



## Article

# Diversity, Environmental Associations, and Co-Occurrence Networks of Mangrove Sediment Yeasts

Lila Kusuma Rahayu <sup>1</sup>, Budi Warsito <sup>1,2</sup>, Muhammad Danie Al Malik <sup>3</sup> and Tri Retnaningsih Soeprbowati <sup>1,4,5,\*</sup> 

<sup>1</sup> PhD Program of Environmental Science, School of Postgraduate Studies, Universitas Diponegoro, Semarang 50275, Indonesia

<sup>2</sup> Department of Statistics, Faculty of Sciences and Mathematics, Universitas Diponegoro, Semarang 50275, Indonesia

<sup>3</sup> Diponegoro Biodiversity Project Laboratory, Integrated Laboratory Unit, Universitas Diponegoro, Semarang 50275, Indonesia

<sup>4</sup> Cluster for Paleolimnology (CPalim), Universitas Diponegoro, Semarang 50275, Indonesia

<sup>5</sup> Department of Biology, Faculty of Sciences and Mathematics, Universitas Diponegoro, Semarang 50275, Indonesia

\* Correspondence: trsoeprbowati@live.undip.ac.id

## Abstract

Mangrove sediments represent dynamic coastal microbiomes that harbor diverse microbial communities under fluctuating environmental conditions. Unlike extensively studied bacterial communities, yeast microbiomes in mangrove habitats remain poorly understood. This study evaluated the diversity, community dynamics, environmental associations, and ecological interaction networks of yeast assemblages in mangrove sediments from Central Java, Indonesia, using ITS metabarcoding and multivariate ecological analyses. Environmental DNA extracted from sediment samples was sequenced using the Illumina NovaSeq 6000 platform, followed by ASV delineation, taxonomic curation, diversity analyses, ordination, variation partitioning, and co-occurrence network inference. *Malassezia*-associated taxa dominated mangrove sediment yeast communities, with pronounced geographic variation in subordinate taxa among ecosystems. Alpha- and beta-diversity analyses revealed heterogeneous community patterns and compositional tendencies across mangrove sediments. Salinity, total dissolved solids, Pb, and Cu were evaluated as potential environmental gradients; however, permutation tests indicated that the measured variables did not significantly explain yeast community composition. Co-occurrence network analysis revealed predominantly positive and modular associations, with several highly connected taxa potentially contributing to network connectivity and ecological organization. These findings expand current understanding of fungal microbiome organization in tropical mangrove ecosystems while emphasizing the need for cautious interpretation of environmental drivers in DNA-based sediment metabarcoding studies.

**Keywords:** community dynamics; ecological network; ITS metabarcoding; mangrove sediments; yeast microbiome



Academic Editor: Bruce S. Seal

Received: 27 May 2026

Revised: 3 July 2026

Accepted: 9 July 2026

Published: 15 July 2026

**Copyright:** © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

## 1. Introduction

Mangrove ecosystems are among the most productive and ecologically important coastal habitats worldwide [1,2], functioning as transitional zones between terrestrial and marine environments. These ecosystems support exceptional biodiversity while providing critical ecosystem services, including carbon sequestration, nutrient cycling, shoreline

stabilization, and long-term blue carbon storage [3,4]. Mangrove sediments, characterized by high organic matter accumulation and reduced decomposition rates, play essential roles in coastal resilience and global climate regulation. However, increasing anthropogenic pressures, including climate change, sea-level rise, industrial pollution, aquaculture expansion, and land-use conversion, continue to threaten the stability of mangrove ecosystems and biodiversity globally [5,6].

Mangrove sediments represent highly dynamic microbial habitats shaped by fluctuating salinity, tidal inundation, hypoxic conditions, and organic-rich substrates [7,8]. These physicochemical conditions support diverse microbial assemblages within highly dynamic estuarine sediments. Sediment-associated microorganisms contribute substantially to organic matter decomposition, nutrient transformation, and biogeochemical cycling, thereby supporting ecosystem functioning and sediment stability [9]. Advances in high-throughput sequencing and environmental microbiology have considerably improved the understanding of mangrove microbiomes. Nevertheless, most studies remain predominantly focused on bacterial and archaeal communities. Zhao et al. [10] found that carbohydrate metabolism genes dominated subtropical mangrove sediments, emphasizing the importance of microbial carbon degradation pathways in coastal ecosystems. Similarly, Padhy et al. [11] reported distinct bacterial and archaeal assemblages associated with greenhouse gas regulation and nutrient cycling. In contrast, Sengupta et al. [12] demonstrated high microbial diversity and potential for secondary metabolite-associated genes in estuarine mangrove sediments. Despite these advances, fungal communities, particularly yeasts, remain comparatively underexplored.

Yeasts constitute an ecologically important component of mangrove fungal diversity due to their strong physiological adaptability and tolerance to environmental stress [13,14]. As unicellular fungi, yeasts are capable of surviving under fluctuating salinity, tidal disturbance, oxygen limitation, and organic-enriched sediment conditions characteristic of mangrove ecosystems [15]. These traits enable yeasts to occupy diverse ecological niches within coastal sediments [16]. Ecologically, mangrove-associated yeasts contribute to carbon degradation, nutrient recycling, and microbial interactions that support the functioning of sediment ecosystems. Their metabolic versatility and environmental resilience further suggest potential ecological importance in maintaining microbiome stability under dynamic coastal conditions [17,18].

