Research article

Purification effect and microbial community structure of bio filter on rural dispersed sewage

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A R T I C L E   I N F O

A B S T R A C T

Taking rural dispersed sewage for research objects, the treatment effect and microbial community structure characteristics of a bio filter (BF) reactor was studied. At fixed time and location, the removal efficiencies of common pollutants were investigated. By using high-throughput sequencing method, the heterogeneities of microbial community structure in fillers and plant roots were analyzed. The results showed that the average annual removal rates of COD_{Cr}, NH_3–N, TN, and TP by the BF were 83.10 %, 65.67 %, 60.25 %, and 80.32 % respectively, and the effluent could reach the first grade of the water pollutant discharge standard of rural sewage treatment facility (DB51/2626-2019). During the sewage treatment process, Scindapsus could effectively establish complex and stable microbial communities, and could better degrade pollutants, especially nitrogen removal. The dominant microbial communities were more than 11 phyla and 19 classes. At the genus level, the dominant bacteria included *Nitrospira, Arthrobacter, Rhodoplanes,* etc.

1. Introduction

After the construction and operation of centralized sewage treatment plants in urban areas throughout China, rural dispersed sewage has emerged as a significant source of water pollution. This issue has hindered the continuous improvement of water quality in China, especially in the comparatively underdeveloped western regions [1]. Rural sewage possesses the characteristics of decentralized pollution sources, complicated collection procedures, small treatment scales, and frequently varying in water quantity and quality [2]. As a result, constructing large-scale centralized wastewater treatment plants with high investment is unfeasible, making decentralized treatment the primary solution [3]. At present, small scale and low-cost sewage treatment technology mainly include ecological and biological methods [4,5]. The bio filter (BF) is a typical ecological treatment technology developed based on the practice of sewage irrigation. Due to its uncomplicated structure, low energy consumption, ease of operation and maintenance, and superior capacity to resist the impacts of loads [6,7], the BF is very suitable for rural sewage treatment.

Domestic and foreign scholars have conducted extensive research on the treatment of Sewage by BFs [8,9]. Among them, the operating conditions of the BF received widespread attention. In the improvement and commissioning of pulsed biofilter process for rural domestic wastewater treatment, Liu et al. [10] found that the overall removal rates of COD_{Cr}, NH_3–N and TP in the improved bio filter were the highest under the 12 h working mode. Ray et al. [11] investigated the effect of reducing aeration at the city of Windsor's

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Lou Romano water reclamation plant, and found that the BOD and ammonium removal were satisfactory at 1300 m^3/h airflow rate. There were also studies proposing requirements for the influent of the BF. Ren et al. [12] highlighted that maintaining a pH between 7 and 8 was essential for efficient denitrification. Deviations from the ideal pH range, such as pH > 8 or pH < 7, reduced the denitrification and ultimately hinders the denitrification process. According to Nagaoka et al. [13], a C/N ratio of 5.0 or more in wastewater guaranteed an adequate carbon source for the denitrification process. Increasing the external carbon source dosage was necessary for C/N ratios less than 5.0. Some studies also focused on the components of the BF. In the experimental study on treatment of domestic sewage by biological aerated filter, Wang [14] proposed the principles of filler selection for the treatment process and obtained the optimal filler and hydraulic retention index. Tao et al. [15] constructed a combined system composed of anaerobic filter-aerobic filter-constructed wetland to realize decentralized rural wastewater treatment, and found that lettuce, cress, leek and tomato preferred nitrate, while garland chrysanthemum, water spinach and spinach preferred ammonium. These studies provided great help for us to analyze the pathways of pollutant degradation in the BF. The BF purified sewage through the combined action of plants and microorganisms. The microorganisms in the BF played a crucial role [16,17], especially during the cold season of plant withering. However, lots of researches on the microorganisms in the BF focused on the microbial biomass [18,19], few studies investigated the relationship between microbial community and pollutant degradation in the BF. This study aimed to analyze the structure of microbial communities and characterize the interdependence among plant roots, fillers, and microorganisms, in the hope of revealing the relationship between purification effect and microbial community structure in the BF.

