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Intertidal zonation affects diversity and functional potentials of bacteria in surface sediments: A case study of the Golden Bay mangrove, China

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ABSTRACT

The combined effects of intertidal zonation and mangrove vegetation on benthic bacterial communities and ecological functions were studied. MiSeq sequencing of the 16S rRNA gene and PICRUSt predictive functional profiles were used to investigate the diversity, community structure and potential metabolic functions of benthic bacteria in surface sediments of a mangrove ecosystem. Compared with the lower intertidal zone, bacterial alpha diversity and richness were significantly higher in the upper intertidal zone and highly associated with sediment organic matter. The upper zone was occupied by higher proportions of heterotrophic bacteria involved in the degradation of organic compounds. These included Desulfobacterales, Anaerolineae and Acidobacteria, while the proportion of Rhodobacterales and Xenococcaceae was greatly increased in the lower zone. No significant difference of either alpha diversity or community composition was found between rhizosphere and bulk sediments, except that higher relative proportions of Rhizobiales and Actinobacteria occurred in rhizosphere sediments. The shift in bacterial community structure was mainly driven by changes in sediment Pb and NH_4^+ concentrations. Among the major carbon, nitrogen and sulfur cycling processes examined, higher potentials of cellulose and hemicellulose degradation, dissimilatory sulfate reduction, and nitrate or nitrite reduction occurred in the upper intertidal zone. Assimilatory sulfate reduction and sulfur oxidation potentials were higher in the root-associated sediments than in the bulk sediments. This study indicated that intertidal zonation was more important than root effects in modulating benthic bacterial diversity and functional potentials in a mangrove ecosystem.

1. Introduction

The intertidal ecosystem is the interface of ocean, atmosphere and terrestrial environments and is important due to its biological productivity and economic value (Ortega-Morales et al., 2010). A typical characteristic of the intertidal zone is periodic inundation, which leads to intertidal zonation with strong environmental stress gradients along the vertical axis perpendicular to the shore. These include desiccation, UV irradiation, nutrients and dissolved oxygen availability (Decho, 2000; Cefalì et al., 2016). The intertidal zone is typically divided into two distinctive habitats, the upper intertidal zone and the lower intertidal zone, based on inundation time (Ortega-Morales et al., 2010). The upper intertidal zone is only flooded by high tides. It is exposed to air for longer periods and experiences prolonged periods of desiccation and decreased sediment water content. The lower tidal zone is submerged most of the time and only exposed during low tide. Longer inundation in the lower tidal zone promotes anaerobic conditions in sediments that affects organic matter degradation and nutrient cycling (Schuur and Matson, 2001; Neatrour et al., 2004). Frequent flooding can enhance the transfer of organic matter from the lower tidal zone to adjacent coastal environments and accelerate the leaching loss of organic matter in sediments (Dittmar et al., 2006; Kristensen et al., 2008).

Tidal zonation has significant impacts on the distribution and ecological functions of microorganisms, which are the primary drivers of matter cycling and energy metabolism (Decho, 2000). Zhu et al. (2018) used MiSeq 16S rRNA gene sequencing to show that the diversity of microeukaryotes was higher in the upper intertidal zone than in the lower intertidal zone. The cyanobacteria composition was dissimilar and had lower diversity in the upper intertidal zone as revealed by DGGE fingerprinting (Abed et al., 2007; Rigonato et al., 2013). Lv et al. (2016) used high-throughput sequencing of 16S rRNA genes to study bacterial communities and their metabolic potentials in tidal flat

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sediments of the Yellow River Delta. A general pattern of tidal zonation was seen, but the effects of plants inhabiting the tidal zones, which might influence the bacterial diversities, was not studied.

Mangroves are typical intertidal ecosystems (Decho, 2000). Compared to bare intertidal sediments, mangrove sediments contain large amounts of organic carbon generated from mangrove litter, root exudates and deposition of phytoplankton debris. These sediments provide suitable niches for the development of taxonomically and functionally diverse microbial assemblages (Bouillon et al., 2004; Sahoo and Dhal, 2009). Mangrove roots excrete active organic compounds to rhizosphere sediments, and higher bacterial abundance and activities are usually found in rhizosphere sediments compared to adjacent bulk sediments. Mangrove roots also release photosynthesis-produced O_2 into sediments, which increases the oxidizing conditions of the rhizosphere sediments (Alongi, 2005; Reef et al., 2010) and influences the community composition of bacteria (Gomes et al., 2010, 2014). Jiang et al. (2013) found a large variation in bacterial diversity between inner and outer mangrove sediments and between the bulk sediments and the rhizosphere. However, the mechanisms underlying the organization and zonation of bacterial communities are poorly understood. For example, which environmental factors are responsible for the variations of bacterial diversity among tidal zonation patterns? Will tidal zonation outweigh the rhizosphere effect on bacterial diversity in intertidal systems?

We investigated a mangrove system and studied the major factors driving the tidal zonation of benthic zonation. We explored the relative importance of tides and rhizosphere effects on benthic bacterial diversity and community composition. MiSeq sequencing was used to reveal the benthic bacterial diversity and community composition in the upper and lower tidal sediments. Based on 16S rDNA information, we predicted a differentiation of functional potentials (e.g., genes involved in nitrogen and sulfur cycling and enzyme activity of cellulose and hemicellulose degradation) among tidal zones and areas derived from mangrove roots.