The emergence of internal transcribed spacer ITS metabarcoding and high-throughput sequencing technologies has transformed fungal microbiome research by enabling detailed characterization of fungal diversity across environmental gradients [19,20]. Contemporary fungal ecology increasingly incorporates alpha diversity, beta diversity, ecological ordination, and co-occurrence network analyses to evaluate microbiome structure and environmental associations [21,22]. Environmental filtering has frequently been proposed as an important driver of fungal community organization in sediment ecosystems. Bahram et al. [23] demonstrated that environmental variables strongly influence fungal community structure across ecosystems, while Wang et al. [24] reported that environmental disturbance can alter microbial interaction networks and ecological connectivity within sediment microbiomes.

Despite growing interest in fungal ecology, studies specifically investigating mangrove-associated yeast microbiomes remain limited. Pothayi et al. [25] reported diverse fungal assemblages in mangrove sediments from India; however, their study primarily focused on broad fungal taxonomic diversity and did not address yeast-specific microbiome dynamics or ecological interactions. Current mangrove microbiome studies also rarely integrate diversity analysis, environmental association modeling, and ecological network approaches within a yeast-focused framework [26]. Consequently, the spatial organization, environ-

mental associations, and interaction structure of mangrove sediment yeast microbiomes remain poorly understood.

Accordingly, this study aimed to characterize the diversity and taxonomic composition of mangrove sediment yeasts, evaluate spatial variation in yeast community structure across mangrove ecosystems, assess environmental associations with yeast microbiome dynamics, and identify ecological interaction patterns among dominant yeast taxa.

## 2. Materials and Methods

### 2.1. Study Area

This study was conducted in five mangrove ecosystems in the northern coastal region of Central Java, Indonesia, namely Demak (DMK), Jepara (JPR), Tegal (TGL), Semarang (SMG), and Brebes (BRB). These ecosystems represent tropical estuarine mangrove habitats characterized by tidal influence, fluctuating salinity, and organic-rich sediments [27]. The selected sites differed in physicochemical conditions and anthropogenic influence, providing environmentally heterogeneous habitats suitable for evaluating yeast community diversity and dynamics across mangrove ecosystems.

Mangrove sediments within the study area are periodically exposed to seawater intrusion and sediment deposition, creating dynamic ecological conditions that support diverse microbial assemblages. Geographic coordinates and spatial distribution of sampling locations are presented in Supplementary Figure S1.

### 2.2. Sediment Sampling

Sediments were collected at low tide to assess intertidal mangrove zones. At each site, three spatially separated points were sampled to capture local environmental variation. Surface sediments (5–10 cm depth) were aseptically collected using sterile tools. Samples (~200–300 g) were placed in sterile polypropylene containers, kept cool during transport to the lab, and stored at  $-20^{\circ}\text{C}$  until DNA extraction and physicochemical analysis [28].

### 2.3. Environmental Parameter Measurements

Environmental parameters were measured to characterize the physicochemical conditions that potentially influence yeast community structure in mangrove sediments [23,29]. The measured variables included pH, temperature, salinity, total dissolved solids (TDS), and heavy metal concentrations of lead (Pb), cadmium (Cd), and copper (Cu) [30,31].

Sediment pH and temperature were measured in situ with a portable multiparameter meter, while salinity and TDS were determined with a calibrated handheld conductivity meter. All measurements were conducted in triplicate at each sampling point, and mean values were used for subsequent ecological analyses.

For heavy metal analysis, sediment samples were transported under controlled conditions to the Laboratorium Balai Besar Standardisasi dan Pelayanan Jasa Pencegahan Pencemaran Industri for laboratory-based quantification of Pb, Cd, and Cu concentrations. Sample preparation followed United States Environmental Protection Agency (USEPA) Method 3051A (2007), which uses microwave-assisted acid digestion to extract trace metals from sediment matrices. Quantification of heavy metals using USEPA Method 3051A (2007) and American Public Health Association (APHA) Standard Method 3111B (2023). Concentrations below the analytical detection limit were replaced with half the detection limit before statistical analyses. Detailed environmental measurements for each sampling replicate are presented in Table S1.

## 2.4. DNA Extraction, ITS Amplification, and Sequencing

Environmental DNA (eDNA) was extracted from mangrove sediment samples using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, Tustin, CA, USA) according to the manufacturer's protocol. DNA concentration and purity were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) [32].

Yeast community profiling was performed using high-throughput sequencing targeting the fungal internal transcribed spacer (ITS1–ITS2) region, a widely used molecular marker for fungal metabarcoding analyses [33]. Amplification of genomic DNA (gDNA) samples was conducted using the primer pair ITS1 (Forward: 5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (Reverse: 5'-TCCTCCGCTTATTGATATGC-3'). Library preparation was subsequently performed from the final polymerase chain reaction (PCR) amplicons, and sequencing was carried out by PT Genetika Science Indonesia (Tangerang, Indonesia) using the Illumina NovaSeq 6000 platform to generate paired-end raw reads [34]. Raw sequencing reads were subjected to quality assessment prior to downstream bioinformatics processing. Detailed sequencing quality statistics, read-filtering summaries, and raw sequencing data are presented in Supplementary Table S2.

