity, structure, composition, and predictive function of microorganisms in the SBBR process differ significantly between working conditions (Welch *t*-test; $P < 0.05$). By comparing different reactors, linear discriminant analysis (LDA) effect size (LEfSe) analysis showed that pool 1 is significantly enriched of *Propioniciclava*, *Gemmobacter*, *Simplicispira*, *Rhodocyclaceae*, *Intrasporangiaceae*, and *Rhodocyclaceae*. Pool 2 is significantly enriched of *Candidatus Moranbacteria*, *Vicinamibacteraceae*, *Meiothermus*, *Reyranella*, *SC-I-84*, *Microtrichaceae*, *Caldilineaceae*, *Rhizobiales* (LDA > 3). In addition, all samples are enriched of *Propioniciclava*, *Gordonia*, *AKYH767*, *Bacteroidetes_vadinha17*, and *Romboutsia*. About 40% of the dominant microflora are significantly correlated with each change in operating conditions, while about 60% of the dominant microflora are not significantly correlated with changes in the corresponding operating conditions. The results of this study provide theoretical support for the SBBR process's operation mechanism in plateau habitat.

Keywords: Tibetan habitat; Microbiota; Microbial function; High throughput sequencing; KEEG

1. Introduction

Tibet is renowned as the world's third pole, with an average altitude of more than 4,000 m. Tibet's distinct environment is characterized by a wide temperature range between day and night, alpine areas, low atmospheric pressure, and high ultraviolet intensity. In recent years, a large number of sewage treatment facilities have been built to deal with residential wastewater, however, these facilities focus mostly on activated sludge treatment processes such A^2O and AO [1–4] with little research on the sequencing biofilm batch reactor (SBBR) process [1–3].

At present, by using single-factor control of A^2O process in accordance with the unique ecological environment of the Tibetan region, Hao et al. [2] have analysed the efficiency of the A^2O process in disposing of wastewater under a stepwise temperature variation working condition. Meanwhile, Zong et al. [3] used Illumina MiSeq high-throughput sequencing technology to further explore the microbiota structure and its response mechanism in the

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A^2O process under different temperature variations [1]. For the strong UV environment, Hao et al. [4] explored the effect of UV irradiation intensity and duration on water quality changes in the wastewater treatment by using UV lamps of different intensities at the same wavelength and stepped control of the irradiation duration for septic tank effluent. Zong et al. [5] explored the changes in the microbiota structure of the A^2O process under different durations of UV irradiation at fixed intensities by exposing the aerobic tank to UV lamps of 40 W intensity with durations of 0, 5, 10, 30 and 180 min. So far, most studies in the plateau region have been conducted with the A^2O process, while few dedicated to SBBR treatment process. The SBBR process is significantly different from the A^2O process in terms of process and test device, which may lead to microbiota structure differences between the SBBR process and the A^2O process.

SBBR is a sequencing batch biofilm treatment process based on activated sludge SBR which increases the formation of filler film and has better impact resistance than SBR [6]. The biofilm reactor's periodic discontinuous intermittent mode operation leads to a more uniform biomass distribution throughout the reactor and is able to handle a higher impact load [7]. Specific SBBR reactors are able to provide continuous biodegradation of phenol [8]. SBBR facilitates anoxic conditions where the biofilm can absorb and store carbon, allowing for efficient nitrification and denitrification [9]. Feng et al. [10] explored the simultaneous nitrification-denitrification mechanism and the microbiota structure within the system under various intermittent microaeration cycles (IMCs) and oxygen-limited intermittent conditions with COD/N ratios by establishing an SBBR system with partially coupled rice hulls. Liu et al. [11] investigated the denitrification effect of SBBR system on low C/N wastewater through acid and alkali pretreatment of corn cobs as an external carbon source and found that alkali pretreatment with corn cobs as carbon source effectively improved the denitrification performance of low C/N ratio wastewater. Wu et al. [12] explored the domestication and identification of cold-tolerant microorganisms in SBBR reactors by applying gradient-type temperature reduction control to the SBBR reactors. These studies show that the SBBR process offers promising prospects in various areas such as nitrification denitrification, special wastewater treatment, shock load resistance and low temperature resistance. In summary, the study of the microbiota structure of the SBBR process under different working conditions in the plateau region is of remarkable significance for the practical exploration of the SBBR process.

In this study, we built an SBBR reactor under plateau conditions and used 16S rRNA gene high-throughput sequencing and metabolomics techniques to characterize the diversity and functional potential of SBBR process microbiota in plateau habitats, providing theoretical support for the mechanistic study of SBBR process in plateau habitats.

The purposes of this study are: (1) Characterize the structure and diversity of microbiota in SBBR reactor at ambient and low temperature, and UV irradiation. (2) Explore the correlation between the impact of microbiota under different working conditions of SBBR process. (3) Analyze the predictive function of the microbiota of the SBBR process under different working conditions. The results of the study provide theoretical foundation for the mechanistic study of SBBR reactors in plateau habitats.

2. Material and methods

2.1. Test device

The SBBR device in this test is made of Plexiglas, and is set up with two reaction devices. The size of the reactor is 400 mm × 400 mm × 600 mm with an effective volume of 64 L. The reactor uses activated carbon particles with a radius of 1.5 mm and a length of 20 mm as the biofilm filling material, with a filling rate of 50%. The bottom of the reactor is aerated by an air pump connected to a microporous aeration disc. The overall structure of the reactor is shown in Fig. 1.

2.2. Sample collection

The campus domestic sewage is used as the test influent, and sludge from the campus sewage pipeline is added to reactor 1 to ensure that the biofilm flora formed by the SBBR process can reflect actual growth. The SBBR process operates on a cycle of 5.5 h aeration, 4 h agitation, 2 h sedimentation and 0.5 h idling, for a total of 12 h, with *T*: 25°C ± 0.2°C, pH: 6.86~7.87, DO: 2.2~4.69 mg/L and UV: 0 W as environmental factors for culture and film hanging. On day 54 of operation, the system was observed to be running well with a more visible biofilm presence. The biofilm in the system was eluted and sampled according to Zhou's method [13], and three parallel samples were taken from each reactor. The measured biofilm amounts in cell 1 were 35.6, 35.2 and 34.8 mg/g with a mean value of 34.87 and a standard deviation of 0.33, while the biofilm amounts in cell 2 were 34.9, 35.4 and 35.1 mg/g with a mean value of 35.13 mg/g and a standard deviation of 0.21. When operating normally, the biofilm amounts in both reactors reached 34.7 mg/g or more [4].

This test designed with three operating conditions, which are as follows: working condition 1 is set to operate at ambient temperature. The SBBR process was run at 25°C, UV: 0 W, irradiation duration 0 s and the microbiota structure of the SBBR process was analysed at ambient temperature. Working condition 2 is set to operate at a lower temperature. The SBBR process was run at a temperature setting of 15°C without UV irradiation and the microbiota structure of the SBBR process was analysed at ambient temperature. Working condition 3 is set to operate at ambient temperature and the microbiota structure of the SBBR process was analysed at a lower temperature. The microbiota structure of the SBBR process changes under fixed UV irradiation. UV irradiation is performed using a UV lamp with a radiation wavelength of 253.74 nm. The UV irradiation intensity is set to 10 W, the irradiation time set to 5 s, and the operating temperature set to 25°C.

Following the method of the relevant literature [13], six samples of biofilm elution were taken from randomly selected fillers of the two reactors under 25°C, 15°C and UV 10 W 5 s operating conditions to form T_25_1, T_25_2, T_15_1, T_15_2, UV_10_1 and UV_10_2, respectively.

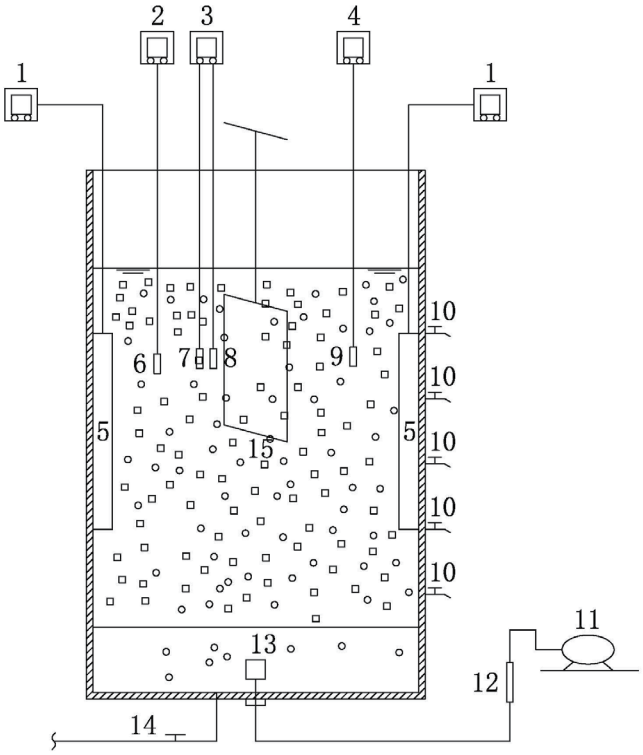


Fig. 1. Process diagram. 1 - UV Controller; 2 - pH probe; 3 - Temperature probe; 4 - Dissolved oxygen probe; 5 - UV lamp; 6 - pH probe; 7 - Temperature probe; 8 - Temperature controller; 9 - Dissolved oxygen probe; 10 - Intake; 11 - Aeration pump; 12 - Float flow meter; 13 - Aeration tray; 14 - Drain; 15 - Stirrer.

