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Original Research

Comparative Assessment of 16S rRNA and Metagenomic Sequencing for Characterizing Microbial Communities across Mangrove Water-Sediment Interfaces

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Abstract

Understanding microbial communities in mangrove ecosystems presents unique methodological challenges due to complex physicochemical gradients and varying biomass levels between water and sediment compartments. We systematically evaluated 16S rRNA amplicon and shotgun metagenomic sequencing approaches using the Dongzhai Port mangrove ecosystem as a model system. Metagenomic sequencing demonstrated superior taxonomic resolution, identifying 82 phyla compared to 52 phyla by 16S rRNA sequencing, and revealed distinct habitat-specific patterns in community structure. Both methods consistently showed higher bacterial diversity in sediment samples, reflecting enriched nutrient conditions and a stable physicochemical environment. While 16S rRNA sequencing effectively captured core community structures, metagenomic analysis provided deeper insights into functional capabilities, particularly in energy metabolism and nitrogen cycling pathways. Notably, metagenomic sequencing uncovered unique distribution patterns of key nitrogen metabolism genes (*gltB, glnA/GLUL, gltD*) between water and sediment compartments. Technical considerations, including PCR inhibition in sediments and variable biomass in water samples, significantly influenced method performance. Our findings provide a quantitative framework for method selection in mangrove microbiome studies, recommending 16S rRNA sequencing for broad community surveys and metagenomic sequencing for detailed functional analysis or rare taxa detection.

Keywords: mangrove ecosystem, microbial ecology, DNA sequencing methods, functional gene, habitat-specific diversity, nitrogen cycling

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Introduction

Marine ecosystems, particularly mangrove-dominated coastal zones, represent critical sites for understanding microbial diversity and function. The unique complexity of these systems stems from their constantly shifting physicochemical gradients, including tidally driven salinity fluctuations, alternating redox conditions, and variable nutrient pulses [1-3]. These dynamic conditions not only shape microbial communities but also challenge our analytical methods. Mangrove sediments contain high levels of PCR inhibitors and environmental DNA from diverse sources, while water samples vary in biomass and salt content across tidal cycles [4, 5]. Such environmental complexity makes mangroves ideal testing grounds for evaluating sequencing methodologies.

Given these environmental challenges, microbial research in mangrove ecosystems has evolved rapidly with technological advances [6, 7]. High-throughput methods have revolutionized our understanding, revealing previously hidden diversity that early culture-based studies missed. Two approaches now dominate the field: 16S rRNA gene amplicon sequencing and shotgun metagenomic sequencing. Their complementary strengths are well-documented in terrestrial and freshwater systems, yet their effectiveness in the challenging mangrove environment remains unclear [8] – a critical gap given these microbiomes' central role in coastal biogeochemistry.

In mangrove research, each method offers distinct advantages while facing specific challenges. 16S rRNA sequencing provides cost-effective community profiling [9], but encounters three major obstacles: PCR inhibition from humic substances, amplification bias under high-salt conditions, and abundance distortion from variable gene copy numbers [10-12]. Metagenomic sequencing overcomes these limitations through direct DNA sequencing, enabling strain-level identification and rare species detection [13, 14]. However, its application faces practical constraints: higher costs, complex bioinformatic requirements, and challenges in handling halophilic and extremophilic sequences [15].

Recent studies have demonstrated the value of combining these approaches [10, 12], particularly for understanding functional processes like nitrogen cycling in mangrove ecosystems [16]. Despite growing research interest, systematic method comparisons in marine systems remain limited [17]. To address this gap, we selected the Dongzhai Port mangrove ecosystem for our comprehensive evaluation, capitalizing on its well-documented environmental gradients and representative Indo-Pacific mangrove community structure. Based on these methodological challenges, we identified three critical parameters for evaluation: taxonomic resolution across salinity gradients, functional gene detection in nitrogen cycling pathways, and rare species identification. This systematic evaluation aims to establish a practical framework for method selection in future mangrove microbiome studies, ultimately enhancing our understanding of these vital coastal ecosystems.

Materials and Methods

Sample Processing and DNA Extraction

Five sampling sites were established in the Dongzhai Port Mangrove Nature Reserve, representing different habitat types (e.g., water and sediment). Samples from each of the five sites were collected in triplicate during the ebb tide using a grab dredger for surface sediment (0-20 cm depth). The sediment samples from each site were thoroughly mixed, divided into three equal parts, and placed in aseptic plastic bags. During the high tide, water samples were collected near the same points and then thoroughly mixed before being divided into three equal parts for collection in aseptic sample bottles. All water and sediment samples were placed in an incubator with ice packs and transported to the laboratory within 4 hours for DNA extraction.

For water samples, macro-organisms and debris were removed using 20 μm mesh filters, and bacterial cells were concentrated on 0.22 μm sterile membrane filters. Genomic DNA was extracted from the processed water filters and sediment samples using an optimized in situ pyrolysis protocol [18]. DNA quality was assessed through three steps: spectrophotometric analysis using a NanoDrop 2000 to confirm A260/A280 ratios of 1.8-2.0, molecular integrity confirmed by 1% agarose gel electrophoresis, and concentration measured with the Qubit™ dsDNA HS Assay Kit (range: 0.2-100 ng/μL). Only samples displaying clear high-molecular-weight bands, DNA concentrations >10 ng/μL, and appropriate A260/A280 ratios were selected for further analysis. The qualified DNA samples were sent to Shanghai Meiji Biopharmaceutical Technology Co., Ltd. for concurrent metagenomic and 16S rRNA gene sequencing.

Library Preparation and Sequencing Strategies

We amplified the V3-V4 hypervariable regions using validated universal primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [19]. To minimize amplification bias, we performed PCR reactions in triplicate with the following thermal cycling conditions: initial denaturation at 95ºC for 3 min; 27 cycles of denaturation (95ºC, 30 s), annealing (55ºC, 30 s), and extension (72ºC, 30 s); followed by a final extension at 72ºC for 10 min. The resulting amplicon libraries (350-450 bp) underwent quality assessment before sequencing on the Illumina MiSeq platform using 300-bp paired-end chemistry.

For metagenomic analysis, we fragmented genomic DNA to approximately 400 bp using a Covaris

M220 ultrasonicator. We constructed libraries using the NEXTFLEX Rapid DNA-SEQ kit, following protocols optimized for complex environmental samples. To detect rare species, we performed high-throughput sequencing on the Illumina NovaSeq platform with 150 bp paired-end chemistry, targeting 15 Gb of data per sample.

Bioinformatic Analysis Pipeline

We processed raw sequence data through Fastp (v0.19.6) for quality filtration and clustered sequences into Operational Taxonomic Units (OTUs) at 97% similarity. Using RDP Classifier (v2.11) [20] against the Silva database (v138), we assigned taxonomic classifications with a 70% confidence threshold. We then predicted functional potential using PICRUSt2 (v2.2.0) with COG and KEGG databases.

Using MEGAHIT (v1.1.2), we assembled high-quality sequences into contigs with a minimum length of 300 bp. MetaGene [21] identified open reading frames (ORFs) from these contigs. We selected genes ≥100 bp and translated them into amino acid sequences. For functional annotation, we aligned these sequences against NR, eggNOG, and KEGG databases using Diamond (v0.8.35) [22] with an E-value cutoff of 1e-5.