## 2.5. Sequence Processing and Taxonomic Assignment

Raw paired-end sequencing reads were processed using a Quantitative Insights Into Microbial Ecology 2 (QIIME2)-based bioinformatics workflow, while downstream statistical analyses and visualization were conducted in RStudio v4.4.0. Adapter sequences and PCR primers were removed using Cutadapt [35]. Sequence denoising, quality filtering, chimera removal, and amplicon sequence variant (ASV) inference were subsequently performed using the Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline [36].

The resulting ASV dataset was subjected to taxonomic classification against the UNITE fungal ITS reference database [37,38]. Because the initial ITS dataset contained both filamentous fungi and yeast-associated taxa, subsequent taxonomic filtering was performed in RStudio to retain only yeast-related ASVs for downstream ecological analyses. Comprehensive taxonomic annotation of retained yeast ASVs is presented in Supplementary Table S3.

Sequence processing was implemented within the QIIME2 environment. Downstream microbiome analyses and data visualization were conducted in RStudio v4.4.0 using the phyloseq, vegan, ggplot2, and dplyr packages [39].

## 2.6. Diversity Analyses

### 2.6.1. Alpha Diversity

Within-sample yeast diversity was evaluated using multiple alpha diversity metrics, including Observed ASVs, Shannon diversity index, and Simpson diversity index [40]. Prior to analysis, sequence abundance data were normalized to minimize biases arising from uneven sequencing depth across samples.

Differences in alpha diversity among mangrove sites were statistically assessed using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test to identify pairwise differences between sampling locations. Statistical assumptions of normality and variance homogeneity were evaluated prior to analysis [41].

### 2.6.2. Beta Diversity

Beta diversity metrics, including Bray–Curtis dissimilarity and Jaccard distance matrices, were employed to quantify inter-sample variation in yeast community composition [42]. Bray–Curtis dissimilarity was used to emphasize abundance-weighted differences in dominant taxa, whereas Jaccard distance was used to evaluate presence–absence turnover,

including rare or site-restricted taxa. Principal Coordinate Analysis (PcoA) was applied to visualize site-specific dissimilarities, with distinct ordination plots generated for each matrix to compare abundance-weighted and occurrence-based community patterns.

Permutational multivariate analysis of variance (PERMANOVA) was conducted to test for significant compositional differences across sites, while permutation-based multivariate dispersion analysis (PERMIDISP) confirmed homogeneity of group dispersions, validating PERMANOVA assumptions. The dual-matrix approach ensured a robust assessment of beta diversity patterns.

#### 2.6.3. Relative Abundance Analysis

Relative abundance analysis was performed to evaluate the compositional distribution of dominant yeast taxa across mangrove ecosystems [43]. Taxonomic abundance profiles of yeasts were calculated at the genus level and visualized as stacked bar plots to compare community composition among sampling sites.

#### 2.6.4. Shared Taxa Analysis

Shared and unique yeast taxa among mangrove sites were evaluated to identify common and habitat-specific community members. Taxa overlap was visualized using Venn diagrams/UpSet plots based on presence–absence data.

### 2.7. Community Structure and Environmental Association

#### 2.7.1. Redundancy Analysis (RDA)

Before conducting ordination analyses, the yeast ASV abundance matrix was subjected to Hellinger transformation to mitigate the effects of dominant taxa and enhance the compatibility of sequencing-derived compositional data with linear multivariate methods. Environmental variables were z-score standardized before RDA to place predictors measured in different units and on different scales on comparable scales. Redundancy Analysis was then applied to examine associations between standardized environmental variables (pH, temperature, salinity, total dissolved solids, Pb, and Cu) and yeast community composition across mangrove ecosystems [44]. The significance of the overall model and individual predictor variables was evaluated using permutation tests (999 permutations). When no predictor was statistically significant, the RDA biplot was interpreted only as an exploratory visualization and not as evidence of environmental control.

#### 2.7.2. Variation Partitioning Analysis (VPA)

Variation Partitioning Analysis (VPA) was conducted to estimate the relative contributions of two predefined environmental groups to variation in the yeast microbiome: sediment properties (pH, temperature, salinity, and TDS) and trace element concentrations (Pb and Cu). VPA fractions were expressed as percentages of adjusted  $R^2$ . Because VPA is complementary to constrained ordination, and because the corresponding RDA permutation tests were not significant, the results were interpreted descriptively rather than inferentially.

### 2.8. Ecological Network Analysis

Co-occurrence network analysis was performed to elucidate putative interactions among yeast taxa in mangrove sediment communities. Prior to network construction, yeast community data were summarized at the genus level and converted to relative abundances to mitigate biases from uneven sequencing depths and improve cross-sample comparability. Low-abundance and low-prevalence taxa were filtered out to reduce spurious correlations and enhance network stability.