2. Materials and methods

2.1. Experimental equipment and materials

The BF reactor was a rectangular parallelepiped tank made of polypropylene with the size of 1500 mm × 500 mm × 500 mm (Fig. 1). The depth of sewage in the reactor was 400 mm, and the porous ceramic particles, 8 mm in diameter, were packed into the reactor with a filling depth of 350 mm. The ceramic particle was often used for wastewater treatment, due to its large specific surface area, high porosity, low cost, and long service life [20]. The reactor tank was equally divided into three internally connected treatment units (Unit 1, 2 and 3) by two partitions. Canna, Scindapsus, and Spathiphylum were planted in sequence on the ceramic particles, with the planting density of 60 plants per square meter. The sewage flowed through three units successively, showed the status of wavy flow.

The influent sewage of the BF reactor was taken from the effluent through the treatment by the fine-screen of the sewage treatment plant in Southwest University of Science and Technology. This part of sewage mainly came from some intramural graduate apartments and surrounding self-built houses, with the characteristics of low water quantity (80 m^3/d), high water quality fluctuations, and strong biodegradability, similar to rural dispersed sewage.

2.2. Sample collection and naming

In January 2022, the debugged BF reactor began to treat sewage, with a hydraulic load of 1 m^3/m^3·d, and water quality monitoring was carried out simultaneously. In December 2022, water quality monitoring was completed, with a cumulative duration of 15 months. The sampling point was set at the sewage inlet and outlet of the BF reactor. The sampling frequency was 2–3 times a week on average, and the sampling time was fixed from 9:30am to 10:30am. Each sample was tested for 4 regular water quality indices, with 3 replicates.
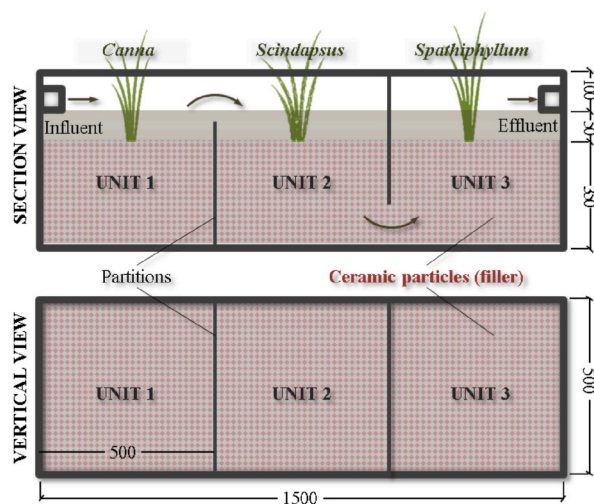

Fig. 1. Structure and size (measured in mm) of the BF.

In December 2022, after 12 months of stable operation of the BF reactor, all microbial samples, including plant root and filler samples, were collected at once. The root sample of Canna, named as CA, was obtained by mixing all 9 Canna root fragments in treatment Unit 1. Using the same method, Scindapsus and Spathiphylum root samples were acquired, named as SC and SP respectively. The filler microbial samples were collected in surface and bottom layers according to different treatment units, and a total of 6 samples were obtained. From surface to bottom layers according to unit numbers, they were named as 1S, 1B, 2S, 2B, 3S and 3B one by one.

2.3. Detection and analysis

2.3.1. Water quality indices
Test methods [21] of regular water quality indices were listed below (Table 1).

2.3.2. Genome sequencing
The DNA of microbial samples was extracted by using DNA isolation kit, then separated and purified by agarose gel electrophoresis. Using sterile water diluted genomic DNA (1 ng/μL) as a template, sequencing region 16SV3-4 was selected for polymerase chain reaction (PCR) sequencing. The upper and lower primers with barcode used in the PCR experiment were 314 F (5 ′-CCTAYGGGRBGCASCAG - 3′) and 806 R (5 ′- GACTACNNGGGTATCTAAT- 3′), respectively, with a PCR amplification product length of approximately 200 bp.

2.3.3. Data processing
Low-quality partial cropping was performed on sequencing results by Cutadapt (Version 1.9.1). According to barcode of characteristic primers, sample data were separated from the obtained sequencing results. After truncating the barcode and primer sequences, "Raw Reads" were obtained. By comparing the sequence with the species annotation database, the chimeras were removed form the"Raw Reads" and the final valid data "Clean Reads" were obtained by using Usearch (V 7.1). The valid data sequence was divided into operational taxonomic units (OTUs) with 97 % similarity, and then compared with the SSUrRNA database of SILVA132. The Mothur method [22] was used for species annotation analysis, with a confidence threshold of 0.8–1.