Statistical Analysis

We conducted comprehensive statistical analyses using R software (version 4.0.3) to compare the two sequencing approaches. Using the *vegan* package, we calculated multiple α diversity metrics – species richness (Sobs), Shannon-Wiener diversity index, Simpson diversity index, and Pielou's evenness index – after normalizing sequences to the minimum sequencing depth. Student's t-tests determined inter-method differences.

Through the *pheatmap* package, we performed hierarchical clustering to visualize patterns of Clusters of Orthologous Groups (COG) functions and nitrogen cycle-related genes. We compared PICRUSt2-predicted functions (16S data) with directly observed functions from metagenomic sequencing. Student's t-tests ($P<0.05$) revealed differences in bacterial COG functional composition and nitrogen cycle gene abundances between water and sediment samples.

For multiple comparisons, *P*-values were adjusted using the False Discovery Rate (FDR) method, and adjusted $P<0.05$ were considered statistically significant. Additionally, Linear Discriminant Analysis Effect Size (LEfSe) was utilized to identify differentially abundant taxonomic and functional features between water and sediment samples, with an LDA score threshold of 2.0.

Data Availability

The 16S rRNA and metagenomic sequences from this study are available in the NCBI Short Read Archive under accession numbers SRP249356 and SRP249901, respectively.

Results

Quality Assessment and Performance Comparison of Sequencing Methods

Quality control processes, including sequence filtering and chimera removal, generated 250,158 valid sequences from both water and sediment samples. Using a 97% similarity threshold for OTU clustering, we identified 5,367 OTUs in water samples and 12,003 OTUs in sediment samples. The Sobs richness index rarefaction curves reached clear asymptotes at the OTU level, confirming adequate sequencing depth for capturing bacterial community composition in the Dongzhai Port mangrove ecosystem (Fig. 1).

Metagenomic sequencing produced an average of 14.95 Gb and 15.00 Gb of data from water and sediment samples, corresponding to 296,952,268 and 297,953,390 initial raw reads. The data showed exceptional quality, with water and sediment samples losing only 1.02% and 0.68% of reads during quality filtering. The final clean read counts reached 293,927,739 for water and 295,937,727 for sediment samples. Despite their distinct physicochemical properties, both habitat types maintained comparable data quality.

Comparative Analysis of Taxonomic Resolution

The 16S rRNA gene sequencing established a baseline taxonomic profile, identifying 52 phyla, 139 classes, 278 orders, 511 families, 910 genera, and 1,801 species across both habitats. This method captured core community structures effectively, revealing 1,051 shared species between water and sediment samples. The Venn diagram (Fig. 2a)) revealed 1,051 shared species between habitats, with 265 species unique to water and 485 to sediment samples, highlighting habitat-specific patterns. Metagenomic sequencing showed slightly lower detection rates at intermediate taxonomic levels – class (2.88%), order (9.71%), and family (8.22%). Yet, it achieved superior resolution at other levels, detecting 57.69% more phyla and nearly doubling genus-level identifications. At the species level, metagenomic sequencing proved particularly powerful for mangrove ecosystem characterization, identifying 11,877 species total – a 5.6-fold increase over 16S rRNA sequencing. The Venn diagram (Fig. 2b)) revealed 9,737 shared species between habitats, with 1,598 species unique to water and 542 unique to sediment samples.

Student's t-test analysis showed higher diversity indices in sediment samples compared to water samples across both sequencing platforms, except for the Sobs richness index. Each method revealed distinct strengths in capturing mangrove microbial diversity patterns. Metagenomic sequencing captured community richness

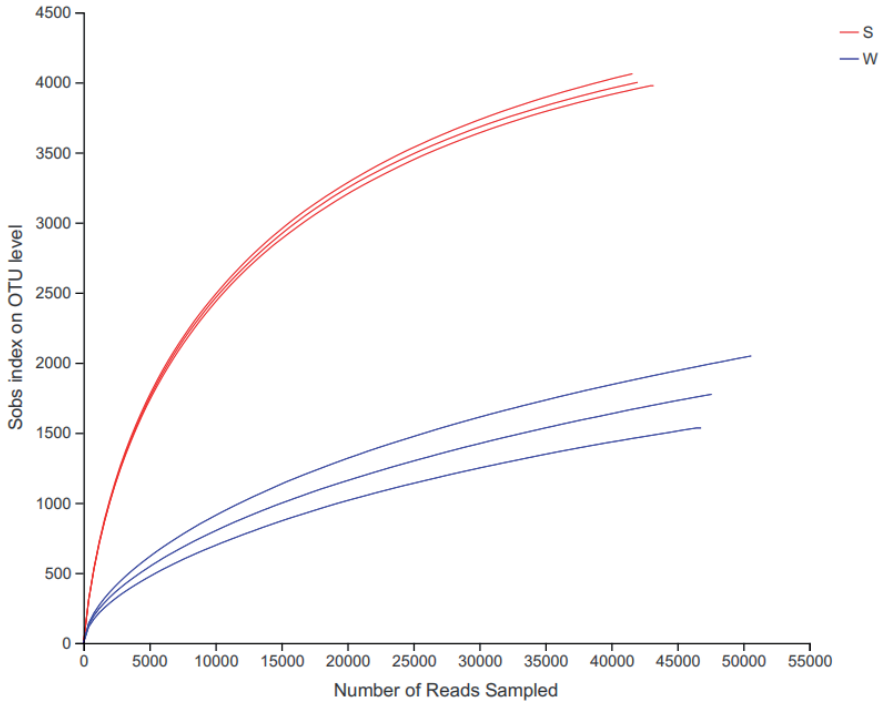


Fig. 1. Rarefaction curves showing bacterial OTU accumulation patterns between mangrove water (W, blue lines) and sediment (S, red lines) samples.