2.3. Test method

Three groups of samples (two from each group depending on the reactor, for a total of six samples) were formed on days 74, 102 and 123 in the SBBR process system under different working conditions in the plateau habitat. The DNA of the extracted samples was extracted with a genome extraction kit and PCR amplification was performed. The PCR products were purified using a DNA gel extraction kit, quantified by Qubit and qPCR, and sequenced on the Illumina MiSeq platform with the aid of Meguiar's Biologicals Ltd. for 16S rRNA sequencing.

2.4. Analytical method

The raw data obtained from Miseq sequencing were first spliced based on overlap relationships, while sequence quality was quality controlled and filtered. Clustering was performed by the USEARCH7-uparse algorithm, with sequences grouped according to operational taxonomic units (OTUs) and a 97% threshold (UPARSE (7.0.1090)) (Caporaso et al, 2010), using the SILVA bacterial database (Bremen, Germany). Information on the abundance, cover and diversity of species in the communities was obtained through diversity index analysis. To investigate the differential relationship between different sample community structures, a cluster analysis was performed on the sample community distance matrix to construct a sample hierarchical clustering tree. β-diversity was mapped using the R Language (version 3.3.1). The sample hierarchical clustering alculated the beta diversity distance matrix via the software Qiime and the sample distance Heatmap map uses FastTree (version 2.1.3 http://www.microbesonline.org/fasttree/) to construct an evolutionary tree based on the maximum likelihood method. Then FastUniFrac (http://UniFrac.colorado.edu/) was used to analyse the inter-sample distance matrix, and PCoA analysis was performed using the R Language (version 3.3.1) for PCoA statistical analysis and graphing. LEfSe (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload) performed linear discriminant analysis (LDA) on samples from different grouping conditions based on taxonomic composition to identify communities or species that have a significant differential impact on sample delineation. PICRUSt function prediction starts by normalising the OTU abundance table by PICRUSt and then obtaining the COG family information and KEGG Ortholog (KO) information corresponding to the OTU by using the greengene id corresponding to each OTU. Then the abundance and KO abundance of each COG were calculated. For Pathway, using PICRUSt, information on the 3 levels of the metabolic pathway can be obtained and a table of the abundance of each level is obtained separately.

3. Results

3.1. Diversity and structure of bacterial community

We obtained 311,350 effective lengths from 6 samples after filtering the raw data. After random resampling, we clustered the non-repetitive sequences (excluding single sequences) premised on 97% similarity and removed the chimera all across the clustering process. We obtained 1495 representative OTU sequences. The Simpson and Shannon curves were stable and the Rank-Abundance curve decreased with smooth changes (As shown in S1, S2). These results show that the most of the biodiversity in the sample has been detected.

In this study, the estimated abundance of Sobs and Chao and the Shannon > Simpson's diversity index (Fig. 2A–D;) were used to evaluate the abundance and diversity of the bacterial community in six samples (T_25_1, T_25_2, T_15_1, T_15_2, UV_10_1, UV_10_2) from two reactors under three working conditions respectively. It's worth noting that the abundance and diversity indices show that the bacterial community in pool 1 after inoculation of the original substrate was slightly lower than the pool 2 sample under all three working conditions. In addition, the richness and diversity are greater at 25°C in the same reactor than at 15°C, and both are less abundant and diverse under the influence of ultraviolet light than under temperature control. The four diversity indices were compared with studies related to SBR reactors under low C/N ratio operating conditions in non-plateau habitats. The Chao of SBBR reactors in plateau habitats ranged from 1,206.68~1,320.13, significantly higher than the Chao of SBR reactors in non-plateau habitats which range from 624~922 [14], indicating that the SBBR process in plateau habitats may have a higher total number of species. Meanwhile, the Simpson

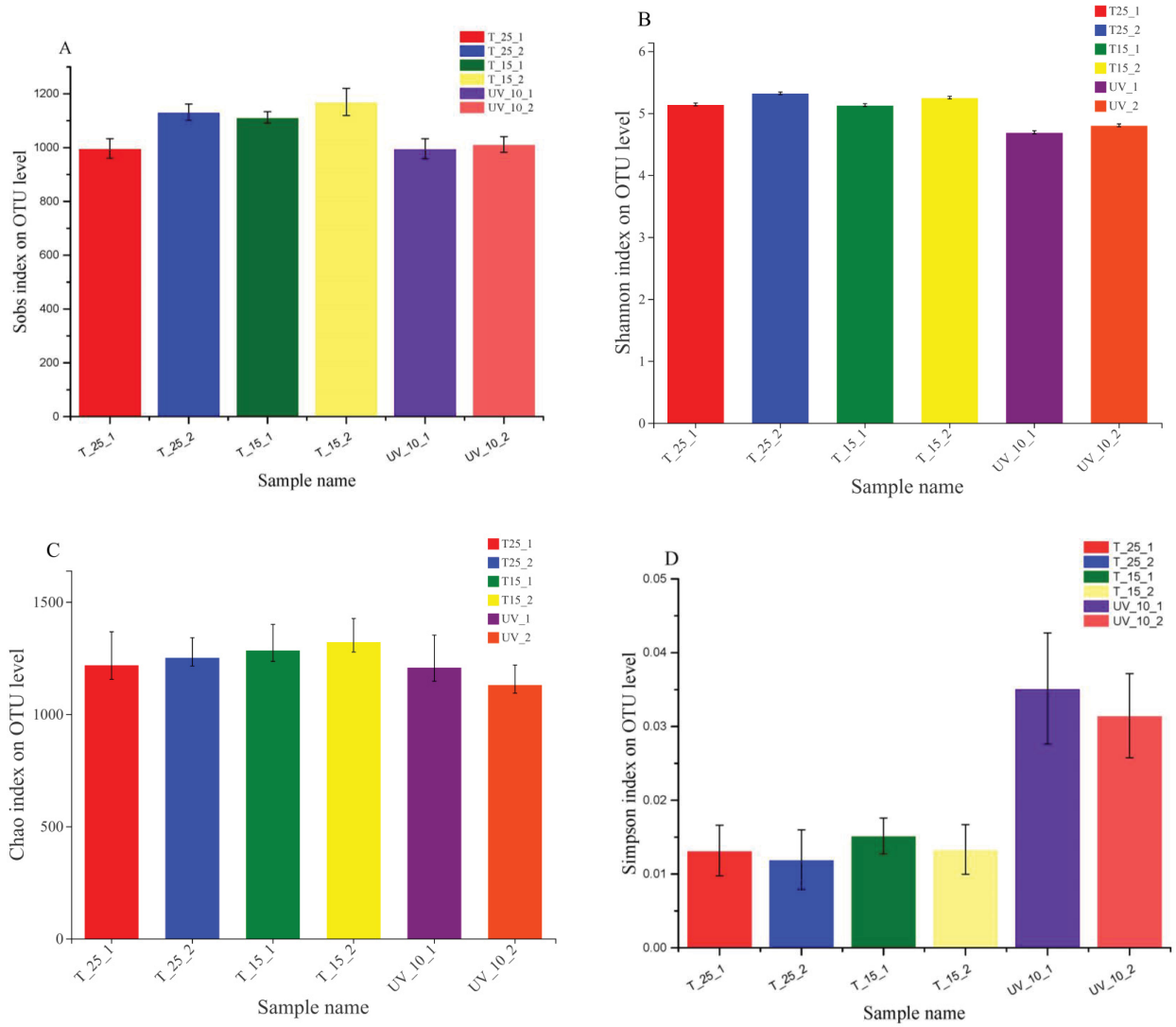


Fig. 2. Comparison of α diversity indices of bacterial communities under different working conditions. (A) Comparison of bacterial indices of bacterial communities in different working conditions. (B) Comparison of Shannon indices of bacterial communities in different working conditions. (C) Comparison of Chao indices of bacterial communities in different sites. (D) Comparison of Simpson's index for bacterial communities at different sites. Higher Sobs and Chao values indicate higher abundance, while higher Shannon values and lower Simpson values indicate higher diversity.

index (0.012~0.035) is higher than that of SBR reactors in non-plateau habitat (0.82~0.96), while the Shannon index (4.68~5.31) was in the range of SBR reactors in non-plateau habitat (4.59~6.76) [14], indicating that SBBR in plateau habitat may have good biodiversity. The Simpson index (0.012~0.035) was also higher than that of SBR reactors in non-upland habitats (0.82–0.96), while the Shannon index (4.68~5.31) was between SBR reactors in non-upland habitats (4.59~6.76) [14], then suggesting that SBBR in upland habitats may have excellent biodiversity.

Multi-factor statistical analysis was carried out to compare the overall structure of the communities at different sites for different working conditions. The PcoA plot visualisation showed a significant separation of the bacterial communities in the two reactors at different working conditions (Fig. 3A). Overall, PCoA's two principal coordinates explained of the variation between all samples. The hierarchical clustering tree at the out level (Fig. 3B) shows that bacterial communities under different working conditions first form one branch with temperature, and then form another branch with ultraviolet influence. The dominant OTUs in the six samples were OTU1160, OTU862, OTU227, OTU1197 and OTU1141. Among them, OTU1197 and OTU1141 have a significantly larger share under the influence of UV compared to the temperature conditions.