ACE index, Chao1 index, Shannon index, Simpson index and sequencing coverage were calculated by Qiime (Version 1.9.1). The rarefaction curve was drawn by R (Version 2.15.3) to verify whether the sequencing data of the sample was sufficient.

3. Results and discussion

3.1. Pollutants removal efficiency of the BF

From the removal effect on pollutants (Table 2), after the stable operation of the BF reactor, the annual average concentration of pollutants in the effluent could reach the first grade of the water pollutant discharge standard of rural sewage treatment facility (DB51/2626-2019). With the great variations in the influent pollutants concentration of the BF, the water quality of the treated effluent also fluctuated, but within the emission limits of the standard. The BF had a good removal effect on COD_{cr} and TP, with an average annual removal rate of 83.10 % and 80.32 %, respectively. The removal effect of nitrogen was also acceptable, with an annual average removal rate of over 60 %, 65.67 % of NH_3 -N, and 60.25 % of TN. This indicated that the BF could effectively treat rural dispersed sewage, displayed good resistance to shock loads of pollutants.

From the interannual changes in sewage treatment efficiency, the BF presented seasonal variation (Fig. 2), with high removal rate from May to September and relatively low in other months. However, the monthly variations of four pollutants are not unanimous. The removal rate of COD_{Cr} first increased then maintained high level and finally decreased, which was similar with a "saddle type" curve (Fig. 2a). The main reason was that the degradation of COD_{Cr} in the BF mainly relied on filler microorganisms. From January to April, with the temperature gradually increasing, microbial activity increased, and degradation ability enhanced. Conversely, from October to December, as the weather grew chillier, the pollutant degradation ability of the BF weakened, with the microbial activity decreasing. From May to September, the temperature remained high and the removal rate could be maintained at above 85 %.

The change trend of NH_3 -N removal rate was similar to that of COD_{Cr}, first increasing, then maintaining at a high level, finally decreasing (Fig. 2b). The main reason was slightly different. The removal of NH_3 -N by the BF not only relied on microbial degradation (nitrification), but also on plant absorption. From May to September, the vigorous plant growth promoted the maintenance of high NH_3 -N removal rate. The removal rate variation during other months was dominated by the activity of filler microorganisms.

Fig. 2c and (d) showed that the variation trend of removal rate was similar between TN and TP. Nitrogen and phosphorus in sewage are both nutritional pollutants, and their removal by the BF is mainly achieved through various functions, such as filler adsorption,

Table 1
Regular water quality indexes and measurement methods.

Water quality index	Test methods	Approval number
COD_{Cr}	Fast digestion-Spectrophotometric method	HJ/T 399-2007
NH_3 -N	Nessler's reagent spectrophotometry method	HJ 535-2009
TN	Alkaline potassium persiflage digestion-UV spectrophotometric method	HJ 636-2012
TP	Ammonium molybdate spectrophotometric method	GB 11893-89

Table 2
Removal effect of the BF on pollutants.