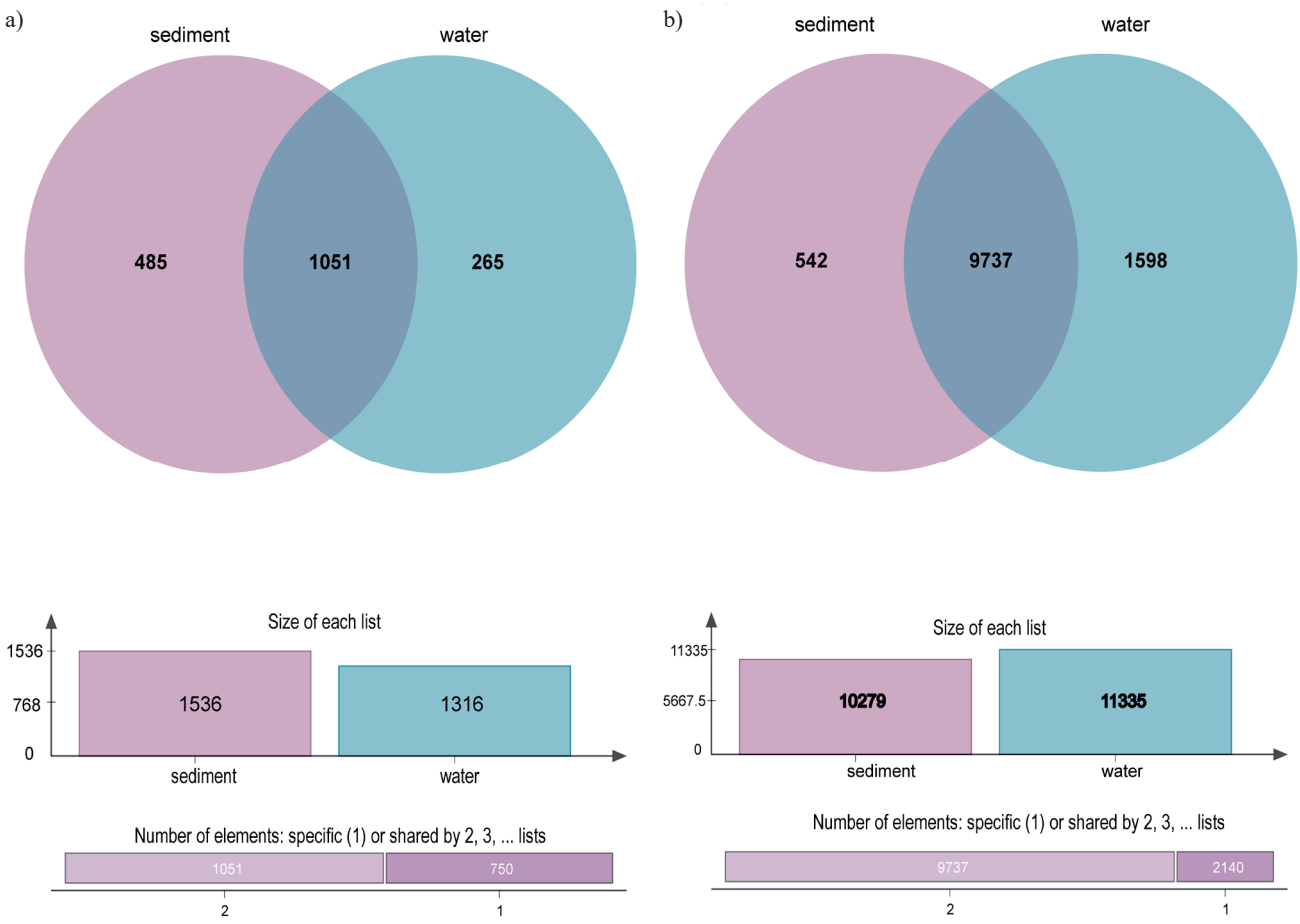


Fig. 2. Distribution of bacterial species between mangrove water and sediment samples based on a) 16S rRNA gene amplicon and b) metagenomic sequencing, showing shared and unique species in Venn diagrams (top) and total species counts (bottom).

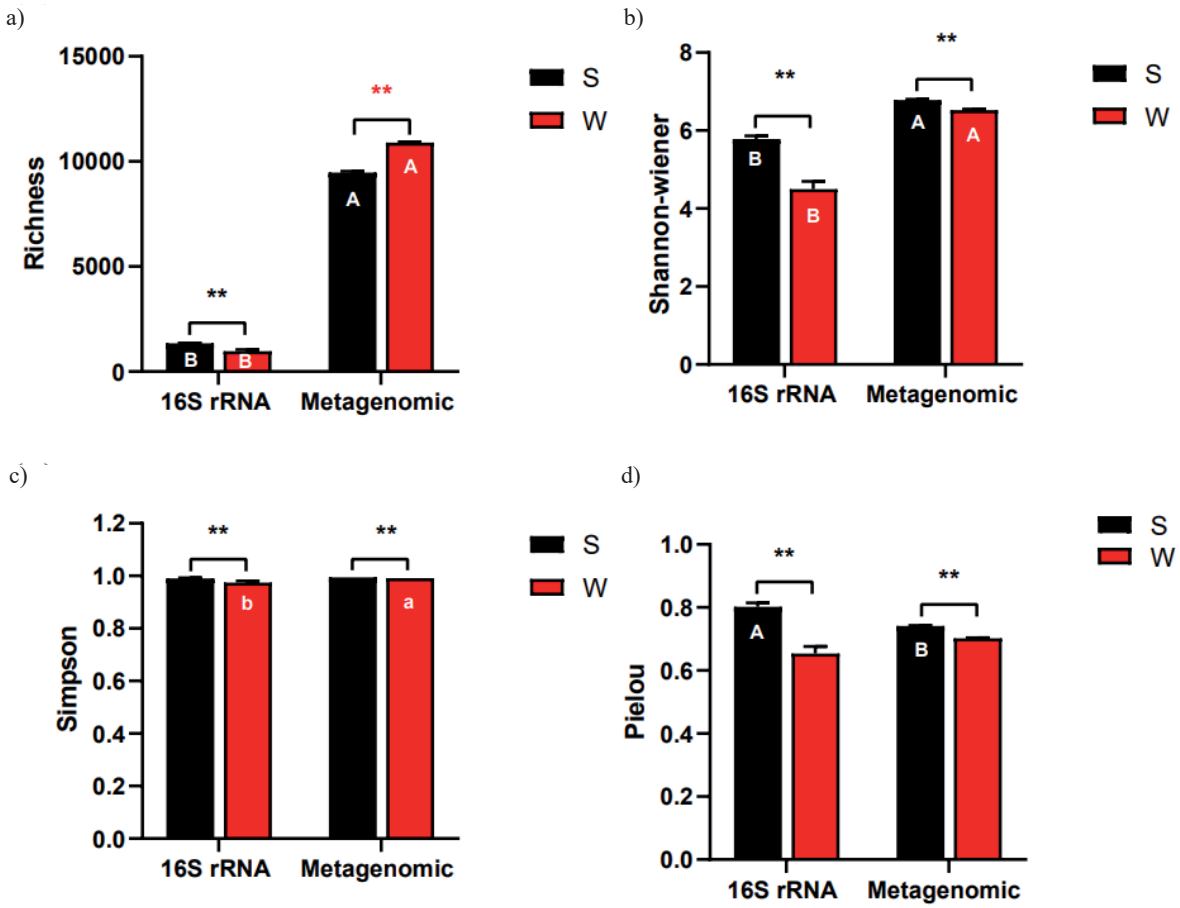


Fig. 3. Bacterial community alpha diversity metrics comparing mangrove water (W, red) and sediment (S, black) samples using 16S rRNA and metagenomic sequencing approaches: a) Sobs richness index, b) Shannon-Wiener diversity index, c) Simpson diversity index, and d) Pielou's evenness index. Letters above the bars denote significant differences between sequencing methods, where uppercase letters indicate a significant difference at $P<0.01$, and lowercase letters indicate a significant difference at $P<0.05$. Asterisks indicate significant differences between habitats (** $P<0.01$).

and diversity with greater sensitivity, showing higher Sobs richness and Shannon-Wiener index values in both sample types (Fig. 3a) and 3b)). This method excelled at detecting rare taxa and showed elevated Simpson diversity index values in water samples, though sediment samples showed no significant difference (Fig. 3c)). The 16S rRNA approach proved particularly effective for measuring community evenness, yielding higher Pielou evenness index values in sediment samples. In water samples, both methods produced similar evenness values (Fig. 3d)).

