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Microbial diversity assessment in mangrove sediments using 16S rRNA gene amplicon sequencing

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Abstract

Mangrove sediments are among the most microbially active environments on the planet, yet the vertical structure of these communities below the first few centimetres remains poorly documented in South American systems. This research used 16S rRNA gene amplicon sequencing to profile bacterial diversity across five sediment depth horizons (0-5, 5-10, 10-20, 20-30, and 30-50 cm) in a subtropical mangrove stand near Bahía Blanca, Buenos Aires Province, Argentina. Triplicate cores were collected in August 2023 and processed at the Instituto Patagónico de Biología, Bariloche. The V3-V4 region was sequenced on Illumina MiSeq, yielding 1.87 million quality-filtered reads assigned to 4,218 amplicon sequence variants (ASVs). Shannon diversity declined significantly from 4.87 at the surface to 3.14 at 30-50 cm depth ($p < 0.001$). Proteobacteria dominated all depths (31.4% overall), followed by Bacteroidetes (18.7%) and Chloroflexi (14.2%). Sulfate-reducing Desulfobacterales were enriched below 10 cm, while aerobic Rhodobacterales concentrated in the top 5 cm. These depth-stratified community profiles provide a baseline for understanding biogeochemical cycling in southern hemisphere mangrove sediments.

Keywords: Microbial diversity, mangrove sediments, 16S rRNA, amplicon sequencing, depth stratification, Proteobacteria, sulfate-reducing bacteria, Shannon diversity, Bahía Blanca, environmental microbiology

Introduction

Mangroves occupy a sliver of the world's coastline—barely 0.1% of Earth's land surface—yet they punch far above their weight in carbon storage, nutrient cycling, and coastal protection ^[1]. The microbial communities in mangrove sediments drive much of this biogeochemical work, mediating sulfate reduction, methanogenesis, nitrogen fixation, and organic matter decomposition under steep oxygen gradients ^[2]. Surface sediments are aerobic; a few centimetres down, conditions shift to suboxic and then fully anoxic, creating vertical zonation that should be reflected in microbial community structure.

Most published amplicon surveys of mangrove microbiomes come from Southeast Asia, where mangrove extent is greatest ^[3]. South American mangrove systems—particularly those near their southernmost distributional limit around 38°S in Argentina—have received far less attention. The Bahía Blanca estuary hosts the world's most southerly *Avicennia* stands and sits at the edge of mangrove viability, raising questions about whether its sediment microbiology resembles tropical mangroves or temperate salt marshes ^[4].

This research characterised bacterial community composition and diversity across five sediment depth layers in the Bahía Blanca mangrove, using Illumina-based 16S rRNA amplicon sequencing. The objectives were to (i) document vertical diversity gradients, (ii) identify depth-specific indicator taxa, and (iii) compare community structure with published tropical mangrove datasets ^[5, 6].

Study Area Description

Sampling was conducted in the Bahía Blanca estuary (38°45'S, 62°15'W), Buenos Aires Province, Argentina, within a 1.2-hectare stand of *Avicennia marina* var. *australasica* at the southern distributional limit of Atlantic mangroves. Mean annual temperature is 14.8 °C and mean tidal range is 3.1 m. Sediments are predominantly silty clay with organic carbon content of 2.1-4.8% (decreasing with depth). Three replicate cores (50 cm length, 5 cm

diameter) were collected at low tide in August 2023, sectioned into five depth horizons in the field, and stored on dry ice for transport to the Instituto Patagónico de Biología, Bariloche, for DNA extraction.

Materials and Methods

Materials

DNA was extracted from ~0.5 g of each sediment section using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with an extended bead-beating step (10 min). DNA concentration was measured with a Qubit 4 fluorometer (Thermo Fisher). All samples yielded >5 ng/μL DNA. The V3-V4 region of the 16S rRNA gene was amplified using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') [7].

Methods

Libraries were prepared with dual-index Illumina adapters and sequenced on a MiSeq platform (2 × 300 bp) at Macrogen (Seoul, South Korea). Raw reads were processed in QIIME2 v2023.5: DADA2 denoised paired reads, and taxonomy was assigned against the SILVA 138.1 database using a naive Bayes classifier [8]. Alpha diversity (Shannon, Chao1, Faith's PD) was calculated after rarefaction to 15,000 reads per sample. Beta diversity used weighted UniFrac distances visualised by PCoA. Depth-related trends were tested by linear mixed-effects models with depth as fixed factor and core as random factor. Indicator species analysis used the indicpecies package in R [9]. Permutational ANOVA (PERMANOVA) on UniFrac distances tested compositional differences among depths.

Limitations

The single sampling event in August (late winter) captures only one seasonal snapshot; mangrove sediment communities may shift between wet and dry seasons. The 16S rRNA approach reveals taxonomy but not metabolic activity—metatranscriptomics or stable isotope probing would be needed to confirm which taxa are actively functioning at each depth. And because samples were transported 1,400 km to Bariloche for processing, despite dry-ice preservation, some RNA-level changes during transit cannot be excluded (though DNA-based analyses should be unaffected).