Pairwise correlations with Spearman's correlation coefficient values of  $\rho |\rho| \geq 0.35$  and false discovery rate (FDR)-adjusted  $p \leq 0.25$  were retained for network construction. Networks were constructed by retaining only robust, statistically significant correlations. Positive correlations were interpreted as potential co-occurrence patterns, whereas negative correlations were interpreted as indicative of niche differentiation or ecological exclusion.

Topological attributes of the networks, encompassing node degree, clustering coefficient, modularity, density, and betweenness centrality, were computed to quantify structural complexity and interconnectivity. Highly connected taxa were identified by their elevated topological significance. Network construction and analysis were performed in RStudio v4.4.0 using the igraph, Hmisc, and ggraph packages, with visualizations produced via ggraph and refined to maximize interpretative clarity.

### 3. Results

#### 3.1. Environmental Gradients Across Mangrove Sediments

Environmental measurements were used to define physicochemical gradients for subsequent yeast community analyses rather than as a primary outcome for site comparisons. Summary statistics for pH, temperature, salinity, total dissolved solids (TDS), Pb, Cd, and Cu were presented in Supplementary Table S1.

The main gradients retained for ecological interpretation were salinity, TDS, Pb, and Cu, because these variables varied among sites and were biologically relevant to sediment-associated yeasts. Tegal showed comparatively higher pH and temperature, Jepara showed elevated salinity and TDS, and Jepara and Semarang showed higher Pb concentrations. These patterns provide environmental context for interpreting yeast diversity and community composition.

Cu concentrations also varied among mangrove ecosystems, with higher values in Jepara and Brebes, whereas Cd showed minimal variation across all sampling locations and was therefore less informative for multivariate interpretation.

The standardized environmental heatmap illustrates these gradients across mangrove sampling sites (Figure 1). The figure was retained to provide environmental context for downstream analyses of yeast diversity, ordination, and variation partitioning.

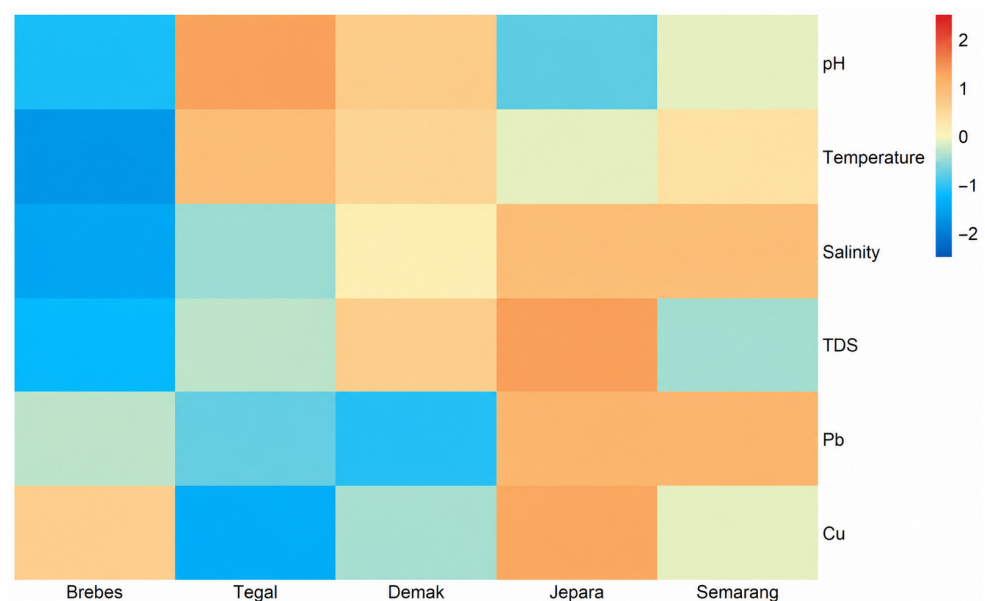


**Figure 1.** Environmental parameter profiles across mangrove sediment ecosystems.

Cd was omitted from the heatmap visualization because its concentration variation across sampling sites was minimal after data standardization, thereby limiting its discriminatory power for site clustering. Consequently, inclusion of Cd did not substantially improve the depiction of environmental heterogeneity among mangrove sediments.

Detailed distributions of individual environmental variables across sampling replicates are provided in Supplementary Figure S2. Collectively, these measurements define the environmental gradients tested against yeast community variation, but they are interpreted cautiously because downstream permutation tests did not identify significant environmental predictors.

### 3.2. Sequencing Overview and Yeast Community Composition

High-throughput ITS metabarcoding yielded sufficient sequencing depth across all mangrove sediment samples for downstream ecological analyses. Sequencing depth summaries were shown in Supplementary Table S2, while detailed processing statistics and filtered yeast ASV summaries are presented in Supplementary Table S3. Complete taxonomic annotation of all retained yeast-associated ASVs is provided in Supplementary Table S4.

Taxonomic profiling revealed that mangrove sediment yeast communities were strongly dominated by the genus *Malassezia* across all sampling locations (Figure 2). The relative abundance of *Malassezia* exceeded 70% in several sites, particularly in Brebes and Jepara, indicating broad ecological dominance of this genus within mangrove sediment ecosystems. In contrast, Tegal and Semarang exhibited lower *Malassezia* abundance and a greater representation of secondary yeast taxa.