3.2. Taxonomic composition of bacterial community

We classified the phylogeny of all sample sequences into 37 phyla, 94 classes, 192 orders, 325 families, 637 genera, 1032 species, and 1495 OTU based on measured data. *Actinobacteria*, *Proteobacteria*, and *Firmicutes* are

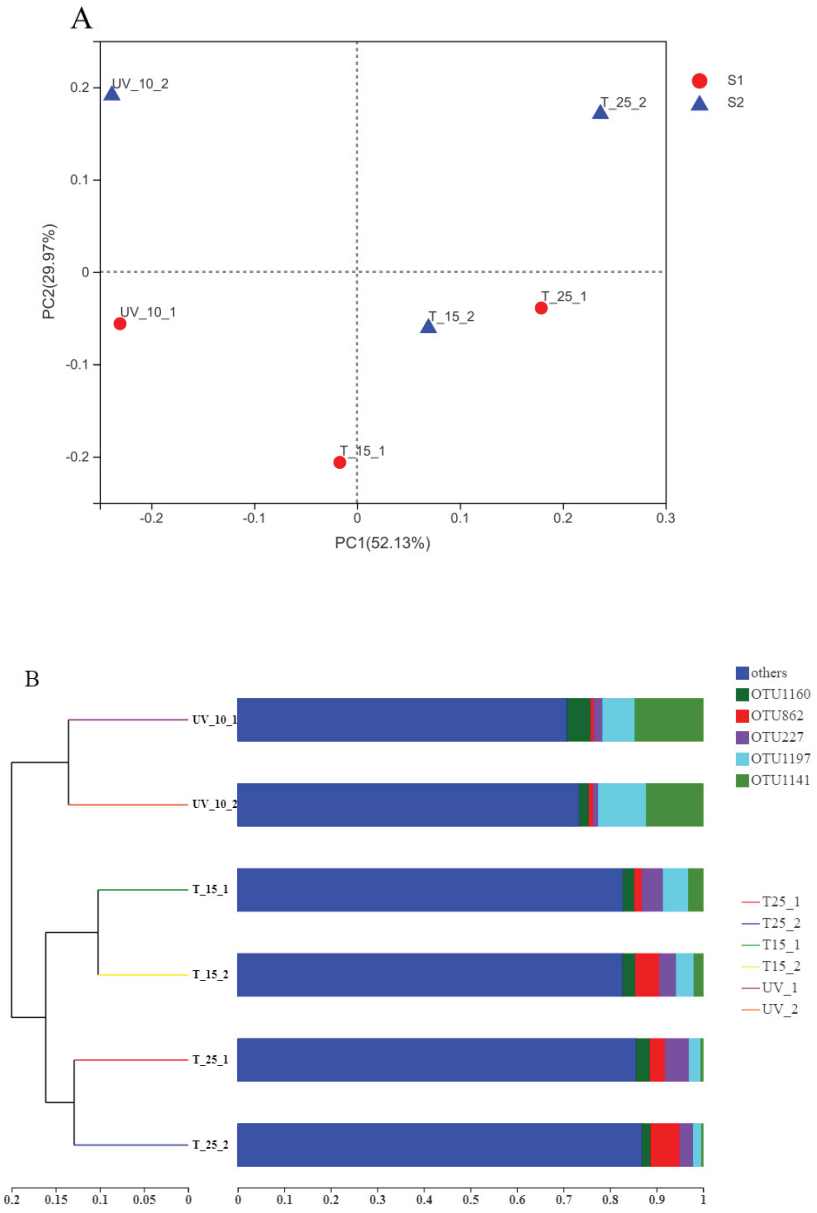


Fig. 3. Structural analysis of the bacterial community under different working conditions. (A) Principal coordinate analysis based on the Bray–Curtis metric for bacterial communities. Percentages indicate the relative contribution of principal components. (B) Hierarchical clustering tree based on the Bray–Curtis metric for bacterial communities.

dominated at the phylum level, accounting for 21.6%~30.4%, 16.1%~37.9% and 8.1%~27.4% of the six samples, respectively (Fig. 4A). It's worth noting that *Proteobacteria* are the dominant bacteria when the temperature is 25°C. The proportion of *Proteobacteria* decreases as the temperature drops, while *Firmicutes* increases. The proportion of *Firmicutes* increases when exposed to ultraviolet light. *Desulfobacterota* (*Desulfovibrio*) in particular is highly sensitive to ultraviolet light and an increase in the percentage can be clearly seen in the graph. But it is less responsive to changes in temperature. In addition, as comparing the inoculated substrate culture samples with the non-inoculated samples, we found that the proportion of *Actinobacteria* (30.4%) and *Firmicutes* (12%) in the inoculated sediment samples was greater in the inoculated substrate samples than in the non-inoculated samples (*Actinobacteriota* 21.6%, *Firmicutes* 8.1%).

At genus level, the larger proportion of all six samples was *Propioniclava*, followed by *Gordonia*, *norank_f__AKYH767*, *norank_f__Bacteroidetes_vadinHA17*, and *Romboutsia* (Fig. 4B). *Desulfobulbus*, *Propioniciclava*, *Romboutsia* and *Christensenellaceae_R-7_group* dominated the two samples both under UV conditions, with *Desulfobulbus* and *Propioniclava* making up the largest proportion of the samples. These bacterial genera have been reported to have the ability to produce, for example, propionic acid, decompose organic matter, and act as natural polymers. Thus these

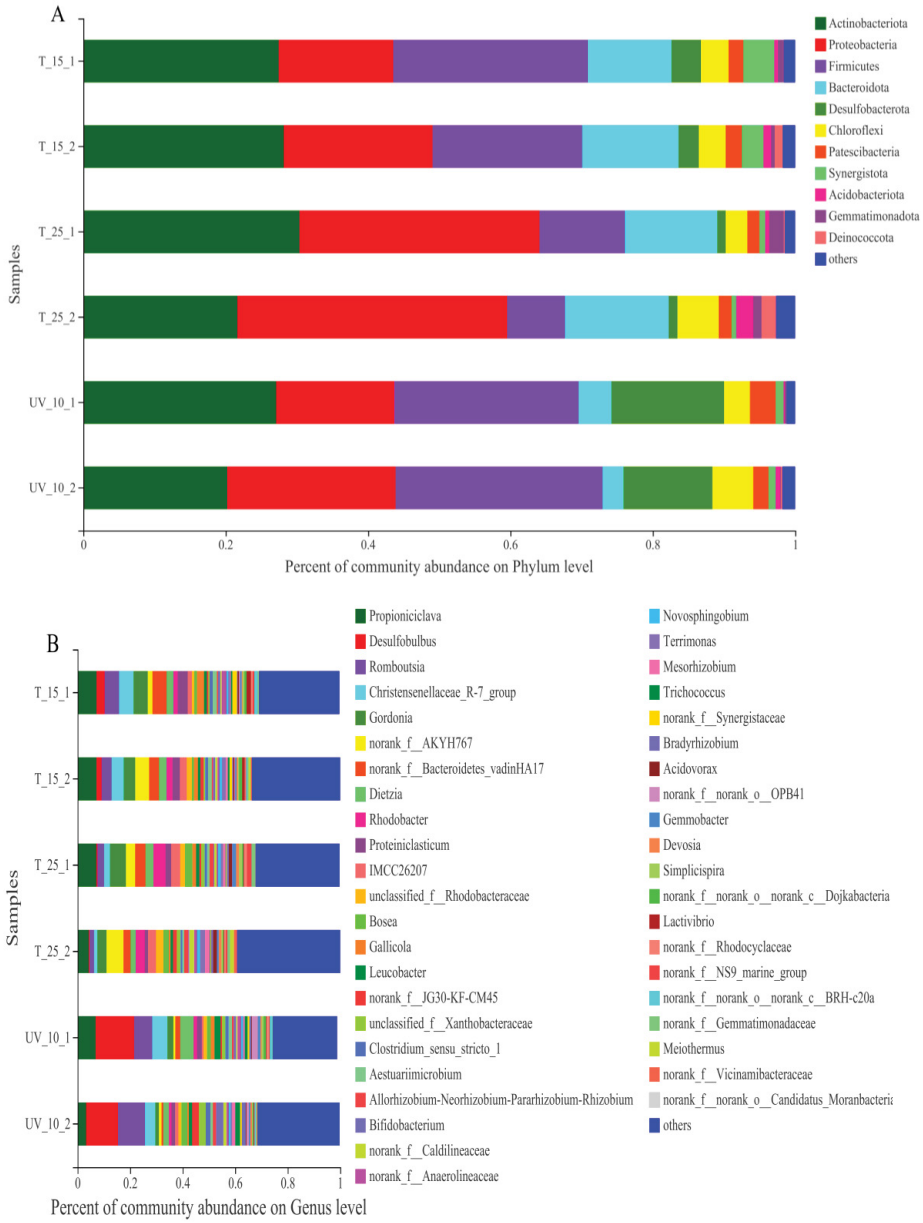


Fig. 4. Distribution of bacterial community in all 6 samples at (a) phylum level or (b) genus level.

genera may have a large contribution to make in carbon metabolism.

3.3. Differences of bacterial communities under different working conditions

The microflora of six working conditions is distinguished by collinear network analysis at the OTU level (abundance 50). (Fig. 5A). It is clear that the most of the OTUs are shared. Reactor 1 at 15°C are similar to the OTU of pool 2 under ultraviolet light, while at 25°C, it is similar to pool 1, suggesting that these three samples have similar core species that are different from pool 2 at 25°C, pool 1 at 15°C, and pool 1 under ultraviolet light.