2. Materials and methods

2.1. Sampling procedures

The southern China study area and sampling methods were described previously (Zhu et al., 2018). The mangrove forest is dominated by *Avicennia marina* with an area of 4 km^2, and has a subtropical monsoon climate. The annual average surface water temperature is approximately 24 °C. Briefly, surface sediments (top 5 cm) of ten sites, which were located in the upper tidal zone (UTZ: sites U1-U5) and the lower tidal zone (LTZ: sites L1-L5), were collected from the Golden Bay Mangrove Reserve (21°24′9.72″N, 109°09′1.51″E), Guangxi Province in June 2014. At each site, five near-root (within 3 cm from the roots) sediment samples were collected using a custom-made corer (1-cm diameter) after the ebb tide. These samples were pooled as were the bulk sediments (1.5 m away from the roots). The samples were cooled on ice and transported to the laboratory for processing. Geochemical variables of the sediments, such as total organic carbon (TOC) and total organic nitrogen (TON) contents, nutrients (NH_4^+, NO_3^- and NO_2^-), sulfate (SO_4^{2-}), concentrations of metals (i.e., Pb, V and Cr), and sediment grain sizes were determined (Zhu et al., 2018).

2.2. DNA extraction, PCR amplification and high-throughput sequencing

Genomic DNA was extracted from the sediment samples utilizing the FastDNA Spin Kit for soil (MP Biomedical, USA) following manufacturer instructions. The V4 hypervariable region of 16S rDNA was amplified using the bacterial universal primers 515F (5′-GTGCCAGCM GCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011). Each sample was distinguished by a unique paired barcode at the beginning of the forward primer and the end of the reverse primer. PCR reaction mixture (30 µl) contained 0.2 µM of each primer, 10 ng of genomic DNA, and 15 µl of Phusion High-Fidelity PCR Master Mix (New England Biolabs). The PCR was performed with 98 °C for 1 min, 30 cycles of (98 °C for 10 s, 50 °C for 30 s, 72 °C for 30 s) and a final extension step at 72 °C for 5 min. Amplicon sequencing was performed on Illumina MiSeq platform (Illumina, USA) at a sequencing company (Novogene, Beijing, China).

2.3. Sequence processing and diversity analysis

Raw sequences were quality filtered and analyzed with QIIME (v.1.8.0, Caporaso et al., 2010) and the MOTHUR v.1.34.4 software package (Schloss et al., 2009). After a series of quality controls, the clean sequences (with quality score > 20; lengths between 249 and 460 bp; with no ambiguous bases and no primer sequences mismatches; homopolymers no longer than 6 bp) were retained. Putative chimeric sequences were detected with reference to the Greengenes database (released Aug. 2013) using USEARCH v.61. Sequences were assigned to operational taxonomic units (OTUs) at a cutoff of 97% sequence similarity using UCLUST v.1.2.2 (Edgar, 2010). Singletons were discarded before analysis. Taxonomy was assigned with UCLUST according to the Greengenes database. The reads assigned to Archaea, Chloroplast, or unassigned were removed before subsequent analyses. We rarefied all sequences at the number of 13,000 (the lowest number of quality sequences among all of the 20 samples) to assess bacterial alpha diversity estimators (OTU richness, Simpson, Shannon and Chao1 indices). Beta diversity was analyzed based on a normalized OTU table using the *edgeR* package (Robinson et al., 2010), without rarefying the sequence data as suggested by McMurdie and Holmes (2014). Beta diversity was calculated using Bray-Curtis distances and visualized using non-metric multidimensional scaling (NMDS) and implemented using PRIMER v.6 (Primer-E, UK). In addition, bacterial beta diversity was also assessed using the weighted UniFrac distance, a metric considering phylogenetic distance. Principal coordinate analyses (PCoA) were conducted using the *vegan* v.2.4–3 package in R v. 3.2.3 to visualize differences in the bacterial community structure.

2.4. Predicted functional profiles

Based on the bacterial 16S rDNA data, the functional profiles were reconstructed to infer the functional traits of bacterial communities in the mangrove sediments using PICRUSt (Langille et al., 2013). To prepare the data for PICRUSt analysis, we performed closed-reference 97% OTU picking against the Greengenes database (released May 2013). The 16S OTU table was normalized by PICRUSt's predicted 16S copy number for each OTU. Predicted gene family counts for each sample were obtained from metagenomic predictions based on KEGG orthology (KO). The nearest sequenced taxon index (NSTI) is an indicator of predictive accuracy, with lower values indicating better accuracy. Predicted KO counts were grouped into KEGG Pathway maps at level 3. KEGG pathways present in the datasets related to enzyme production potentials involved in cellulose and hemi-cellulose degradation, nitrogen and sulfur metabolism were classified, by hand, according to their KEGG identification.

2.5. Statistical analyses

The Student's *t* test was used to test the differences in relative abundances of microbial group, alpha diversity estimators and predicted function genes between the upper and lower tidal sediment and between near-root and bulk samples. Analysis of similarity (ANOSIM) was conducted to test the dissimilarity in community structure among sample groupings (Clarke, 1993). To investigate the relationship between environmental parameters and bacterial community structure, redundancy analysis (RDA) was performed using the normalized OTU table in R with the vegan package. Spearman's correlation was