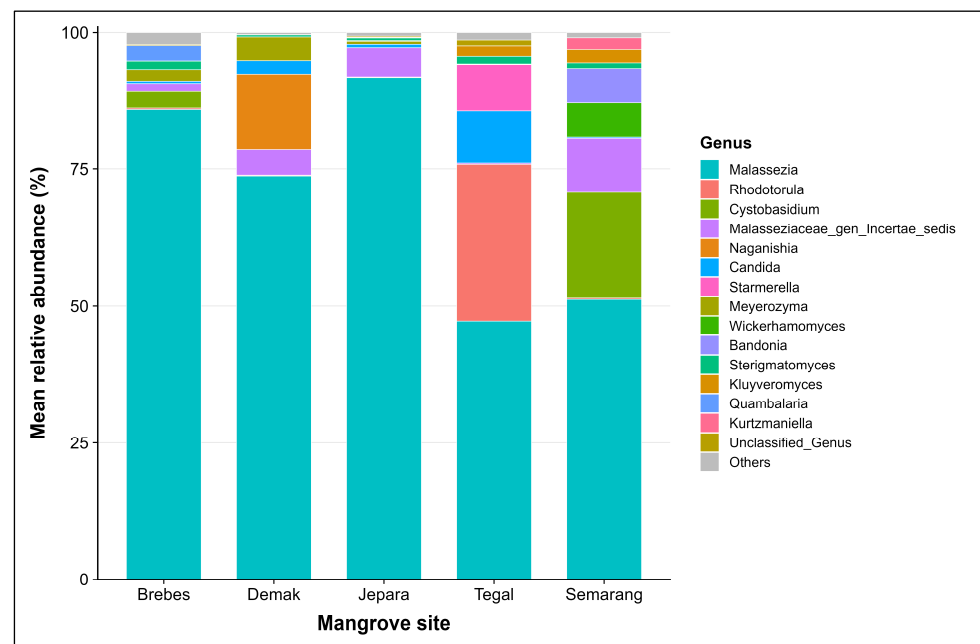


**Figure 2.** Relative abundance profiles of dominant yeast genera across mangrove ecosystems.

Distinct site-specific community compositions were observed among mangrove ecosystems. Tegal sediments showed a substantial increase in *Rhodotorula* and *Candida*, whereas Semarang was characterized by a higher relative abundance of *Cystobasidium*, *Wickerhamomyces*, and *Malasseziaceae\_gen\_Incertae\_sedis*. Demak sediments exhibited an elevated abundance of *Naganishia*, whereas Jepara communities remained strongly dominated by *Malassezia*, with relatively limited contributions from secondary taxa. Several additional genera, including *Meyerozyma*, *Starmerella*, *Bandonia*, and *Kluyveromyces*, were detected

at lower relative abundance levels across multiple sites, indicating moderate taxonomic heterogeneity among mangrove ecosystems.

At broader taxonomic levels, yeast communities were consistently dominated by the class *Malasseziomycetes* across all mangrove sediments (Supplementary Figure S3). However, Tegal and Semarang showed higher proportions of *Saccharomycetes*, *Microbotryomycetes*, and *Cystobasidiomycetes*, suggesting greater taxonomic diversification than in other locations. Similar compositional patterns were observed at the order level, where *Malasseziales* represented the predominant taxonomic group across sites (Supplementary Figure S4). Nevertheless, elevated contributions of *Saccharomycetales*, *Sporidiobolales*, and *Cystobasidiales* were observed in Tegal and Semarang sediments.

Family-level taxonomic composition further demonstrated substantial spatial variation among mangrove ecosystems (Supplementary Figure S5). *Malasseziaceae* was identified as the dominant family across all locations, whereas increased abundances of *Sporidiobolaceae*, *Cystobasidiaceae*, and *Saccharomycetaceae* were detected in sediments from Tegal and Semarang. Demak sediments additionally showed relatively higher representation of *Filobasidiaceae*, indicating localized taxonomic differentiation among mangrove habitats.

Overall, these findings demonstrate that mangrove sediment ecosystems harbor distinct yeast community compositions, characterized by strong dominance of *Malassezia*-associated taxa, alongside spatial variation in secondary yeast assemblages across environmentally heterogeneous mangrove sites.

### 3.3. Shared and Unique Yeast Taxa

Comparative analysis of yeast taxa distribution revealed both substantial ecological overlap and site-specific community differentiation among mangrove sediment ecosystems (Figure 3). A total of 18 yeast taxa were shared across all sampling locations, indicating a common core yeast community broadly distributed throughout mangrove sediments. These shared taxa likely represent environmentally tolerant yeast groups capable of adapting to fluctuating estuarine conditions characteristic of mangrove ecosystems.