We further confirmed the existence of different OTUs in samples from different reactors. LEfSe identified 19 distinguishing features (LDA threshold > 3) between the two reactors, of which 13 OTUs were significantly enriched in pool 2 (Fig. 5B) and conversely only 6 were significantly enriched in pool 1. Samples from pool 1 were characterized by the predominance of *Propioniclava* OTU1443, *Gemmobacter* OTU1152, *Simplicispira* OTU97, OTU1267 of *hocyclaceae*, OTU1272 of *Intrasporangiaceae*, and OTU755 of *Rhodocyclaceae*. Pool 2 samples were characterized by OTU877 from *JG30-KF-CM45*, OTU11 from *Candidatus Moranbacteria*, OTU573 from *Vicinamibacteraceae*, OTU1337 from *Meiothermus*, OTU412, OTU1284 from *Reyranella*, OTU1284 from un OTU1248 of ungrouped *SC-I-84*, OTU779

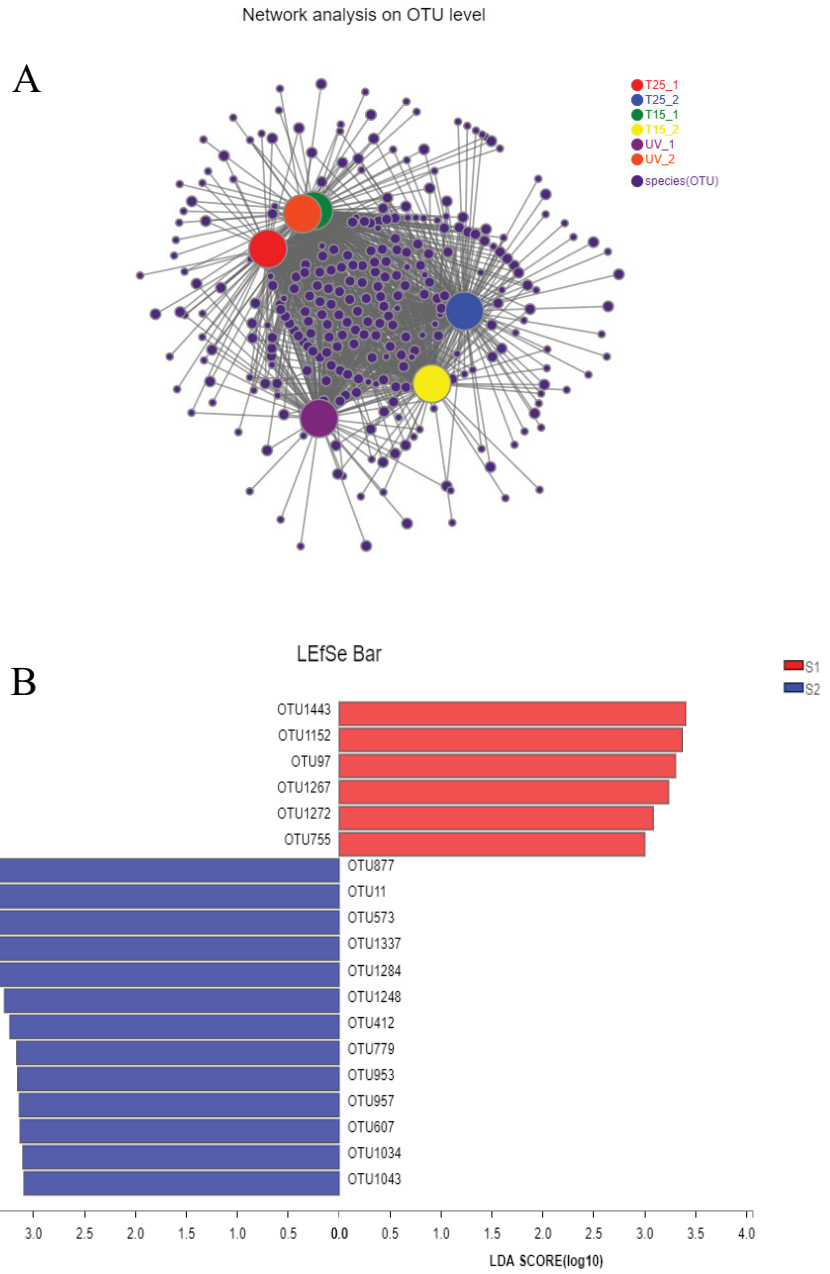


Fig. 5. (A) Co-linear network map for different operating conditions and (B) LEfSe showing differences in bacterial communities between reactors at the OTU level different reactors.

of ungrouped *Microtrichaceae*, OTU953 of ungrouped *Caldilineaceae*, OTU957 of ungrouped *Rhizobiales* dominate.

3.4. Correlation between reactor microorganisms and working conditions

The SBBR reactor was controlled by four variable in the study: temperature, DO, pH, and UV. The top 50 taxonomic abundance species were analyzed under the genus level. The relative abundance of the top 50 species in terms of abundance at each working condition is shown in S3 (see the supplement for details). The sum of the relative abundance of these bacterial genera accounted for 60.16% of the total sample. And the majority had a role in decomposing organic matter, suggesting that the SBBR reactor may have a strong capacity for carbon metabolism.

Unclassified *Bradyrhizobium*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Xanthobacteraceae*, *Leucobacter*, and *Bosea* were significantly positively correlated with temperature, per a Spearman correlation thermogram. Temperature were negatively correlated with *Propionibacterium*, *Gordonia*, *Bacteroidetes_vadinha17*, *Proteiniclasticum*, *Gallicola*, *Aestuariimicrobium*, *Dojkabacteria*, *Lactivibrio*, *Sporomusaceae*, *Mycobacterium*, *Corynebacterium*, *Brooklawnia*.

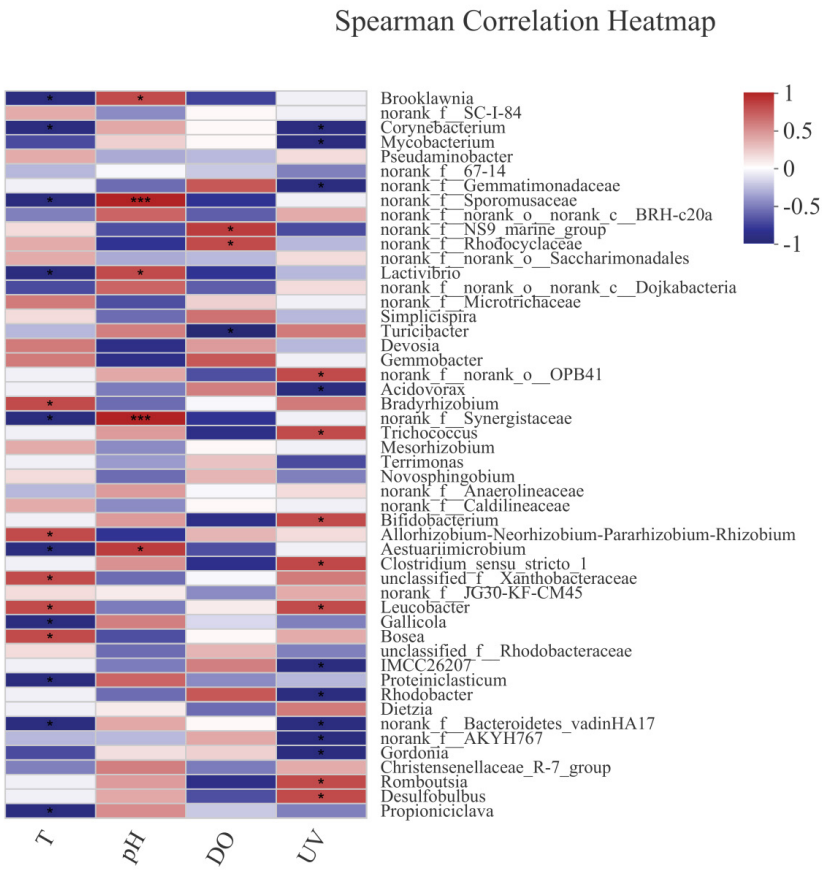


Fig. 6. Correlation of the Top 50 genera in terms of relative abundance at the genus level for different working conditions. *indicates significant negative or positive correlations (***$P < 0.001$). Through picrust2, Fig. 6 shows a heat map of the bacterial community's functional categories (levels 1 and 2 of the Kyoto Encyclopedia of Genes and Genomes (KEGG)). The color intensity in the heat map represents the relative abundance (%) of the functional categories, and 6 samples indicated.

Propioniciclava, *Christensenellaceae_R-7_group*, *Proteiniclasticum*, *Gallicola*, *Clostridium_sensu_stricto_1*, *Aestuariimicrobium*, *Synergistaceae*, *Turicibacter*, *Dojkabacteria*, *Lactivibrio*, *BRH-c20a*, *Sporomusaceae* and *Brooklawnia* were positively correlated with pH. *Bosea*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Gemmobacter*, *Devosia*, *Microtrichaceae*, *Rhodocyclaceae* and *NS9_marine_group* was significantly negatively correlated with pH.

Rhodobacter, *IMCC26207*, *Acidovorax*, *Gemmobacter*, *Simplicispira*, *Rhodocyclaceae*, *NS9_marine_group*, *Gemmatimonadaceae* were significantly positively correlated with dissolved oxygen. *Desulfobulbus*, *Romboutsia*, *Clostridium_sensu_stricto_1*, *Aestuariimicrobium*, *Bifidobacterium*, *Trichococcus*, *Synergistaceae*, *OPB41*, *Turicibacter*, *Dojkabacteria*, *Lactivibrio*, *BRH-c20a*, *Sporomusaceae* and *Brooklawnia* were negatively correlated with dissolved oxygen.