Method-specific Performance in Community Composition Analysis

Our 16S rRNA gene sequencing of water samples identified four dominant phyla (>1% relative abundance), accounting for 97.51% of the total community. Proteobacteria dominated the community, followed by Bacteroidetes, Actinobacteria, and Cyanobacteria (Fig. 4a)). At the class level, we found eleven dominant groups, with α-Proteobacteria, Flavobacteria, β-Proteobacteria, Actinobacteria, and γ-Proteobacteria showing the highest abundance (Fig. 4b)). Metagenomic analysis of water samples revealed three dominant phyla comprising 96.15% of the community: Proteobacteria (most abundant), Bacteroidetes, and Actinobacteria (Fig. 4c)). Eight dominant classes emerged, including α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Flavobacteria, and Actinobacteria – three fewer than the 16S rRNA method detected (Fig. 4d)). In sediment samples, 16S rRNA sequencing uncovered eleven dominant phyla representing 93.64% of the community. Five phyla exceeded 5% relative abundance: Proteobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, and Acidobacteria (Fig. 4a)). We detected seventeen dominant classes, primarily Acidobacteria, γ-Proteobacteria, Anaerolineae, and Actinobacteria (Fig. 4b)). The metagenomic approach identified ten dominant phyla in sediment (95.32% of total abundance), with Proteobacteria, Actinobacteria, and Chloroflexi exceeding 5% relative abundance (Fig. 4c)). Eleven dominant classes emerged, including Actinobacteria and various Proteobacteria classes

– six fewer than the 16S rRNA method detected (Fig. 4d)).

16S rRNA sequencing revealed higher abundances of Bacteroidetes and Actinobacteria in water samples, while sediments showed dominance of seven phyla, including Chloroflexi, Acidobacteria, and Nitrospirae (Fig. 5a)). Metagenomic data confirmed these patterns, showing enriched Bacteroidetes in water and elevated levels of six phyla – notably Chloroflexi, Firmicutes, and Gemmatimonadetes – in sediments (Fig. 5b)). At the class level, both methods yielded complementary insights. 16S rRNA analysis showed α-Proteobacteria, Flavobacteria, and Actinomycetes enrichment in water samples. Eight classes characterized sediment communities, including γ-Proteobacteria, δ-Proteobacteria, and Anaerolineae (Fig. 5c)). Metagenomic sequencing reinforced these distribution patterns while uncovering additional subclass variations (Fig. 5d)).

Functional Profile Analysis of Bacterial Communities Using Different Sequencing Approaches

16S rRNA sequencing identified three main functional categories: general function prediction (Category R), unknown functions (Category S), and amino acid metabolism (Category E). Metagenomic sequencing offered finer resolution, highlighting unknown functions (Category S), energy production and conversion (Category C), and amino acid metabolism (Category E). Hierarchical clustering analysis revealed distinct segregation between the functional profiles from these two methods (Fig. 6).

Our 16S rRNA analysis found higher proportions of amino acid metabolism (Category E) and unknown function (Category S) in water samples, while sediment samples showed elevated levels of general function