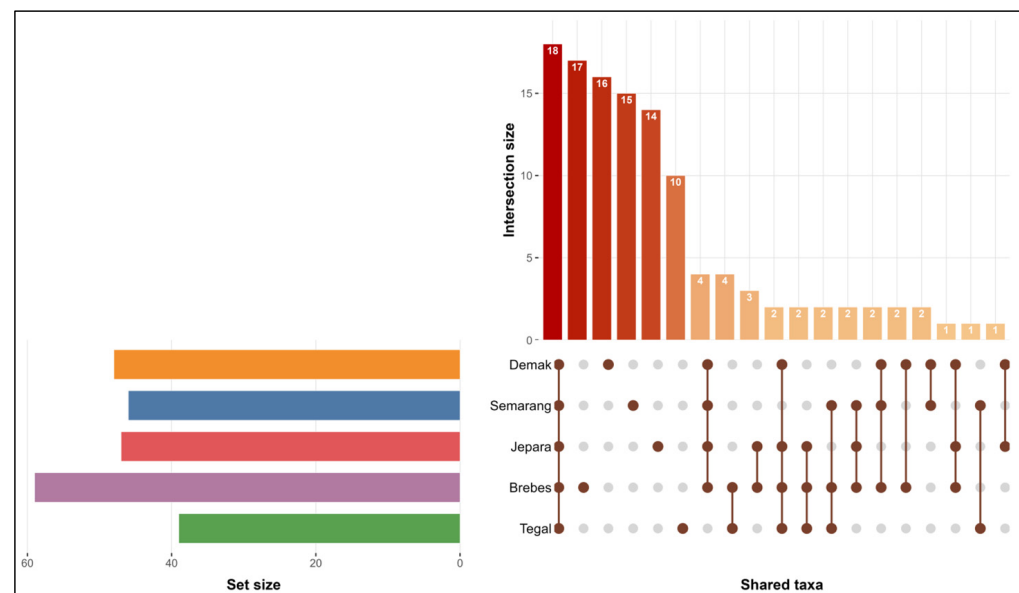


**Figure 3.** UpSet plot showing shared and unique yeast genera across mangrove ecosystems. The plot is based on genus-level presence–absence data and shows both shared and site-specific genera.

Despite the presence of a common core community, considerable variation in unique taxa composition was observed among sites. Brebes exhibited the highest number of unique yeast taxa (17 taxa), followed by Demak (16 taxa), Semarang (15 taxa), Jepara (14 taxa), and Tegal (10 taxa) (Figure 3). These findings indicate pronounced habitat specificity and suggest that local environmental conditions may contribute to the spatial differentiation of yeast assemblages across mangrove sediments.

Additionally, intermediate patterns of taxa sharing were identified among subsets of mangrove ecosystems. Specifically, four taxa were shared across Brebes, Demak, Jepara, and Tegal, whereas other combinations occurred among smaller site groups, signifying moderate ecological connectivity among geographically disparate mangrove habitats. The configuration of shared taxonomic assemblages further illustrates that yeast community composition across sites was not uniformly homogeneous, but rather encompassed both cosmopolitan and habitat-constrained taxa.

The Venn diagram analysis corroborated the UpSet visualization by illustrating complex patterns of overlap among mangrove ecosystems (Supplementary Figure S6). Although a central group of shared taxa was consistently detected across all sites, each mangrove ecosystem also contained distinct site-specific taxa that contributed to local community uniqueness. Brebes and Demak displayed relatively high proportions of exclusive taxa, whereas Tegal exhibited comparatively lower site-specific richness.

Presence–absence analysis of dominant yeast taxa further confirmed spatial heterogeneity in community composition across mangrove ecosystems (Supplementary Table S5). Several dominant genera were consistently detected across all sampling sites, while others exhibited restricted occurrence patterns associated with particular mangrove sediments. These distribution patterns suggest that environmental heterogeneity and local habitat conditions may contribute to the ecological structuring and spatial turnover of yeast communities within tropical mangrove ecosystems.

Overall, the observed coexistence of shared core taxa and site-specific yeast assemblages indicates that mangrove sediment ecosystems harbor both ecologically widespread and environmentally specialized yeast communities, reflecting complex diversity dynamics across heterogeneous coastal habitats.

### 3.4. Alpha Diversity Dynamics

Alpha diversity analyses showed descriptive variation in yeast community diversity among mangrove sediment ecosystems (Figure 4). Diversity patterns differed across observed ASVs richness, Shannon diversity, and Simpson diversity indices, indicating spatial tendencies in taxa richness and community evenness among sampling sites.

Observed ASV richness was higher in sediments from Brebes, Demak, Jepara, and Semarang, whereas Tegal exhibited comparatively lower richness (Figure 4). These patterns indicate possible within-community differences in taxa accumulation, but they should be interpreted as descriptive trends because statistical tests did not detect significant site-level differences.

Shannon and Simpson diversity indices showed similar descriptive tendencies. Demak and Semarang exhibited relatively higher values, suggesting greater community heterogeneity and evenness, whereas Tegal recorded lower values. However, these differences were not statistically significant and therefore do not support strong site-level inference.

Rarefaction analysis indicated that sequencing depth was generally sufficient to capture most of the yeast diversity across mangrove sediments (Supplementary Figure S7). Most rarefaction curves approached asymptotic patterns as sequencing depth increased, suggesting adequate sampling coverage and supporting the reliability of downstream eco-

logical analyses. Although sequencing depth varied among sites, the observed saturation trends indicate that differences in diversity patterns were unlikely to be strongly influenced by insufficient sequencing effort.