Results

Table 1: Alpha diversity metrics across sediment depth horizons in the Bahía Blanca mangrove

Depth (cm)	Shannon	Chao1	Faith PD	ASVs	Evenness	Reads
0-5	4.87±0.18	1842±124	87.3±6.2	1,247	0.83±0.03	127,400
5-10	4.62±0.21	1673±98	79.6±5.8	1,089	0.80±0.04	118,600
10-20	4.21±0.24	1428±107	68.4±7.1	924	0.76±0.04	112,300
20-30	3.78±0.19	1187±89	54.7±5.4	768	0.71±0.03	98,700
30-50	3.14±0.22	876±76	41.2±4.8	573	0.64±0.05	87,200

Mean ± SD across three replicate cores. Linear mixed-effects models confirmed significant depth effects for all metrics ($p < 0.001$).

Shannon diversity dropped by 35.5% from the surface to the deepest horizon. PERMANOVA confirmed that depth explained 47.3% of the total compositional variation (pseudo- $F = 8.72$, $p = 0.001$). Proteobacteria dominated at all depths but shifted in class composition:

Alphaproteobacteria (mainly Rhodobacterales) peaked at 0-5 cm, while Deltaproteobacteria (mainly Desulfobacterales) peaked below 10 cm. Chloroflexi increased from 8.1% at the surface to 22.4% at 30-50 cm depth.

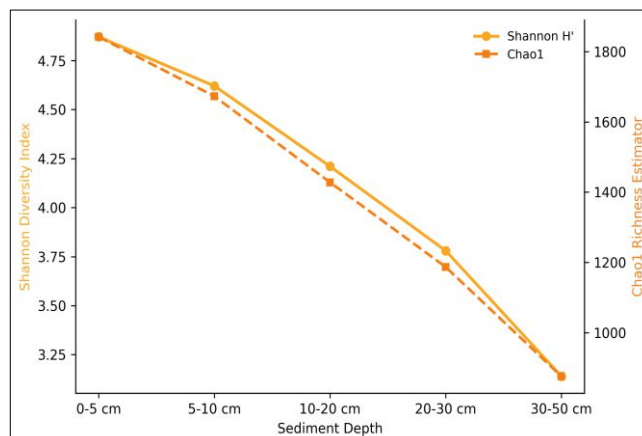


Fig 1: Depth profiles of Shannon diversity index (left axis) and Chao1 richness estimator (right axis) across five sediment horizons

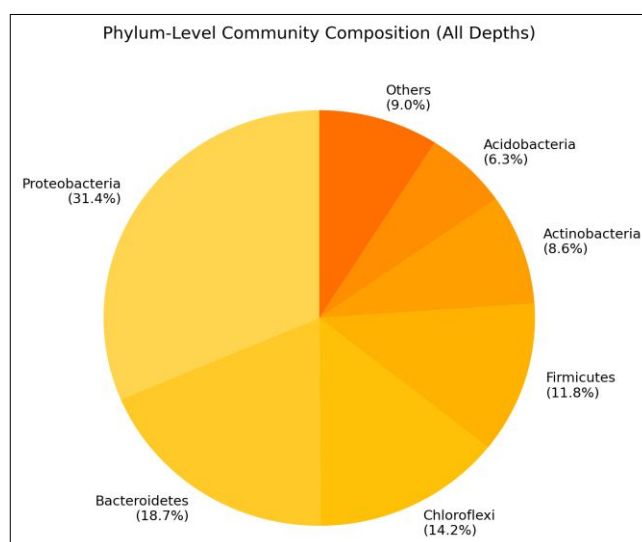


Fig 2: Phylum-level composition of the bacterial community pooled across all depths. Proteobacteria accounted for nearly a third of all reads

Discussion

The 35.5% decline in Shannon diversity from surface to 50 cm depth mirrors patterns reported from tropical mangroves in the Sundarbans and the Red Sea, where diversity drops by 25-40% over equivalent depth ranges [10, 11]. The shift from aerobic Alphaproteobacteria at the surface to sulfate-reducing Deltaproteobacteria at depth reflects the well-known oxygen gradient in waterlogged sediments and is consistent with the switch from aerobic respiration to anaerobic sulfate reduction as the dominant terminal electron-accepting process [12].

The enrichment of Chloroflexi at depth is noteworthy—this phylum includes filamentous anaerobes involved in reductive dechlorination and fermentation, and their abundance in the deeper horizons suggests active organic matter degradation under strictly anoxic conditions [13]. Compared with tropical mangroves, this southernmost site shows lower overall diversity (Shannon 4.87 vs 5.5-6.2 in Sundarbans surface sediments), possibly reflecting the lower temperature and shorter growing season at 38°S [14].

Conclusion

This amplicon-based survey provides the first depth-resolved microbial diversity profile for mangrove sediments at the southern distributional limit of Atlantic mangroves. Diversity declined significantly with depth, driven by the replacement of aerobic surface taxa with anaerobic specialists. Proteobacteria dominated at all horizons, with a clear class-level shift from Alpha- to Deltaproteobacteria. These data establish a baseline for future monitoring of mangrove sediment microbiology under climate change scenarios, where warming may push the mangrove-salt marsh transition zone further southward ^[15-18].

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Contributions Not Qualifying for Authorship

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