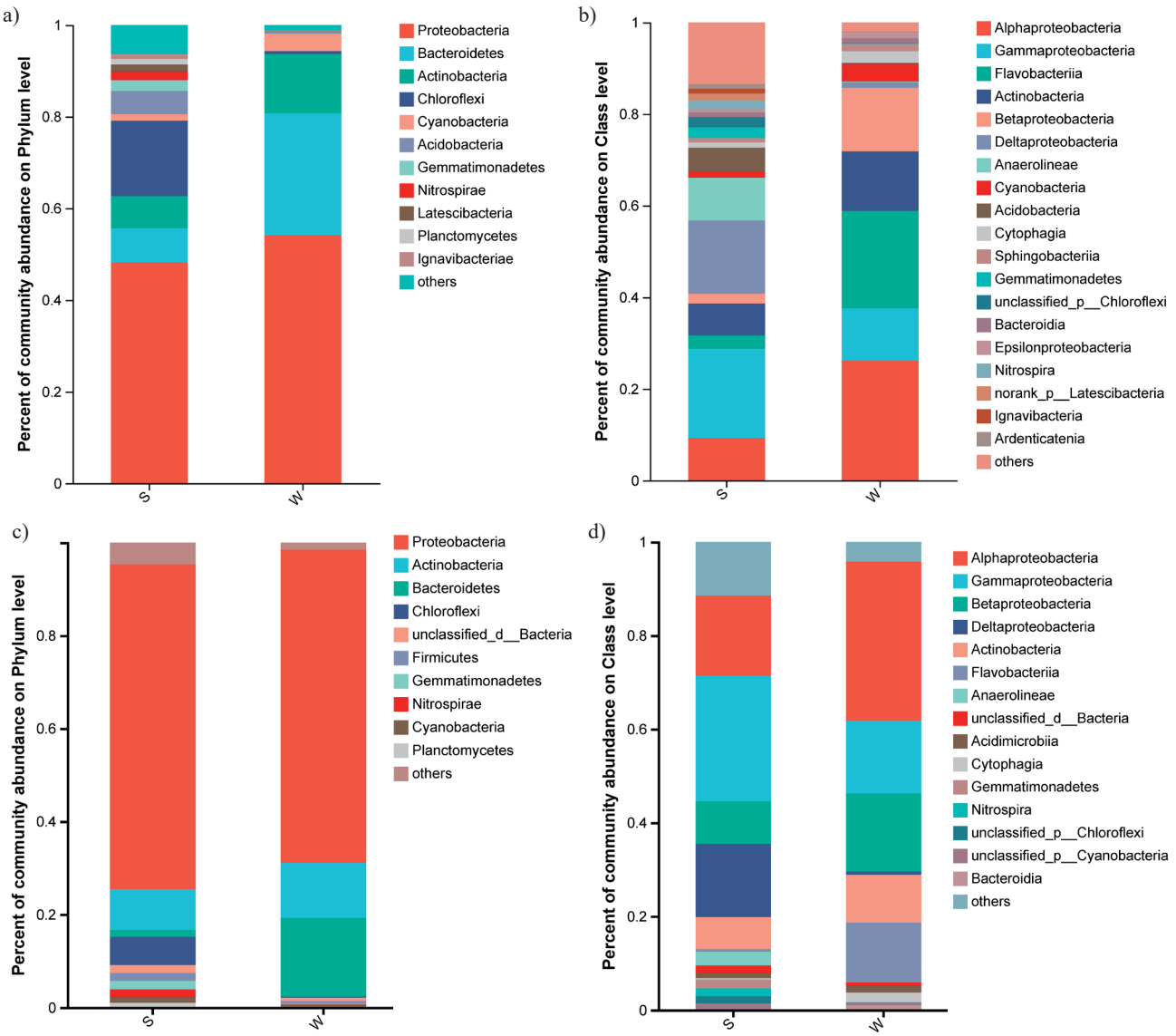


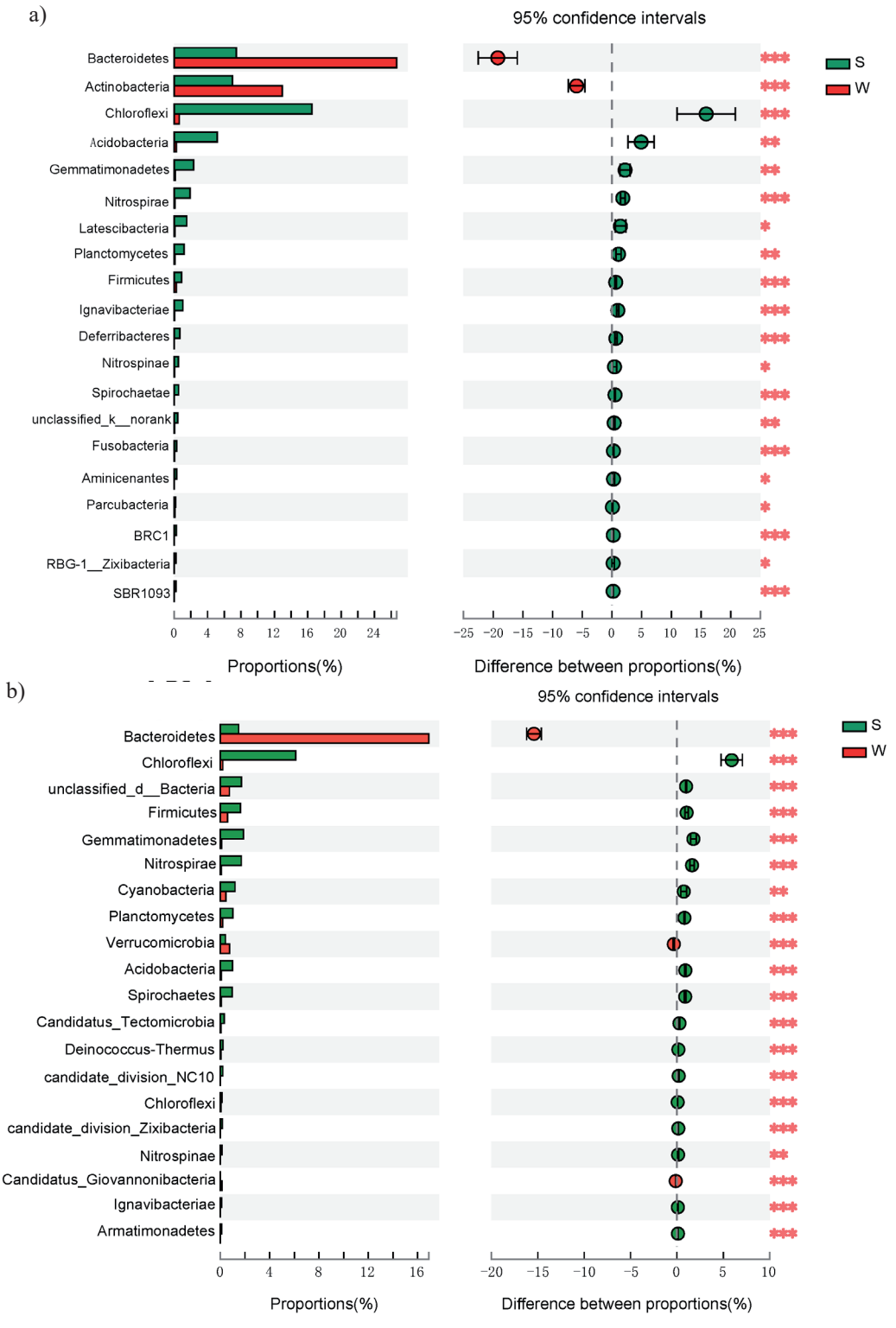
Fig. 4. Bacterial community composition across mangrove water (W) and sediment (S) samples: Phylum-level (a) 16S rRNA, c) metagenomic) and class-level (b) 16S rRNA, d) metagenomic) distributions from 16S rRNA and metagenomic sequencing.

prediction (Category R) (Fig. 7a)). Metagenomic data revealed similar patterns for amino acid metabolism (Category E) and unknown function (Category S) in water samples, but uniquely identified energy production and conversion (Category C) enrichment in sediment samples (Fig. 7b)). These distinct detection patterns underscore the value of combining both approaches for comprehensive functional analysis of mangrove microbiomes.

Comparative Analysis of Bacterial Nitrogen Cycle Gene Composition Using Different Sequencing Methods

Three key nitrogen metabolism genes – *gltB, glnA/GLUL, and gltD* – dominated the profiles across water and sediment samples (Fig. 8). Each sequencing method revealed unique distribution patterns of these genes.

Through functional prediction, our 16S rRNA analysis showed clear differences in relative gene