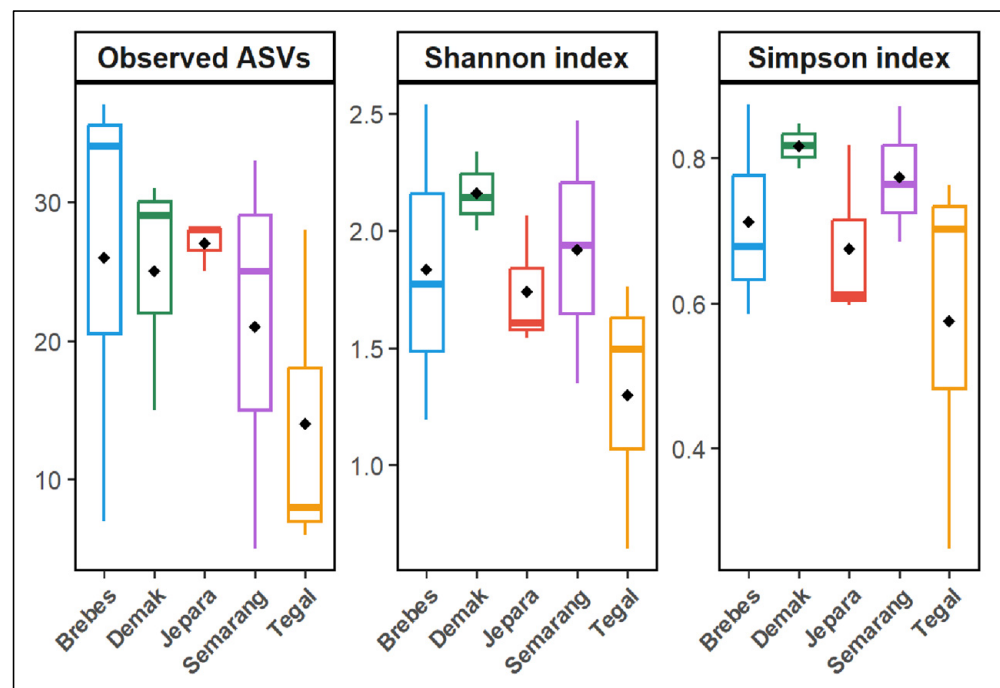


**Figure 4.** Alpha diversity indices of yeast communities across mangrove ecosystems. Observed ASVs are presented as counts, not thousands. In each boxplot, the box indicates the interquartile range; the central horizontal line indicates the median; the vertical whiskers indicate the data range within 1.5 times the interquartile range; and black diamond symbols indicate the mean. Group differences were evaluated using one-way ANOVA, with results presented in Supplementary Table S6.

Statistical analysis using one-way ANOVA did not detect significant differences among mangrove ecosystems for observed ASVs ( $p = 0.671$ ), Shannon index ( $p = 0.357$ ), or Simpson index ( $p = 0.421$ ). The ANOVA results and post hoc Tukey HSD comparisons were relocated to Supplementary Table S6. Therefore, alpha diversity patterns are interpreted as descriptive tendencies rather than statistically supported differences among sites.

Overall, alpha diversity analyses indicate descriptive heterogeneity in yeast richness, diversity, and evenness across mangrove sediment ecosystems, but the available replicate-level data did not provide statistical support for significant differences in alpha diversity among sites (Supplementary Table S7).

### 3.5. Beta Diversity and Community Structure

Beta diversity analyses revealed substantial variation in yeast community composition among mangrove sediment ecosystems, indicating pronounced spatial turnover in community structure across sampling sites. Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarities showed clear separation among several mangrove ecosystems, particularly along the first ordination axis, which accounted for 28.2% of the variation (Figure 5). The second axis represented an additional 15.2% of variation, collectively indicating moderate compositional differentiation among yeast assemblages.