| Water quality index	Influent concentration (mg/L)		Effluent concentration (mg/L)		Average removal rate of pollutants (%)	Emission limits (mg/L)
	Interannual variation	Annual average	Interannual variation	Annual average		
COD_{Cr}	201.92–348.29	256.09	23.42–65.72	43.27	83.10	60
NH_3 -N	11.34–16.21	13.75	2.38–8.03	4.72	65.67	8
TN	26.72–35.07	30.29	8.57–16.29	12.04	60.25	20
TP	2.35–5.19	3.71	0.21–1.68	0.73	80.32	1.5
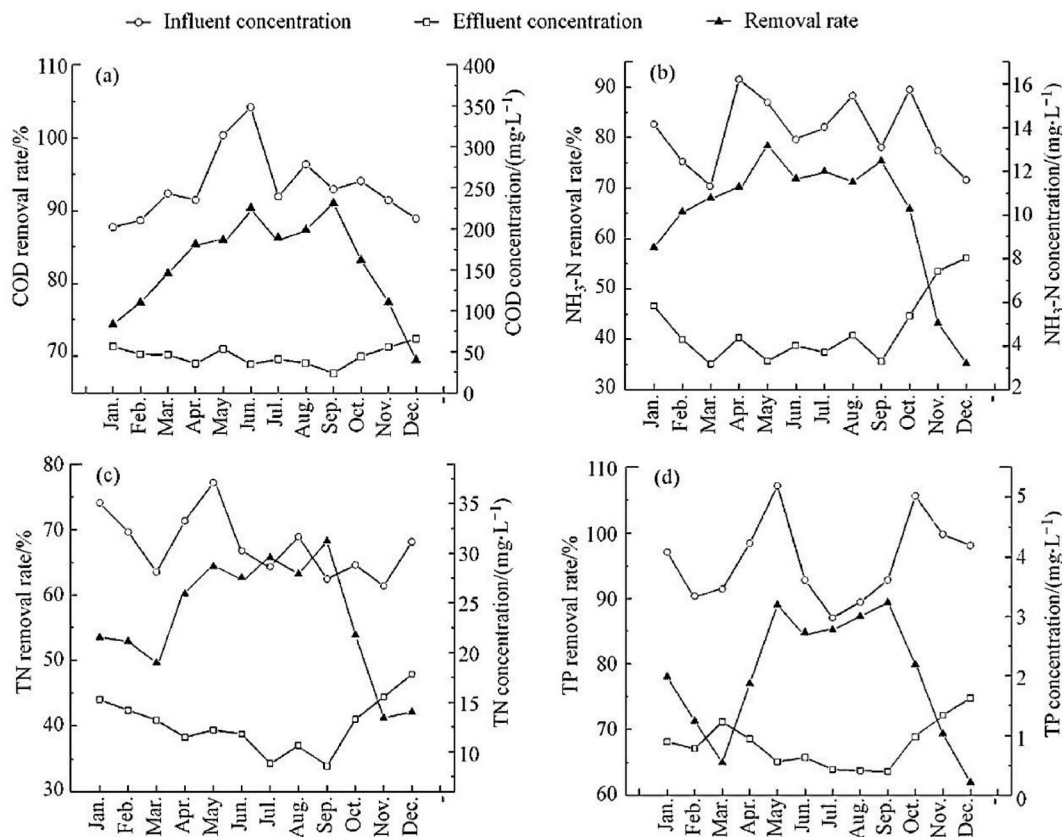
Fig. 2. Interannual variation in pollutant degradation effect of the BF.

plant uptake, and microbial degradation [23]. In the low temperature seasons, because of plants wither and low microbial activity, adsorption of filler dominates. Generally, under adsorption equilibrium conditions, the adsorption capacity is positively correlated with the concentration of pollutants to a certain extent [24]. Therefore, during the colder months of 2022 (From January to April, and From October to December), the removal rates of TN and TP by the BF were both low and varied with their respective concentration of the influent sewage. From May to September, due to the increase in temperature, plant uptake and microbial degradation were enhanced, and the removal rates of TN and TP was maintained at a high level.

3.2. Sequencing results and validation

After cutting and filtering low quality sequences for all samples, a total of 71215 effective sequences were obtained under quality control. Clustering the sequence into OUTs resulted in a total of 4398 OTUs with 97 % similarity. Then, the OTUs sequence was compared and annotated with the Sliva132 database, with a total of 1906 (43.34 %) that can be annotated to the genus level. Randomly extracting a certain amount of sequencing data (sequence number) from the microbial samples, counting the corresponding OTUs species (number), and the rarefaction curves was plotted (Fig. 3). As the sample sequencing amount reached 23045, all curves tended to flatten. More data would only generate a small amount of OTUs, and the sequencing data was reasonable. Therefore, it can be interpreted that the sequencing depth covered the vast majority of species in the microbial samples. In addition to directly reflecting

the rationality of the sequencing data amount, the rarefaction curves can also indirectly reflect the richness of species in the sample [25].Among the microbial samples of three plant roots, the species richness of SC was higher than that of the other two, and the difference between CA and SP was small. This was mainly due to the fact that Scindapsus belong to deep rooted plants with well-developed fibrous roots and strong nutrient absorption ability. The root environment of microorganisms was good, with rapid growth and reproduction. For the species richness of 6 filler samples, the unit differentiation characteristics were obvious, Unit 2 (2S and 2B) > Unit 1 (1S and 1B) > Unit 3 (3S and 3B), and the difference between the two samples in the same unit was small. This indicated that the microbial environment of the filler was greatly influenced by plant root microorganisms.