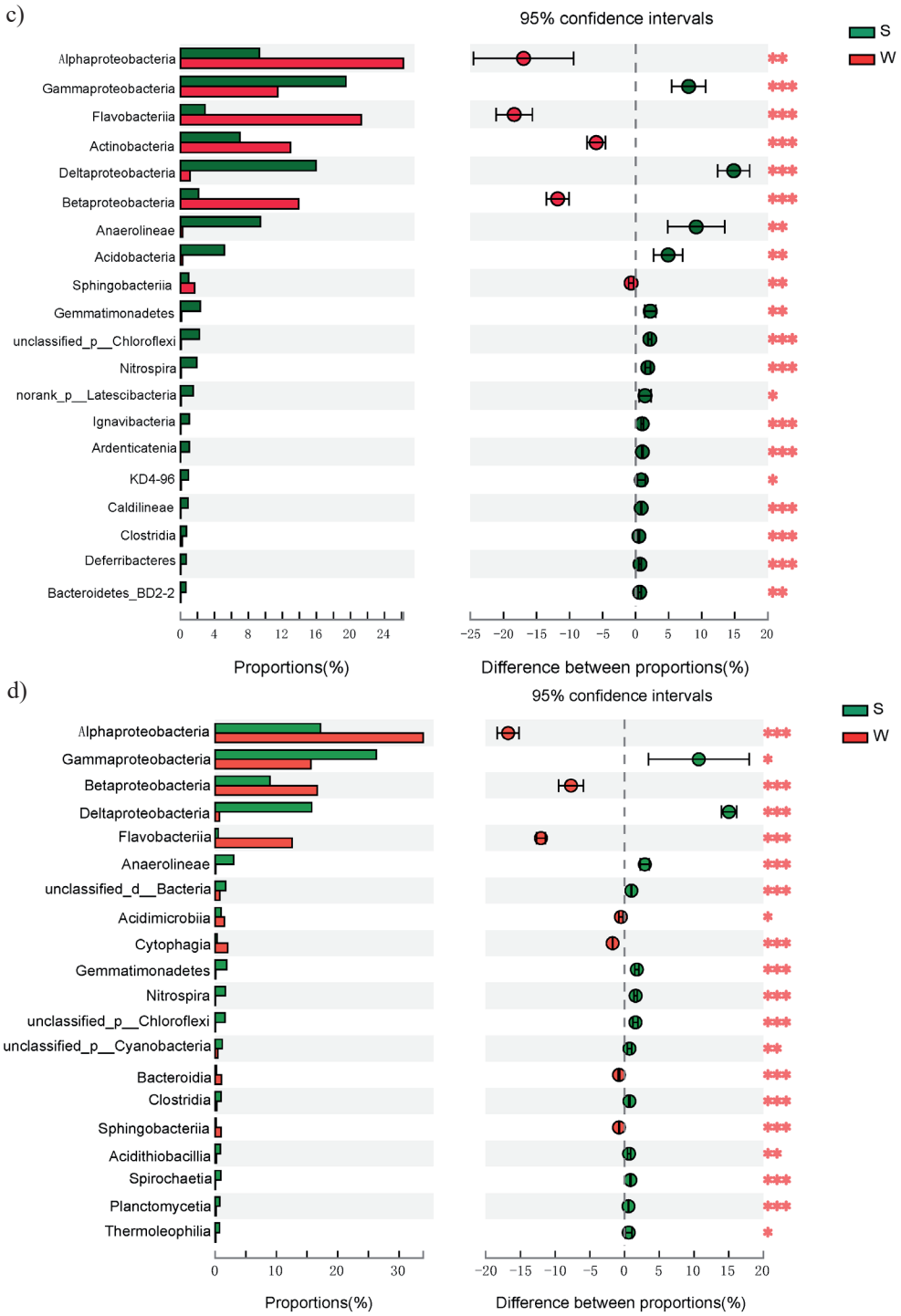


Fig. 5. Distribution of bacterial taxa between mangrove water (W) and sediment (S) samples at the phylum level (a, b) and class level (c, d), based on 16S rRNA gene sequencing (a, c) and metagenomic analysis (b, d). Left panels show relative abundances (%), and right panels indicate differences with 95% confidence intervals. Statistical significance was assessed using false discovery rate (FDR) correction (adjusted $P<0.05$). Linear discriminant analysis effect size (LEfSe) was applied to identify differentially abundant features (LDA score > 2.0). Significance levels: *P<0.05; **P<0.01; ***P<0.001.

abundances between sample types (Fig. 9a)). All three genes exhibited higher abundance in water samples, with *gltB* showing the most striking enrichment. Metagenomic sequencing validated these findings while capturing finer-scale abundance variations (Fig. 9b)). This approach revealed subtle differences in *gltB, glnA/GLU*L, and *gltD* expression patterns between sample types, particularly their enrichment levels in water samples. The metagenomic data also uncovered co-occurrence patterns of these genes within the broader nitrogen cycling network.

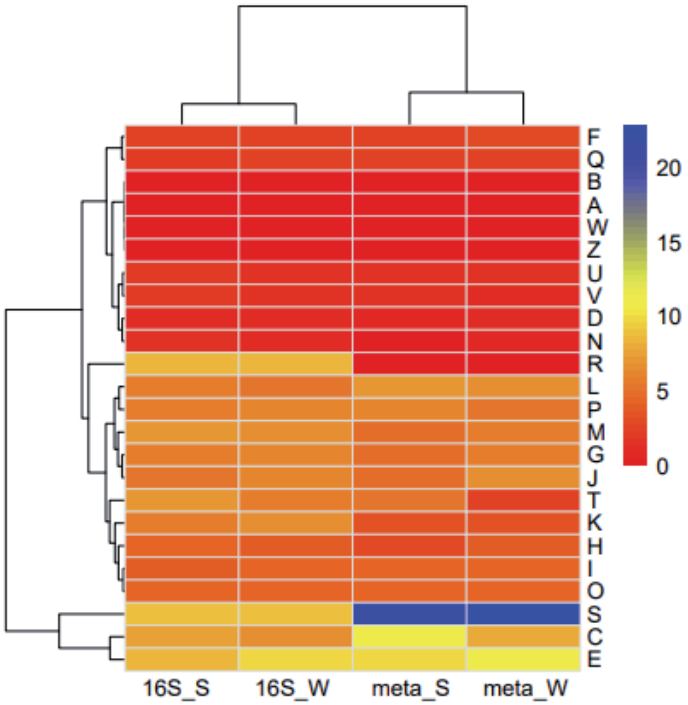


Fig. 6. Hierarchical clustering heatmap showing bacterial COG functional profiles across mangrove water (W) and sediment (S) samples using 16S rRNA and metagenomic (meta) sequencing methods. Color scale indicates relative abundance (0-20%), with letters A-Z representing different COG functional categories.

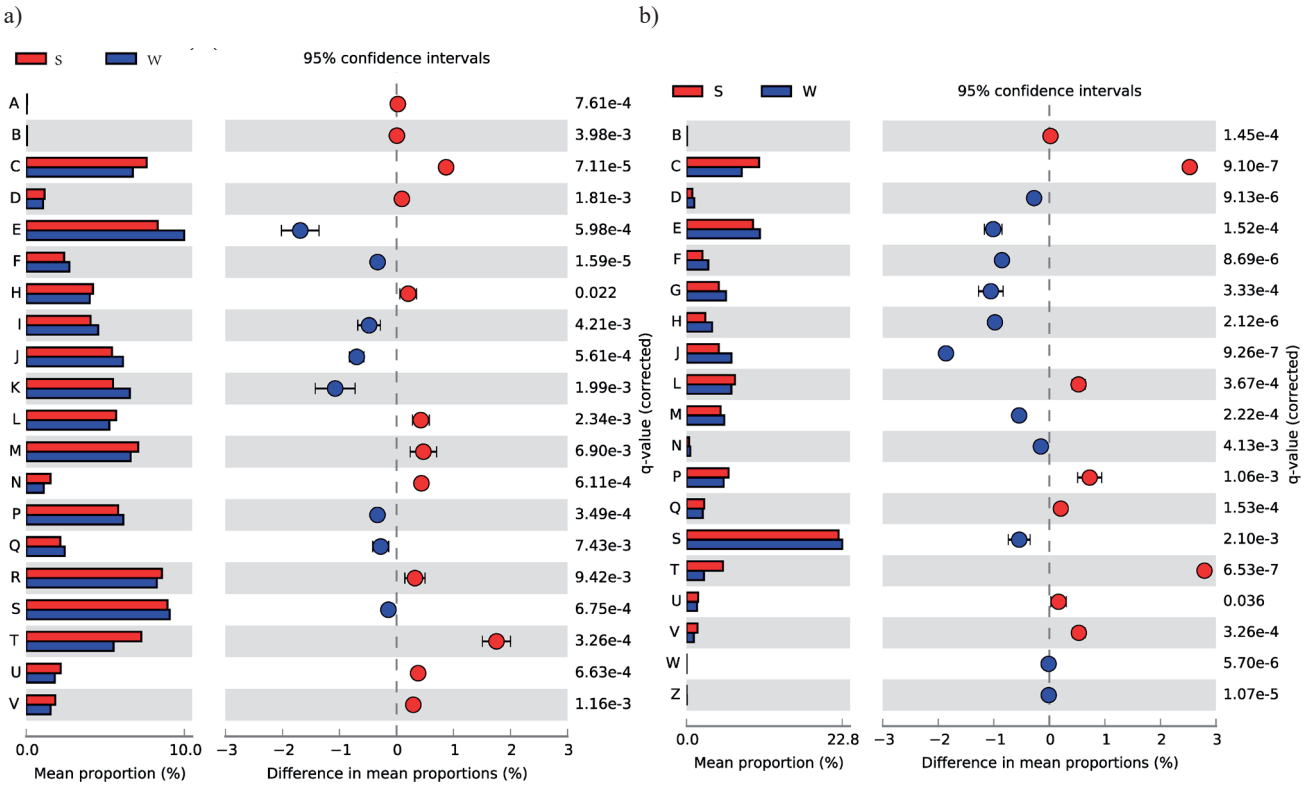


Fig. 7. Distribution of bacterial COG functional categories between mangrove water (W, blue) and sediment (S, red) samples, based on a) 16S rRNA gene sequencing and b) metagenomic analysis. Left panels display the mean relative abundances (%), while right panels show the differences in proportions between sample types with 95% confidence intervals. Differentially abundant functions were identified using LEfSe with a Kruskal–Wallis test ($\alpha = 0.05$) and an LDA score threshold of 2.0. The q-values shown on the far right represent FDR-adjusted significance levels.