Desulfobulbus, *Romboutsia*, *Dietzia*, *Leucobacter*, *Xanthobacteraceae*, *Clostridium_sensu_stricto_1*, *Bifidobacterium*, *Trichococcus*, *Bradyrhizobium*, *OPB41* and *Turicibacter* were significantly positively correlated with UV. *Gordonia*, *AKYH767*, *Bacteroidetes_vadinha17*, hodobacter, *IMCC26207*, *Terrimonas*, *Acidovorax*, *NS9_marine_group*, *Gemmatimonadaceae*, *Mycobacterium* and *Corynebacterium* were significantly negatively correlated with UV.

A total of 21 genera were significantly correlated with temperature, 20 genera significantly correlated with pH, 22 genera significantly correlated with DO and 22 genera significantly correlated with UV. It can be seen that most genera responded to all four conditions at the genus level.

3.5. Functional prediction of bacterial community

According to KEGG pathway analysis, the functional classifications of bacterial communities in all samples are metabolism (77.75%), genetic information processing (6.62%), environmental information processing (5.59%), cellular processes (4.50%), human diseases (3.63%), and biological systems (1.90%) (Fig. 7A). Most microbial functions at KEGG Level 2 are realized in the global map and overview map, carbohydrate metabolism, amino acid metabolism, energy metabolism, cofactor and vitamin metabolism, membrane transport, translation, replication and repair, cell community prokaryotes, nucleotide metabolism, signal transduction, lipid metabolism, xenobiotic biodegradation and metabolism, cell community prokaryotes, nucleotide metabolism, signal transduction, lipid metabolism, xenobiotic biodegradation and metabolism (Fig. 7B). By comparing samples from reactor 1 with reactor

2, we found a number of microbial functions significantly enriched in the reactor 1 samples, including sulphite respiration, chemoheterotrophy, aerobic chemoheterotrophy, fermentation, respiration of sulphur compounds, sulphate respiration, and aromatic compound degradation (Fig. 8). In contrast, reactor 2 samples were significantly enriched for predicted functions associated with phototrophy, animal parasites or symbionts (Fig. 8).

4. Discussion

In recent years, with the development of urbanization and the emerging sponge cities, increasing attention has been paid to urban wastewater treatment. Meanwhile, as a key factor in the biological treatment process, the microbial community has been in the spotlight in fields like treatment effect. However, studies on the characteristics of bacterial communities in wastewater treatment processes at high altitudes in Tibet, especially on the microbiota associated with the SBBR process, are still scarce [1–4]. In this study, first we analysed the microbial composition and function of two reactors, pool 1 inoculated with sludge from the campus sewer network and pool 2 not inoculated with sludge. Then we discussed the relationships between microorganisms and metabolites in the SBBR process under various working conditions. Our results present fresh insights into the microbial community of SBBR processes at high altitudes and highlight the correlation between microbial and metabolism as a result of changes in working conditions. Furthermore, learning about the correlation between inoculated sludge with or without reactor microbes and metabolites could provide a theoretical basis for the improvement of SBBR treatment process efficiency.

In this study, the microbial community structure of the two reactors was significantly differentiated between ambient, lower temperature, and ambient operating UV irradiation conditions, and the significant differences in microbial structure led to significant differences in microbial function. This may be attributed to the microbial structure of Pond 1 inoculated with sludge taken from the campus sewer line. In addition, the two reactors differed significantly in microbial diversity between the working conditions and showed a high microbial diversity.

4.1. Discussion of dominant communities in SBBR systems under different working conditions

The goal of this study is develop our understanding of SBBR process bacteria and microorganisms in high-altitude environment. According to the results of Fig. 4, pool 1 has significantly lower biodiversity and richness than pool 2. This may be attributed to the fact that pool 1 is inoculated with sludge from the campus sewer line and has become more adapted to the municipal wastewater over generations, leading to a more stable diversity and abundance of microorganisms. Microbial structure has attracted a lot of attention as an important part of biological treatment. At the phylum level, *Actinobacteria*, *Proteobacteria*, and *Firmicutes* are the most prominent, with slight variations depending on working conditions. It is in line with previous biological treatment research results. These bacterial phyla are common in the biological treatments. Relevant reports show that these bacterial substances are involved in carbohydrate transport and protein metabolism, which is important for the treatment of contaminated material. It's worth noting that each of the samples contains high-demeanor *Actinobacteria*, *Proteobacteria*, and *Firmicutes*. These three types of bacteria are denitrifying bacteria [15,16]. *Firmicutes* microorganisms can produce short-chain fatty acids and provide nutrients to the

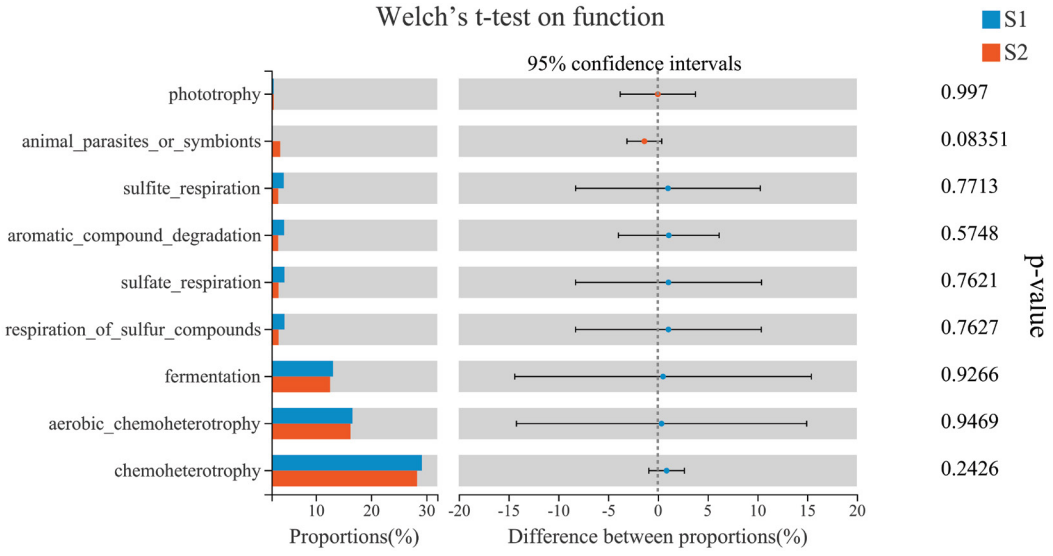
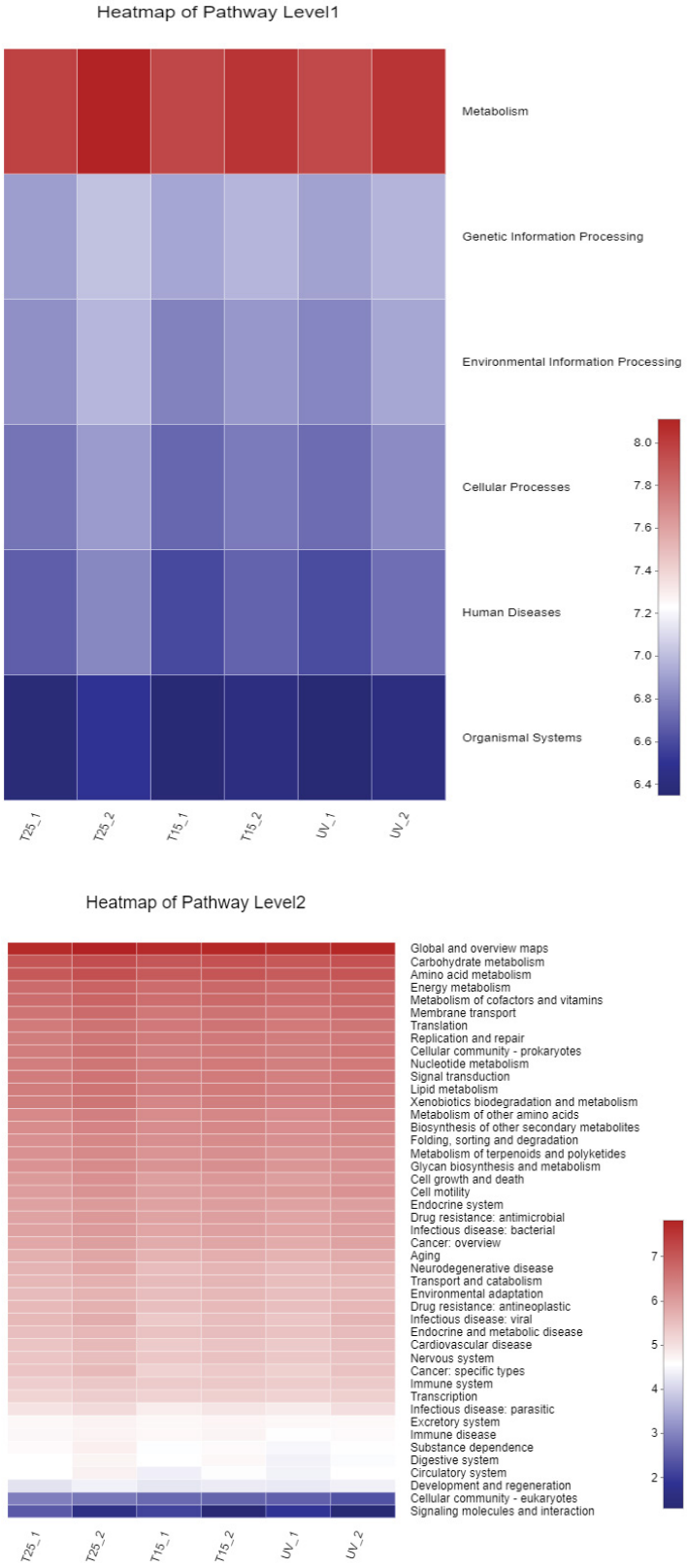


Fig. 8. Predicted different putative functions between bacterial community content and Different reaction pool samples based on PICRUST2 analysis. Columns indicate the KEGG ortho (KO) function for the nine divisions (corrected P < 0.05), and bars in the figure indicate the mean proportion of functional categories. 95% confidence intervals reflect differences in mean proportions (%), and corrected p values are shown on the right side of the figure.