3.3. Microbial community diversity analysis

The minimum coverage index of all samples was 0.996 (Table 3), indicating that the sequencing results could accurately and completely reflect the true condition of the microorganisms in the BF. The ACE index and Chao1 index of SC were both highest, suggesting that the Scindapsus root microorganisms had the highest species richness. 3B had the lowest ACE index and 3S had the lowest Chao1 index, implying that the microbial species richness of the filler in Unit 3 was the lowest. Among the microbial samples of plant roots, SC had the highest Shannon index, and had the richest community diversity. For the filler samples, 2S had the richest microbial diversity with the highest Shannon index of 8.416. By contrast, two filler samples of Unit 3, had low Shannon index and poor microbial diversity. The Simpson index of all samples had small difference, and could not be used to determine differences in microbial diversity.

3.4. Microbial community composition analysis

The differences in microbial community structure of the BF could also be explained by the community composition of microorganisms at different taxonomic levels of phylum, class, and genus (Fig. 4). The microorganisms in the BF are mainly bacteria. The dominant bacterial communities in the filler and plant roots all reached more than 11 phyla, of which Proteobacteria, Acidobacteriota, Chloroflexi, Firmicutes, and Actinobacteria accounted for 86.26 % of the total sequence. At the class level, the dominant bacterial community level reached over 19, Gammaproteobacteria, Acidobacteria-6, Alphaproteobacteria, Bacilli, Betaproteobacteria, Deltaproteobacteria and Anaerolineae were the main classes, with a relative abundance of over 45 %. At the genus level, the dominant bacteria mainly included *Nitrospira*, *Arthrobacter*, *Rhodoplanes*, *Pseudomonas*, *Planctomyces*, *Hyphomicrobium,* etc. Among them, the main Nitrifying bacteria were *Nitrospira*, the main nitrite bacteria were *Arthrobacter*, and the main denitrifying bacteria were *Pseudomonas* and *Hyphomicrobium*. At all different taxonomic levels, SC, 2S and 2B all showed differences from other samples, reaching a high abundance in Proteobacteria (Fig. 4a), Gammaproteobacteria (Fig. 4b) and *Nitrospira* (Fig. 4c). It indicated that the microorganism of Unit 2 has strong assimilation and catabolism effect on nutrient nitrogen in sewage [26]. These bacteria played a decisive role in the microbial degradation of pollutants, and their abundance was related to the removal rate of pollutants in the BF. However, most of them belonged to non culturable bacteria, which provided a direction for further development and utilization of microbial resources in the BF.

There are many determining factors for the differences in the microbial samples, such as taxonomic, richness, diversity etc. For differential comparison, these factors could be seen as dimensions which increased the complexity of the comparison. Nonmetric Multidimensional Scaling (NMDS) can simplify the research objects in multidimensional space to low dimensional space for analysis, while preserving the original relationships between objects [27].The NMDS analysis results of 9 microbial samples were shown in Fig. 5. The stress coefficient was 0.068 (less than 0.2), indicating that this figure could accurately reflect the ranking of microbial samples in terms of differences. For differences in treatment units, the differences between Unit 1 and the other two units were great, while there was little difference between Unit 2 and Unit 3. For samples within the units, the samples of Unit 2 had great differences, while the samples in the other two units had little differences.
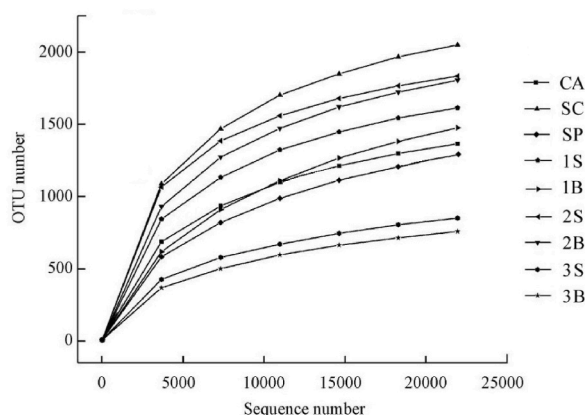

Fig. 3. The rarefaction curves of microbial samples with 97 % similarity.