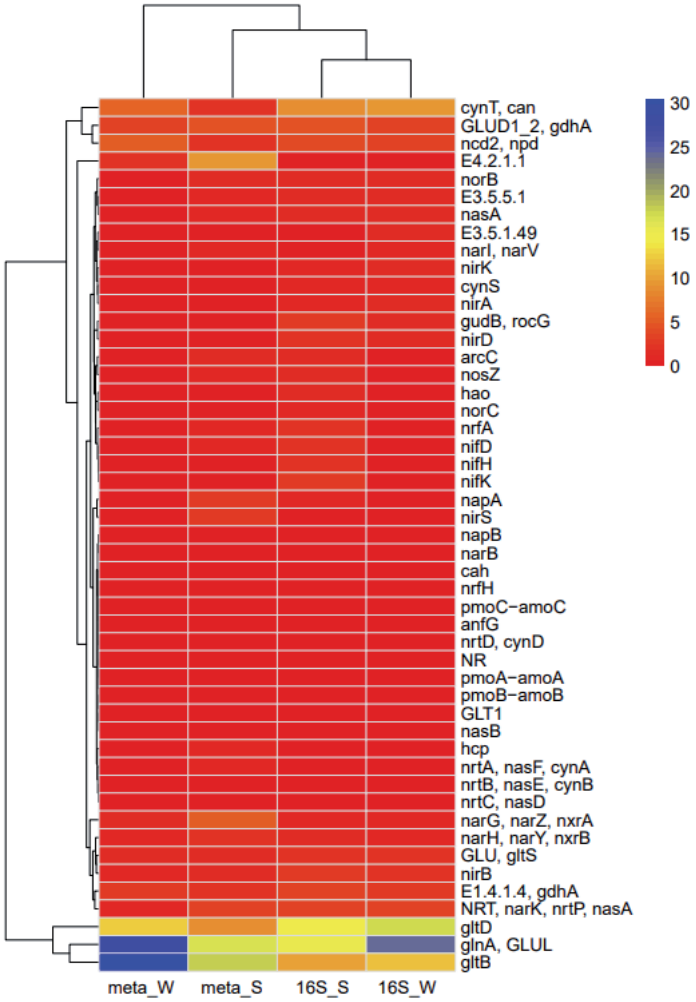


Fig. 8. Hierarchical clustering heatmap showing the distribution of nitrogen cycling genes across mangrove water (W) and sediment (S) samples analyzed by metagenomic (meta) and 16S rRNA (16S) sequencing. The color scale represents relative abundance (0-30%).

Discussion

Methodological Comparison in Taxonomic Resolution and Coverage

Metagenomic sequencing identified 82 phyla in our mangrove samples, exceeding the 52 phyla detected by 16S rRNA sequencing and surpassing previous findings in similar ecosystems [23-26]. This higher resolution reflects metagenomic sequencing's capacity to capture complete genomic content, particularly facilitating the detection of rare taxa [25, 27, 28]. However, differences in annotation thresholds and reference database completeness may also contribute to the observed discrepancy, especially in underrepresented marine lineages.

Our analysis also revealed clear habitat-dependent differences in the performance of the two methods. While 16S rRNA amplicon sequencing detected greater species richness in sediment samples, shotgun metagenomics captured higher microbial diversity in water. These differences likely stem from the interplay between methodological biases and habitat characteristics, including DNA extraction efficiency, community complexity, and environmental inhibitors [29]. Additionally, classification outcomes are inevitably shaped by confidence thresholds – such as the 0.7 cutoff in 16S annotation and the E-value criterion in metagenomics – which may limit taxonomic resolution where reference genomes are scarce.

When benchmarked against similar studies in other dynamic coastal systems such as estuaries and salt marshes [30], these observations emphasize that method-specific strengths and limitations are often habitat-dependent. This comparative perspective underscores the distinctiveness of mangrove microbiomes within broader aquatic interfaces and emphasizes the need for sequencing approaches that align with environmental context and research goals.

Habitat-Specific Community Structure and Method Performance

Both sequencing methods revealed higher bacterial diversity indices (Shannon-Wiener, Simpson, and Pielou)