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other microorganisms. *Firmicutes* enrichment is helpful to the denitrification process and the normal function of microbial structure and the regulation of the micro ecological environment. *Proteobacteria* are the main phylum of microorganisms involved in the biological treatment process, where they are mainly involved in the degradation of organic matter and have a large impact on the removal of COD [15,16]. *Actinobacteriota* is a denitrifying bacterium that participates in the biological treatment's denitrification process. Also, relevant reports show that this phylum can produce a variety of effective antibiotics and also inhibit the growth of some microorganisms [15,16].

Propioniciclava and *Dietzia* are most common core bacterial genera in every working condition. During operation, these bacteria are used to decompose organic matter. This study shows that each reactor has a certain treatment capacity for pollutants under different working conditions. However, further research is needed to explore the similarity of microorganisms under various working conditions. *Propioniciclova* is a gram-negative bacterium which was isolated in 2011 in a methanogenic reactor used to treat cattle farm waste [17]. In the reaction process, it can produce propionic acid through combination with other substances, which is important for the decomposition of organic matter in sewage treatment [18]. *Dietzia* is found in high abundance in all stages. *Dietzia* is shown to produce alkane hydrogenase that can degrade crude oil and long-chain alkanes [19,20]. Thus, *Dietzia* is often used in the treatment of crude oil and may play an important part in the metabolic degradation of organic matter in this study.

In all working conditions, the dominant bacterium in pool 1 is *Romboutsia* and *Christensenellaceae_R-7_group*, *Gordonia* and *norank_f__Bacteroidetes_vadinHA17*, with significantly higher abundances than in the samples from pool 2. *Romboutsia* is a common intestinal bacterium which has been found with in intestines of humans and animals [21]. Some *Romboutsia* bacteria are reported to be able to produce acetic acid by mixing and fermenting with other bacteria to. Therefore, *Romboutsia* in the reactor may be involved in organic substance metabolism [22,23]. The bacterial genus *Christensenellaceae_R-7_group* can be found in the microbial community of animal intestines [24] and biological treatment, and in human intestines. According to previous studies, *Christensenellaceae_R-7_group* bacteria can produce various acids in intestinal reactions, demonstrating that they may be involved in the metabolism of organic matter in sewage treatment. *Gordonia* bacteria are often found in all kinds of environments and are widely used in biological treatment methods. This genus is capable of degrading most xenobiotics, environmental pollutants, or other slowly biodegrading natural polymers as well as transforming or synthesising potentially useful compounds [25]. So it may have a role in the synthesis of various organic substances and the degradation of pollutants in sewage treatment. Some biological treatment methods contain *norank_f__Bacteroidetes_vadinHA17*, and studies have shown that it can improve the activity of enzymes involved in the production of lactic acid and propionic acid during anaerobic digestion [26,27]. Thus, in this study, this type of bacteria may be involved in organic metabolism. The dominant bacterial genera present in all working conditions of the SBBR reactor are likely to be involved in the metabolic processes of organic matter. The results of this study show that all stages of the reactor have effective degradation of pollutants, which is consistent with the predicted results of the bacterial community with 77.75% of the metabolic function expression.

As comparingtop ten bacteria in total abundance in both the two reactor samples, we found that *Proteiniclasticum* (yak rumen bacteria) and *Gallicola* were significantly enriched in pool 1, while *norank_f__AKYH767* and *IMCC26207* were significantly enriched in pool 2. *Proteiniclasticum* is largely symbiotic in the rumen of ruminants such as cattle, sheep, and deer. It has also been reported in the anaerobic stage of biological treatment. It is an obligate anaerobic bacterium competent for breaking complex organic matter into small molecular acids for further degradation. [27]. Thus, this type of bacteria may be involved in the decomposition of organic matter during sewage treatment process. *Norank_f__AKYH767* is an unclassified genus of *Sphingobacteriales*, which is a Gram-negative bacterium that is both active and anaerobic. This bacteria can produce acid with oxidation carbohydrates such as galactose, glucose, lactose, maltose, sucrose, and other sugars, according to some studies [28]. *Actinomycetes*, such as *IMCC26207*, are often found in oligotrophic freshwater lakes and have also been reported in biological treatment methods in plateau habitats. Relevant studies show that this bacteria contains genes encoding proteins, leading to participation in the sulfate reduction and ammonia assimilation cycle. Meanwhile, the *IMCC26207* genome encodes phosphate transporter (pstabc), polyphosphate kinase (PPK), and a two-component regulatory system (senx3-regx3), that can be induced to allow the flow of small concentration of environment friendly inorganic phosphate, indicating that this bacterium can survive in phosphorus-limited freshwater [29,30]. Thus, this microorganisms can be involved in the decomposition of organic matter in wastewater treatment, as well as in the removal of phosphorus. In plateau habitat, *norank_f__AKYH767*, *IMCC26207*, *Proteiniclasticum*, and *Gallicola* are abundant in the sewage treatment process [3,4]. It is therefore assumed that this genus may not be the dominant genus for biological treatment of wastewater in highland habitats.

4.2. Exploring the effect of temperature change on the microbial community of the SBBR process

Temperature is a key factor in the operation of biological treatment methods and its variation affects the structure and composition of the microbial community. By analysing the change in abundance of the same reactor at different temperatures, we found that at 25°C, *Rhodobacter* and *norank_f__AKYH767*, *IMCC26207*, and *Bosea* was significantly higher than at 15°C in pool 1. From the above analysis we can conclude that *norank_f__AKYH767* and *IMCC26207* are dominant bacteria in plateau habitat, and are more capable of adapting to plateau environmental changes. *Rhodobacter* is a Gram-negative bacterium with a vesicular endosperm of photosynthesis. Researches show that this bacteria can grow photoheterotrophically under anaerobic conditions using a wide range of organic matter as a carbon and electron donor [31,32]. *Bosea* gram-negative, Ouattara

et al. [33] were also obtained from anaerobic digester sludge and found to have the capacity to produce cellular fatty acid and to utilize substrate matters. The abundance of this bacteria is positively correlated with temperature, as shown by Spearman correlation heat map, which is consistent with the line that the abundance of this bacteria decreases as temperature decreases. The abundance of *Desulfobulbus*, *Gallicola*, and unclassified *Synergistaceae* are significantly higher when the operating condition is 15°C than when the operating condition is 25°C. It is consistent with the conclusion that the three genera were negatively correlated with temperature in the above study. *Desulfobulbus* is a spore-free anaerobic bacterium, able to convert sulfate compounds to hydrogen sulfide. It can be found in fresh or brackish water anaerobic sediments, cattle rumen juice, and animal feces [34,35]. *Gallicola* is a Gram-positive bacterium and this genus has been reported to use organic matter as energy to produce the non-glycolysis of butyrate [36]. *Synergistaceae* are anaerobic bacteria, which have been reported to ferment a range of carbohydrates and mono- and dicarboxylic acids, with the end products being acetate, hydrogen and CO_2 [37]. All three types of bacteria are involved in the metabolic breakdown of organic matter and other pollutants in the wastewater treatment process, in line with the functional prediction of 77.75% of the metabolic function.

4.3. Exploring the effects of UV exposure on microbial communities

Ultraviolet radiation (UV) is strongly correlated with microbial colony composition and structure. Most studies show that UV has an inhibitory effect on bacteria [38], while some studies illustrate that UV irradiation at certain intensities can cause a growth-promoting effect on some bacteria [39]. We examine the microbial abundance in the same reactor when exposed to ultraviolet light. It was found that the top ten bacterial genera in relative abundance in pool 1 without UV irradiation under 25°C operating conditions were *Gordonia*, *AKYH767*, *Dietzia*, *Propioniciclava*, *Bosea*, *IMCC26207*, *Romboutsia*, *Rhodobacter*, *Bacteroidetes_vadinha17*, *Proteiniclasticum*. In contrast, the top ten bacterial genera in terms of abundance under working conditions of 10 W UV irradiation for 5 s were *Desulfobulbus*, *Romboutsia*, *Dietzia*, *Christensenellaceae_R-7_group*, *Propioniciclava*, *OPB41*, *Gordonia*, *Proteiniclasticum*, *Gallicola*, *Bacteroidetes_vadinha17*. The shared genera included *Gordonia*, *Dietzia*, *Propioniciclava*, *Romboutsia*, *Bacteroidetes_vadinha17* and *Proteiniclasticum*, while four genera, *AKYH767*, *Bosea*, *IMCC26207* and *Rhodobacter*, showed a significant decrease in the proportion of the community under the influence of UV light. Whereas the abundance of six genera, *Desulfobulbus*, *Romboutsia*, *Propioniciclava*, *Christensenellaceae_R-7_group*, *Dietzia*, and *Leucobacter*, was significantly increased. In particular, the change in the abundance of *Desulfobulbus* after UV irradiation in pool 1 was significant, with the relative abundance of *Desulfobulbus* after UV irradiation being 37.6 times higher than before irradiation. However, the four genera *Gordonia*, unclassified *OPB41*, unclassified *Bacteroidetes_vadinha17*, and *Proteiniclasticum* showed a significant decrease in abundance compared to UV irradiation. The unclassified *OPB41* belongs to the *Actinomycetes* group, a genus of bacteria present in sediments and involved in the breakdown of metabolic sugars in related reports [40–42].