Table 3

Diversity index analysis of microbial samples.

| Sample name	Sampling point	Richness index		Diversity index		Coverage
		ACE index	Chao1 index	Shannon index	Simpson index	
CA	Canna root	1251.015	1251.378	8.285	0.992	0.997
SC	Scindapsus root	1372.527	1285.642	8.352	0.992	0.997
SP	Spathiphylum root	1218.266	1157.362	8.015	0.992	0.997
1S	Surface filler of Unit 1	1214.815	1227.271	8.162	0.991	0.997
1B	Bottom filler of Unit 1	1277.065	1267.631	8.157	0.983	0.997
2S	Surface filler of Unit 2	1356.362	1347.374	8.416	0.986	0.997
2B	Bottom filler of Unit 2	1330.036	1219.846	8.183	0.993	0.996
3S	Surface filler of Unit 3	1196.734	1150.182	7.742	0.987	0.997
3B	Bottom filler of Unit 3	1175.642	1217.061	7.931	0.991	0.997
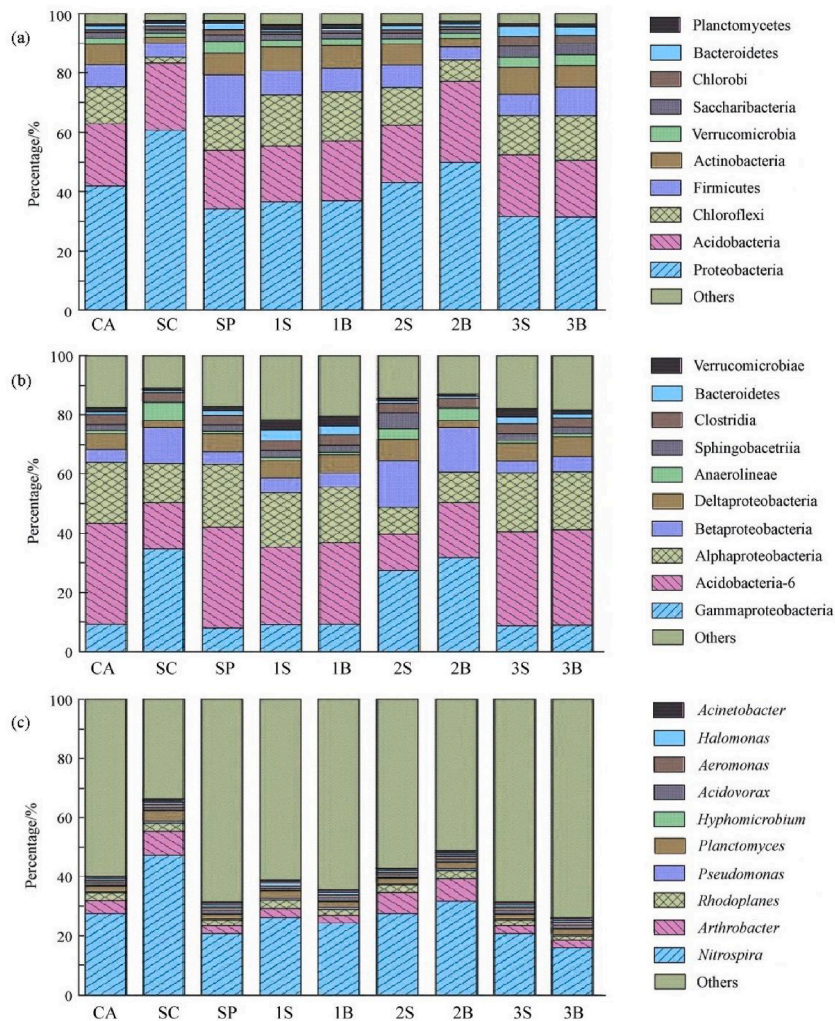
Fig. 4. The microbial community structure at different levels.