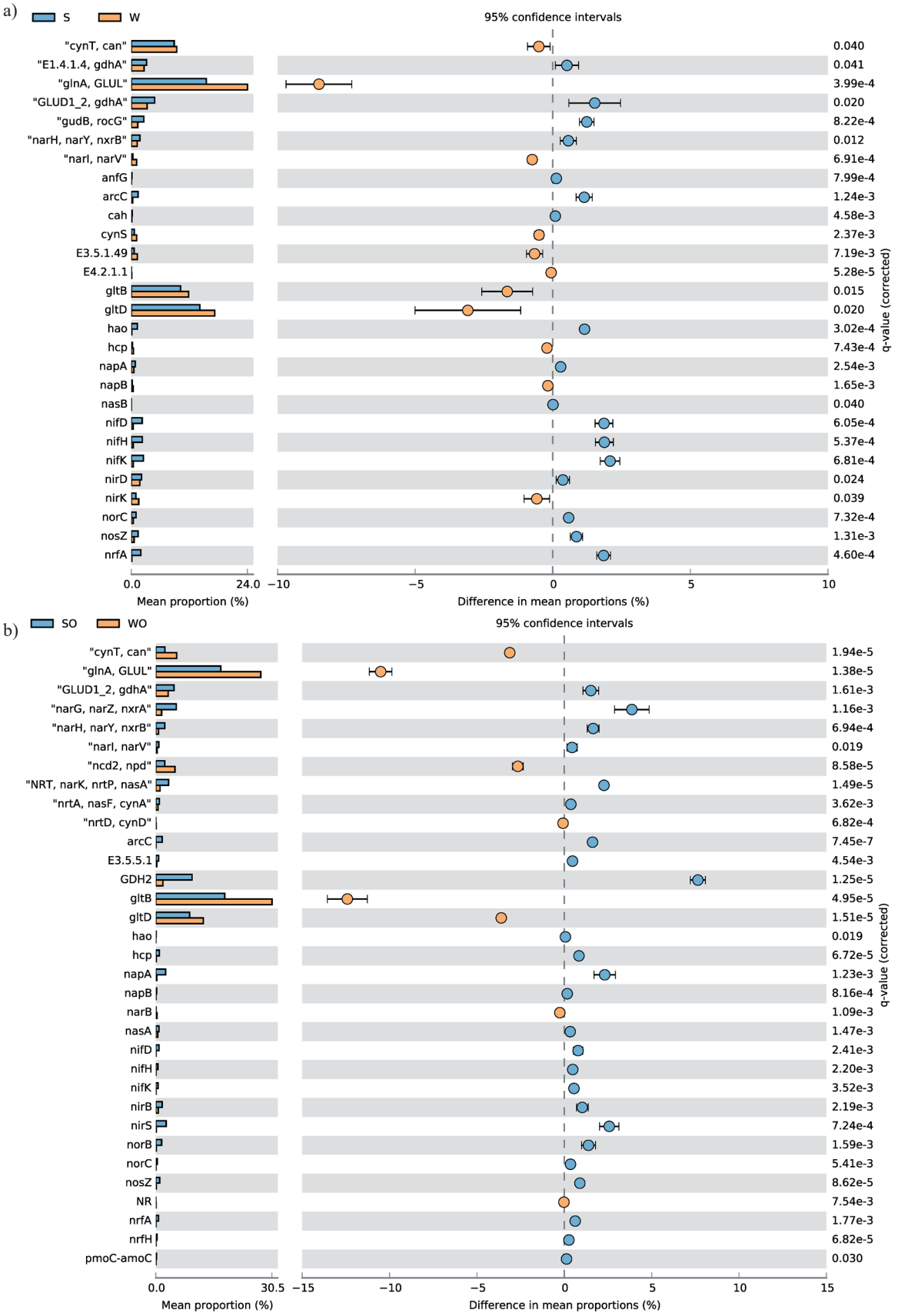


Fig. 9. Distribution of nitrogen cycling genes between mangrove water (W) and sediment (S) samples, based on a) 16S rRNA gene prediction and b) metagenomic analysis. Left panels display the mean relative abundances (%), while right panels show the differences in proportions between sample types with 95% confidence intervals. Differentially abundant genes were identified using LEfSe with a Kruskal–Wallis test ($\alpha = 0.05$) and a linear discriminant analysis (LDA) score threshold of 2.0. The q-values shown on the far right represent FDR-adjusted significance levels.

in sediment compared to water samples, matching patterns found in other wetland ecosystems [10, 31, 32]. This cross-method agreement points to three key ecological drivers: enriched nutrient and organic matter availability in sediments [33, 34]; stable physicochemical conditions buffering against environmental fluctuations [35]; and enhanced microbial niche differentiation. These factors collectively foster greater microbial diversity in sediment habitats.

The core community composition remained consistent across methods: Proteobacteria dominated both habitats, followed by Bacteroidetes and Actinobacteria, supporting previous findings from global mangrove studies [36-38]. Both approaches captured essential biogeochemical players like α-Proteobacteria and γ-Proteobacteria [39-41]. Metagenomic analysis further revealed their functional capabilities, reflecting the intense material and energy flux characteristic of mangrove ecosystems [42]. The high proportion of shared species between water and sediment samples, confirmed by both methods, indicates active material exchange between these connected habitats [26].

Functional Gene Detection and Annotation Efficiency

Each sequencing method showed distinct strengths in detecting functional genes. Both identified similar dominant COG categories (S and E), yet metagenomic sequencing excelled at revealing specific functional pathways, especially those linked to energy production and conversion (Category C) [43]. This advantage stems from capturing the entire genetic content rather than single marker genes. Metagenomic sequencing identified 41 phyla compared to 26 phyla through 16S rRNA analysis, showing particular strength in capturing rare taxa and functional genes across both matrices. This stems from whole-genome coverage rather than single-region amplification.

The power of metagenomic sequencing became particularly clear in analyzing nitrogen cycling genes. We found *gltB, glnA/GLUL*, and *gltD* predominating in both water and sediment samples from the Dongzhai Port mangrove. This pattern differs from previous findings in the Beibu Gulf mangroves [44], where *gs_K00266, nmo, glnA, nasA,* and *ureC* showed the highest abundance. These genes play vital roles in nitrogen cycling [45]. Glutamine and glutamate synthetases create a critical metabolic hub, enabling glutamine synthesis from glutamate and ammonium ions, followed by glutamine-to-glutamate conversion. These pathways are essential for nitrogen utilization by both microorganisms and mangrove vegetation [46]. The robust organic nitrogen cycling in Dongzhai Port mangroves aligns with its rich organic nitrogen pool [47]. While we focused on these specific genes due to their consistent detection and distinct spatial patterns – effectively demonstrating the strength of metagenomics in resolving functional pathways – we acknowledge that a comprehensive understanding of nitrogen cycling requires a broader set of canonical genes, including *amoA* (ammonia oxidation), *nifH* (nitrogen fixation), and *nirS*/*nirK*, *norB*, *nosZ* (denitrification). Given the methodological focus of this study, a full exploration of these pathways was beyond our current scope. Future metagenomic investigations should certainly extend to these key genes to provide a more comprehensive picture of nitrogen cycling potential in mangroves, building upon the methodological insights presented here. Although both methods detected similar numbers of nitrogen cycle genes, they differed in sensitivity to abundance variations between habitats, highlighting their complementary strengths in analyzing complex ecosystems.

Technical Considerations and Method-Specific Limitations

Yet, metagenomic approaches face notable challenges: intensive computation needs, higher costs per sample, and current marine database limitations affecting annotation accuracy [48]. These factors demand careful consideration in large-scale studies. While 16S rRNA sequencing offers proven pipelines and cost-effective profiling, its single-marker constraints limit functional predictions and gene content analysis, particularly affecting habitat-specific adaptation studies. It is important to acknowledge that functional predictions from 16S rRNA data are inherently indirect and prone to false positives, and should thus be interpreted as reflecting potential rather than realized functions. Our findings, by demonstrating the superior functional resolution of metagenomic sequencing, further highlight these constraints. While experimental validation, such as enzyme activity assays, represents the gold standard for confirming metabolic capabilities, such assays were beyond the scope of this comparative study. We therefore recommend that future investigations aiming to conclusively establish functional differentiation integrate such experimental validations alongside in silico predictions.

For optimal mangrove microbiome characterization, combining both approaches is most effective: 16S rRNA for efficient community profiling, and metagenomic sequencing for uncovering functional adaptations and rare taxa [49]. This complementarity becomes crucial in mangrove systems, where understanding both community structure and function across environmental gradients remains essential [50].

Conclusions

Our systematic comparison of 16S rRNA and metagenomic approaches revealed distinct methodological strengths in mangrove microbiome analysis. Metagenomic sequencing identified 82 phyla

compared to 52 phyla by 16S rRNA sequencing, demonstrating superior capacity in capturing taxonomic diversity. Both methods effectively detected dominant bacterial groups, with Proteobacteria, Bacteroidetes, and Actinobacteria consistently dominant across habitats. Method performance showed clear habitat dependencies. In sediment samples, both approaches revealed higher bacterial diversity indices, reflecting the enriched nutrient conditions and stable physicochemical environment. The methods complemented each other in functional analysis – 16S rRNA sequencing provided efficient community profiling, while metagenomic sequencing uncovered detailed functional pathways, particularly in energy metabolism and nitrogen cycling. Notably, metagenomic analysis identified unique nitrogen cycle gene distribution patterns between water and sediment compartments.

Technical considerations emerged as crucial factors in method selection. While 16S rRNA sequencing offers cost-effective and established analytical pipelines, metagenomic sequencing provides comprehensive functional insights despite computational challenges. For future mangrove microbiome studies, we recommend selecting methods based on specific research objectives: 16S rRNA sequencing for broad community surveys and pilot studies, and metagenomic sequencing when detailed functional understanding or rare taxa detection is crucial. In complex projects, the combination of both approaches may provide the most comprehensive understanding of mangrove microbial ecology.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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