The top ten bacterial genera in relative abundance in pool 2 under operating conditions without UV irradiation at 25°C were *AKYH767*, *Gordonia*, *Rhodobacteraceae*, *IMCC26207*, *Rhodobacter*, *Propioniciclava*, *Bosea*, *Dietzia*, *Terrimonas* and *Romboutsia*. Whereas the top ten bacterial genera in terms of abundance under 10 W UV irradiation for 5 s of operation were *Desulfobulbus*, *Romboutsia*, *Christensenellaceae_R-7_group*, *Bifidobacterium*, *Bosea*, *Anthobacteraceae*, *Dietzia*, *JG30-KF-CM45*, *Trichococcus*, *Clostridium_sensu_stricto_1*. Only three genera, *Bosea*, *Dietzia* and *Romboutsia*, were shared under the influence of UV light, while the other seven genera were inhibited. Notably, nine dominant genera, that is, *Desulfobulbus*, *Romboutsia*, *Christensenellaceae_R-7_group*, *Bifidobacterium*, unclassified *JG30-KF-CM45*, unclassified *Xanthobacteraceae*, *Bosea*, *Dietzia* and *Trichococcus* were detected significantly higher than before, with *Desulfobulbus* also showing a significant increase, 42.3 times higher than before.

Bifidobacterium is a common flora of the intestinal tract and studies have shown that this genus has the ability to autoaggregate and surface hydrophobicity to adsorb hydrocarbons [43–45]. *Xanthobacteraceae* are aerobic, chemoenergetic organic trophic bacteria. It can undergo respiratory metabolism and some studies have shown that this genus can use sugars to produce acid but not gas [46,47]. *Trichococcus* is partly anaerobic and extremely cold tolerant. According to relevant studies, the bacteria are present in large numbers in sludge and some genera are responsible for the swelling of sludge [48,49]. All of these genera have been shown to be involved in metabolising organic matter in relevant studies. Therefore, in this study these genera are presumed to be involved in the process of dissipating pollutants in the wastewater treatment process. The analysis showed that the dominant bacterial genus in pool 2 was more variable compared to pool 1. The possible cause is that the inoculated sludge in cell 1 has been exposed to the natural environment of the plateau for a long time and is more adapted to UV irradiation, resulting in the bacteria in cell 1 being more resistant to UV irradiation.

4.4. Functional evaluation forecast

We have analysed a large number of microorganisms in the SBBR treatment process in the plateau region under the respective working conditions to provide well-supported data for the functional evaluation of the microbiota of the SBBR treatment process in the plateau region.

Our results show that the SBBR treatment process microbial functions as global and overview maps, carbohydrate metabolism, amino acid metabolism, energy metabolism, cofactor and vitamin metabolism, membrane transport, translation, replication and repair, cell community-prokaryotic, nucleotide metabolism, signal transduction, lipid metabolism, xenobiotic biodegradation and metabolism, which are generally consistent with the main metabolic pathways predicted to be functional in the microbiota of the biological treatment method. Furthermore, approximately 77.75% of the predicted functions in the microbiota of the SBBR treatment process were associated with metabolic

pathways, particularly global and overview maps, carbohydrate metabolism, amino acid metabolism, energy metabolism, cofactor and vitamin metabolism, which supports the previous consensus that the biological treatment method microbiota may play an important role in the metabolism of pollutants in wastewater treatment. It is worth noting that changes in inoculated sludge and working conditions affect the predictive function of the microbiota. In pool 1, temperature changes had a significant effect on the relative abundance of four predicted functions: replication and repair, nucleotide metabolism, cell community-prokaryotic, and lipid metabolism, and the relative abundance of two predicted functions: signal transduction and cell community-prokaryotic, were significantly affected by UV irradiation. The relative abundance of four predicted functions, replication and repair, nucleotide metabolism, cell community-prokaryotic, and signal transduction, was significantly affected by temperature changes in pool 2, and the relative abundance of two predicted functions, replication and repair, and lipid metabolism, was significantly affected by UV irradiation. In contrast, the number of predicted microbial functions detected in pool 1 was generally lower than in all conditions in pool 2 when compared with or without sludge inoculation. This is consistent with the results of the richness and diversity analysis of the microbiota composition and structure, with pool 2 being richer in microbiota. It may suggest that inoculation of sludge, while enhancing the specific microbial species adapted to the highland environment, reduces the abundance and diversity of organisms and hence the function of the microbiota.

5. Conclusion

This study describes the taxonomic and functional characteristics of the microbiota of the SBBR treatment process in a plateau habitat. It is found that there are significant differences in the prediction of microbial diversity, structure and function of the samples by inoculated sludge under different working conditions. The number of predicted microbial functions detected in pool 1 was generally lower than in all conditions in pool 2 when compared with or without sludge inoculation. *Propioniciclava Gordonia norank_f__Bacteroidetes_vadinHA17* and *Romboutsia* are significantly abundant in all samples, while many bacteria, including *Desulfobulbus*, *Propioniciclava*, *Romboutsia*, and *Christensenellaceae_R-7_group*, are highly abundant in ultraviolet irradiated samples. It's worth noting that about 40% of the dominant bacteria are significantly influenced by changes in each working condition. However, the potential functions of these microorganisms and their response mechanisms to changes in working conditions need to be further investigated.

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Authors' contributions

CXy designed the study and interpreted the results and was a major contributor in writing the manuscript. ZLs carried out the experiments and collect the data. ZHf conducted all the works. WJ was involved in the gene expression experiments. All authors read and approved the final manuscript.

Data availability

Not applicable

Compliance with ethical standards

Conflict of interest

The author declares that they have no conflict of interest.

Consent to participate

All authors mentioned in the manuscript have agreed for authorship and for participation in the manuscript.

Consent to publish

All authors have read and agreed to the published version of the manuscript.

Ethics approval

This article is a research in the direction of sewage treatment and does not involve ethical codes.

Manuscript not submitted on preprint server.

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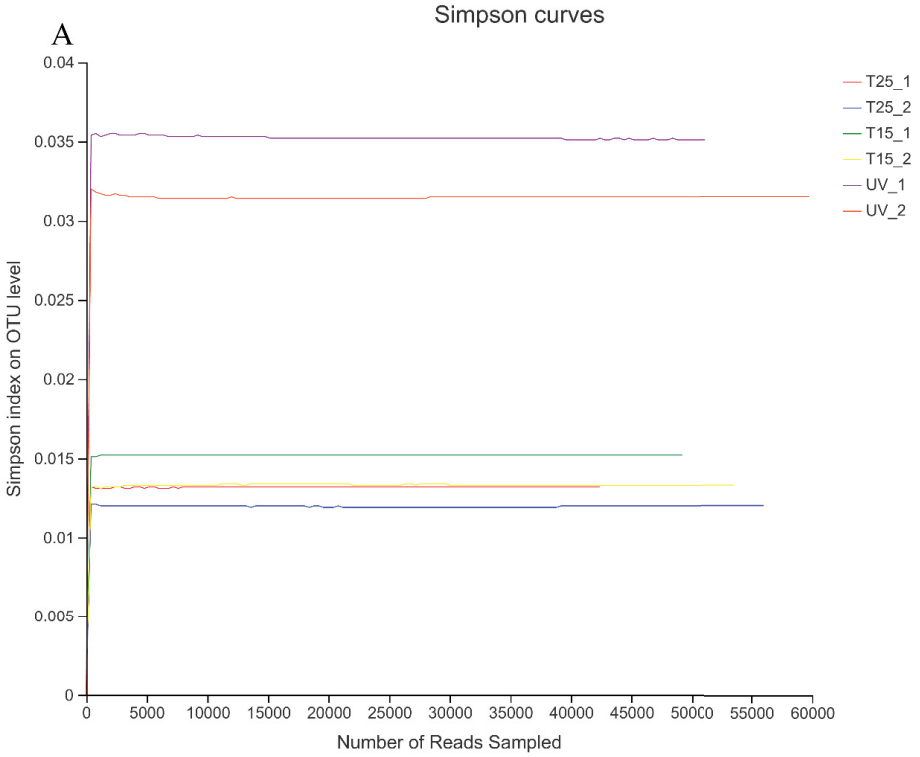
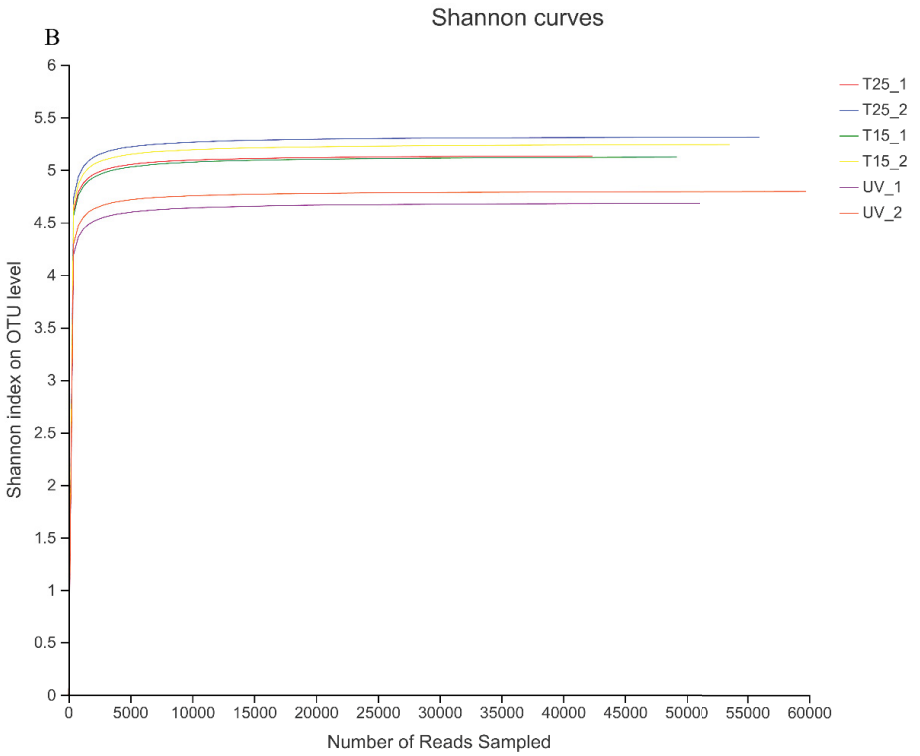
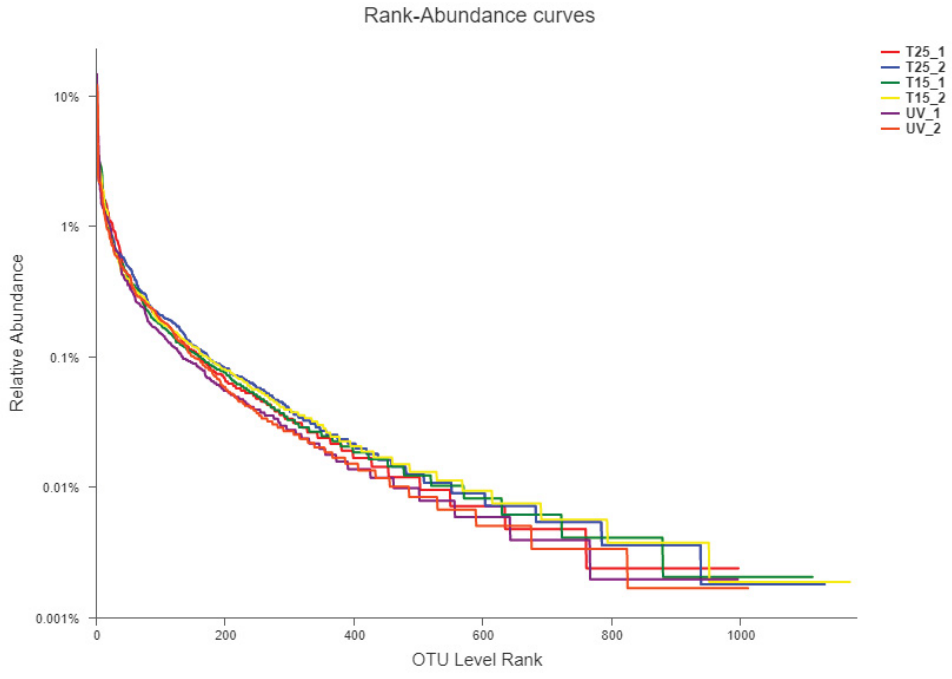
Supplementary information