By using the PICRUSt software package to predict microbial functional characteristics, and comparing it with the KEGG database, microbial functional prediction information was obtained (Fig. 6). The functional gene clusters with high relative abundance were Amino Acid Metabolism, Membrane Transport, Carbohydrate Metabolism, Replication and Repair, Energy Metabolism, etc. Similarly, the microorganisms in the second unit still exhibited advantages in Amino Acid Metabolism and Membrane Transport function.
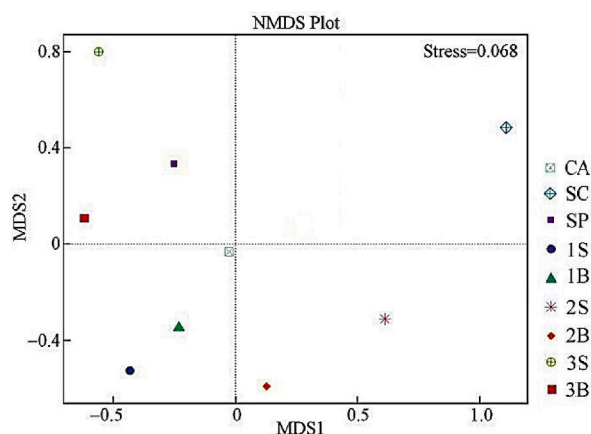
Fig. 5. Non-metric Multidimensional scaling analysis of microbial samples.
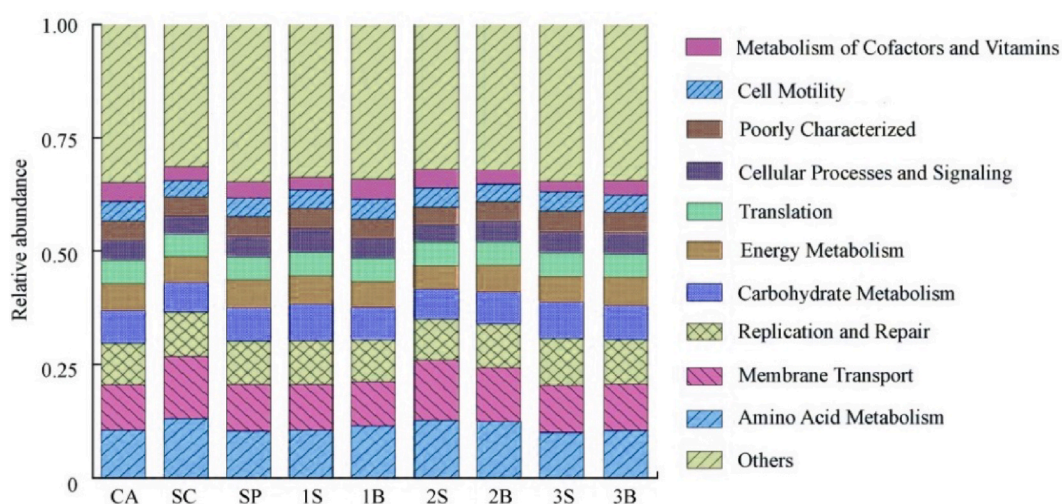

Fig. 6. The microbial functional characteristics.

4. Conclusions

For testing the treatment of BF on rural dispersed sewage, the pollutant purification effect was evaluated and microbial community characteristics were analyzed. Some conclusions could be drawn as follows.

(1) The degradation efficiency of pollutants in the BF showed seasonal changes, and the variation patterns were slightly different. The BF reactor could effectively and stably treat rural dispersed sewage, reaching the standard of charging.

(2) There are differences in the microbial communities of different plant roots and fillers, mainly in richness, diversity, etc. During the sewage treatment process, Scindapsus could effectively establish complex and stable microbial communities, and could better degrade pollutants, especially nitrogen removal.

(3) The dominant bacterial communities in the BF reactor were more than 11 phyla, of which Proteobacteria, Acidobacteriota, Chloroflexi, Firmicutes, and Actinobacteria accounted for 86.26 %. The dominant bacterial community level reached over 19 classes, Gammaproteobacteria, Acidobacteria-6, Alphaproteobacteria, Bacilli, Betaproteobacteria, Deltaproteobacteria and Anaerolineae were the main classes. At the genus level, the dominant bacteria mainly included *Nitrospira*, *Arthrobacter, Rhodoplanes,* etc.

The research results could provide reference and data support for cultivating efficient pollutant degrading bacteria and efficiently treating rural dispersed sewage. Classifying microorganisms according to functions such as nitrification and denitrification, so as to reveal the mechanism of pollutant removal in the BF, need to be estimated in future studies.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

CRediT authorship contribution statement

Songtao Shen: Writing – original draft, Software, Resources, Methodology, Investigation, Funding acquisition. **Junlong Meng:** Writing – original draft, Software. **Tuodi Zhang:** Writing – original draft, Software, Investigation. **Chuanhui Zhou:** Writing – original draft, Investigation. **Yingyi Xu:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Songtao Shen reports financial support was provided by Science and Technology Department of Sichuan Province.

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