Postprint: Spatial Distribution Characteristics of Microbial Communities in the Bosten Lake Constructed Wetland

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Abstract

Bosten Lake artificial wetland is a typical multi-stage surface-flow wastewater artificial wetland treatment system. In this study, water and sediment samples were collected from the inlets and outlets of various subsystems of the Bosten Lake artificial wetland, and the spatial distribution characteristics of microbial communities in water and sediments within the multi-stage wetland system were analyzed using high-throughput sequencing technology. The results demonstrated that overall, microbial diversity and abundance in artificial wetland sediments were higher than in the water column. In the multi-stage wetland system, microbial community abundance and diversity initially increased from the inlet and subsequently decreased after discharge from the subsurface-flow wetland into Bosten Lake. The core phyla in water were Proteobacteria, Firmicutes, Actinobacteria, and Planctomycetes. Among these, Proteobacteria exhibited an overall increasing trend, rising from 59.11% at the initial inlet to 84.80% at the final outlet, whereas Firmicutes displayed an overall decreasing trend, dropping from 24.05% to 6.83%. However, Firmicutes bacteria remained dominant, indicating that this phylum represents a dominant group in wastewater treatment systems. In sediments, Proteobacteria and Firmicutes bacteria remained the dominant groups; the abundance of Firmicutes bacteria also first increased then decreased, while Proteobacteria bacteria showed an overall decreasing trend with increasing treatment depth. Bacteroidetes and Chloroflexi exhibited completely opposite trends throughout the treatment process. Although the dominant bacterial phylum in both water and sediments was Proteobacteria, and the primary functional groups in both were amino acid transport and metabolism, the overall structure and function of microbial communities remained significantly different.

Full Text

Spatial Distribution of Microbial Communities in the Wetland Sewage Treatment System Constructed on Bosten Lake

Introduction

Constructed wetlands represent an important ecological engineering approach for wastewater treatment, with microbial communities playing a central role in pollutant degradation and nutrient cycling [?]. Previous studies have demonstrated the effectiveness of vertical flow constructed wetlands in treating domestic wastewater [?]. The microbial ecology of these systems is complex, with community structure influenced by environmental parameters and treatment processes [?]. Recent investigations have focused on understanding spatial variations in microbial diversity and function within multi-level treatment systems [?].

Materials and Methods

Sample Collection and Preservation. Water and sediment samples were collected from different subsystems of the multi-level wetland sewage treatment system constructed on Bosten Lake. Samples were filtered through 0.22 m membranes and stored at -80°C until analysis.

DNA Extraction and Sequencing. DNA was extracted from the collected samples using the E.Z.N.A. Mag-Bind Soil DNA Kit (OMEGA). DNA concentration and quality were assessed using a Qubit 2.0 Fluorometer. The V3–V4 region of the 16S rRNA gene was amplified using primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). PCR products were sequenced on the MiSeq PE250 platform.

Bioinformatic Analysis. Raw sequences were processed using USEARCH (v2018) at 97% sequence similarity to cluster Operational Taxonomic Units (OTUs). Samples S0 through S6 were analyzed using standard bioinformatic pipelines. ArcGIS was used for spatial mapping of sampling locations. Mothur was employed for additional sequence analysis. Statistical analysis was performed in R version 3.5.3 using ggplot2 [21]. GraPhlAn was used for visualization of microbial community structure [22]. PICRUSt was used for functional prediction of microbial communities [23].

Operational Taxonomic Units. OTUs were clustered at 97% sequence similarity, yielding 35,320 OTUs. Taxonomic classification was performed against reference databases. Functional prediction of microbial communities was conducted using PICRUSt.

Results

Microbial Diversity and Community Structure. Microbial diversity and richness in sediment samples were significantly higher than those in water samples. Richness and diversity increased in initial regions near the sewage inlet and decreased following discharge from the final subsurface wetland into Bosten Lake.

The core bacterial phyla identified in water samples were Proteobacteria, Firmicutes, Actinobacteria, and Planctomycetes. The abundance of Proteobacteria increased from 59.11% at the inlet to 84.80% at the outlet, while Firmicutes decreased from 24.05% at the inlet to 6.83% at the outlet. However, Firmicutes remained the dominant taxon in the sediment samples throughout the treatment system. In sediments, the abundance of Firmicutes first increased and then decreased with depth, whereas Proteobacteria showed the opposite trend. Bacteroidetes and Chloroflexi exhibited completely opposite trends during the entire treatment process.

Analysis of microbial community composition revealed distinct spatial patterns across the treatment system. Bacteroidetes and Chloroflexi showed contrasting abundance patterns, with Bacteroidetes being more prevalent in certain zones while Chloroflexi dominated in others. The relative abundance of these phyla varied significantly between water and sediment compartments, reflecting different ecological niches and functional roles within the wetland system.

Functional Characteristics. Functional analysis revealed that amino acid transport and metabolism, transcription, and cell wall/membrane/envelope biogenesis were the dominant metabolic pathways. The relative abundance of functionally unclassified genes ranged from 6.06% to 7.62%. Proteobacteria, which are primarily involved in amino acid transport and metabolism, dominated both water and sediment samples, though the overall structure and function of microbial communities varied significantly between these compartments.

Figure Captions

Fig. 1 Typical landscape and sampling locations map of the V-shaped constructed wetland of the Bosten Lake. Sampling points S0-S7 are marked along the treatment flow path, with S0 at the sewage inlet and S7 at the discharge point into the lake.

Table Captions

Tab. 1 Alpha diversity indices for water and sediment samples collected from different zones of the constructed wetland system. Diversity metrics include the Chao1 richness estimator and Shannon diversity index.

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