S1. Simpson and Shannon curves





S2. Rank-Abundance



S3. Abundance table of the top 50 bacterial genus by working conditions

Genus	T_15_1	T_15_2	T_25_1	T_25_2	UV_10_1	UV_10_2	SUM
g__*Desulfobulbus*	3.09%	1.94%	0.47%	0.30%	14.61%	12.13%	5.67%
g__*Romboutsia*	5.47%	3.79%	2.50%	1.74%	6.92%	10.32%	5.28%
g__*Gordonia*	4.49%	3.63%	5.14%	2.93%	1.89%	1.08%	3.07%
g__*norank_f__AKYH767*	1.64%	5.14%	3.28%	6.23%	0.65%	0.94%	2.99%
g__*Dietzia*	2.49%	2.74%	3.00%	1.85%	5.08%	2.12%	2.84%
g__*Propioniciclava*	2.98%	3.28%	2.93%	2.08%	3.10%	0.99%	2.50%
g__*Christensenellaceae_R-7_group*	2.90%	2.59%	1.10%	0.58%	3.47%	2.47%	2.20%
g__*Proteiniclasticum*	3.51%	2.54%	2.03%	1.19%	1.47%	1.22%	1.95%
g__*norank_f__Bacteroidetes_vadinHA17*	3.41%	2.34%	2.07%	1.39%	1.38%	0.54%	1.80%
g__*Bosea*	0.86%	0.68%	2.75%	1.96%	1.34%	2.16%	1.61%
g__*unclassified_f__Rhodobacteraceae*	0.85%	1.87%	1.65%	2.72%	1.26%	1.13%	1.59%
g__*IMCC26207*	1.25%	1.88%	2.63%	2.24%	0.45%	0.62%	1.47%
g__*Gallicola*	2.77%	1.58%	1.48%	0.66%	1.46%	0.59%	1.38%
g__*Rhodobacter*	0.73%	1.45%	2.27%	2.14%	0.70%	0.56%	1.28%
g__*Propioniciclava*	1.46%	1.34%	1.48%	0.86%	1.04%	0.79%	1.14%
g__*unclassified_f__Xanthobacteraceae*	0.33%	0.47%	1.11%	1.21%	1.01%	2.13%	1.07%
g__*Propioniciclava*	1.31%	1.30%	1.29%	0.65%	1.21%	0.79%	1.07%
g__*Aestuariimicrobium*	1.55%	1.34%	0.86%	0.58%	1.18%	0.93%	1.07%
g__*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*	0.45%	0.58%	1.61%	1.71%	0.75%	1.25%	1.06%
g__*Clostridium_sensu_stricto_1*	1.02%	0.89%	0.79%	0.58%	1.09%	1.49%	0.99%
g__*norank_f__Bacteroidetes_vadinHA17*	1.65%	1.22%	1.57%	1.12%	0.42%	0.13%	0.98%
g__*Propioniciclava*	1.14%	1.00%	1.11%	0.49%	1.24%	0.53%	0.89%
g__*Novosphingobium*	0.69%	0.99%	1.07%	1.18%	0.73%	0.71%	0.89%
g__*Terrimonas*	0.57%	1.36%	0.69%	1.79%	0.19%	0.58%	0.88%
g__*Bifidobacterium*	0.72%	0.63%	0.17%	0.21%	0.67%	2.19%	0.81%
g__*Trichococcus*	0.57%	0.65%	0.22%	0.23%	1.08%	1.84%	0.80%
g__*Bradyrhizobium*	0.20%	0.31%	0.74%	0.78%	0.71%	1.48%	0.73%
g__*Acidovorax*	0.56%	0.52%	1.26%	1.48%	0.25%	0.32%	0.72%
g__*norank_f__JG30-KF-CM45*	0.15%	0.69%	0.17%	0.92%	0.18%	1.85%	0.71%
g__*Turicibacter*	0.62%	0.72%	0.49%	0.51%	0.64%	0.93%	0.66%
g__*Gemmobacter*	0.54%	0.42%	1.34%	0.50%	0.94%	0.41%	0.66%
g__*Devosia*	0.33%	0.49%	1.07%	1.01%	0.42%	0.60%	0.65%
g__*Leucobacter*	0.36%	0.34%	0.59%	0.41%	1.38%	0.71%	0.63%
g__*Gordonia*	0.90%	0.76%	0.94%	0.55%	0.38%	0.27%	0.61%
g__*Lactivibrio*	1.71%	0.98%	0.30%	0.19%	0.24%	0.23%	0.59%
g__*Mesorhizobium*	0.13%	0.64%	0.33%	1.34%	0.12%	0.80%	0.59%
g__*norank_f__norank_o__norank_c__BRH-c20a*	1.14%	0.45%	0.06%	0.04%	0.98%	0.59%	0.55%
g__*norank_f__Anaerolineaceae*	0.84%	0.35%	0.18%	0.64%	0.62%	0.49%	0.53%
g__*norank_f__NS9_marine_group*	0.60%	0.27%	1.57%	0.62%	0.31%	0.07%	0.53%
g__*norank_f__norank_o__norank_c__Dojkabacteria*	0.68%	1.00%	0.30%	0.24%	0.70%	0.19%	0.51%
g__*Pseudaminobacter*	0.18%	0.37%	0.45%	0.73%	0.29%	0.89%	0.50%
g__*norank_f__Rhodocyclaceae*	0.55%	0.28%	0.78%	0.54%	0.63%	0.08%	0.45%
g__*Simplicispira*	0.51%	0.24%	0.93%	0.23%	0.64%	0.24%	0.44%
g__*IMCC26207*	0.23%	0.60%	0.46%	0.68%	0.12%	0.44%	0.43%
g__*norank_f__norank_o__OPB41*	0.10%	0.07%	0.00%	0.01%	1.90%	0.43%	0.42%
g__*Christensenellaceae_R-7_group*	0.56%	0.40%	0.32%	0.15%	0.62%	0.49%	0.42%
g__*norank_f__Synergistaceae*	0.92%	0.60%	0.11%	0.09%	0.35%	0.43%	0.42%
g__*norank_f__Gemmatimonadaceae*	0.38%	0.31%	1.04%	0.77%	0.07%	0.01%	0.41%
g__*Pseudorhodobacter*	0.39%	0.40%	0.91%	0.47%	0.15%	0.04%	0.37%
g__*Rhodobacter*	0.30%	0.40%	0.77%	0.58%	0.13%	0.11%	0.37%
Total	60.75%	58.82%	60.37%	52.10%	67.13%	62.31%	60.16%