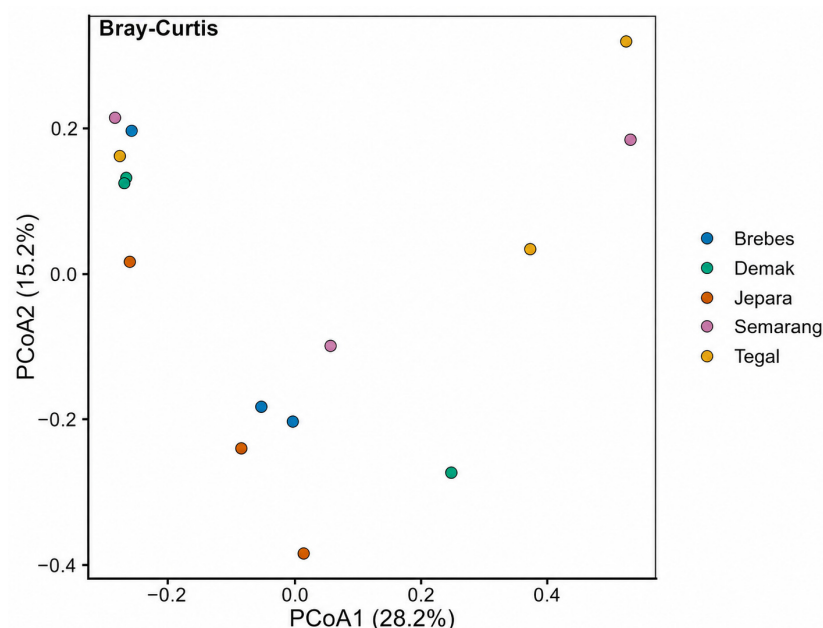


**Figure 5.** Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarity.

Distinct clustering tendencies were observed among several sites. Samples from Jepara and Tegal tended to separate from those of Brebes and Demak along the primary ordination axis, whereas Semarang communities displayed comparatively broader dispersion across ordination space. These patterns suggest differences in relative abundance structure among mangrove sediment yeast communities.

PCoA based on Jaccard dissimilarity produced broadly similar clustering patterns, although with slightly reduced variance explanation compared with Bray–Curtis ordination (Figure 6). The first and second axes explained 23.9% and 11.6% of the total variation, respectively. Presence–absence-based ordination demonstrated that several sites shared comparable taxon composition, while others exhibited distinct occurrence patterns associated with habitat-specific taxon distributions. Tegal and Semarang samples showed relatively greater separation from the remaining mangrove ecosystems, suggesting elevated community turnover and taxon replacement among these locations.

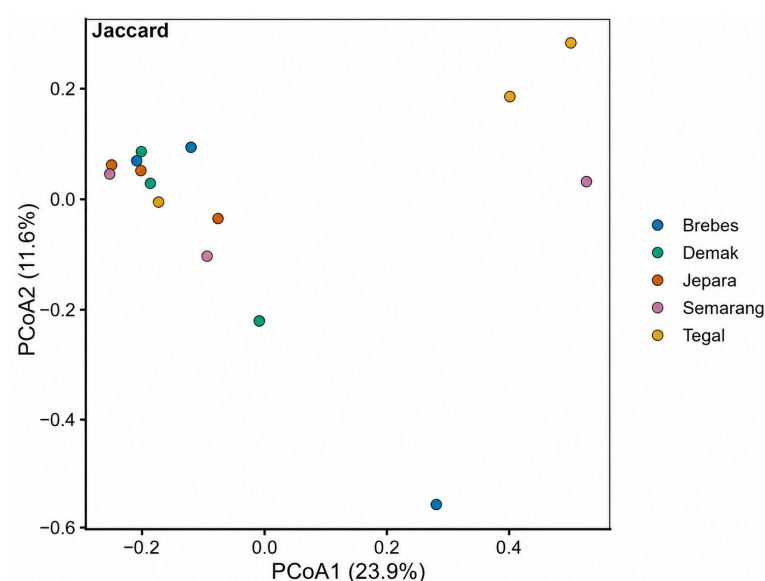


**Figure 6.** Principal Coordinates Analysis (PCoA) based on Jaccard dissimilarity.

Confidence ellipse visualization further illustrated differences in community dispersion among mangrove sites (Supplementary Figure S8). The Brebes, Demak, and Jepara communities exhibited relatively compact clustering patterns, indicating within-site compositional variability. In contrast, Tegal and Semarang showed broader spatial dispersion within ordination space, suggesting higher heterogeneity in yeast community structure among replicate samples.

Multivariate statistical analyses provided a cautious interpretation of the ordination patterns (Table 1). PERMANOVA detected no statistically significant compositional differences among mangrove sites for either Bray–Curtis ( $p = 0.3446$ ) or Jaccard ( $p = 0.4075$ ) dissimilarity matrices. PERMDISP results were also non-significant, indicating homogeneous multivariate dispersion among groups. Therefore, the PCoA patterns are interpreted as visual tendencies rather than statistically supported site separation.

**Table 1.** Multivariate statistical analyses of yeast community structure.

| Analysis  | Distance Metric | R <sup>2</sup> | Pseudo-F | p-Value | Interpretation         |
|-----------|-----------------|----------------|----------|---------|------------------------|
| PERMANOVA | Bray–Curtis     | 0.2979         | 1.0608   | 0.3446  | Not significant        |
| PERMDISP  | Bray–Curtis     | —              | 0.9242   | 0.4903  | Homogeneous dispersion |
| PERMANOVA | Jaccard         | 0.2895         | 1.0185   | 0.4075  | Not significant        |
| PERMDISP  | Jaccard         | —              | 0.9689   | 0.4672  | Homogeneous dispersion |

PERMANOVA and PERMDISP were performed using 9999 permutations. PERMDISP evaluates homogeneity of multivariate dispersion among groups.

Overall, beta diversity analyses showed visual patterns of compositional turnover and abundance-structure variation among mangrove sediments. However, the non-significant PERMANOVA results indicate that these patterns should be interpreted descriptively and not as statistically confirmed community separation among sites.

### 3.6. Environmental Drivers of Yeast Community Structure

Environmental association analyses were used to test whether measured physico-chemical variables significantly explained variation in yeast communities across mangrove sediment ecosystems. Redundancy Analysis was retained as an exploratory ordination because permutation tests indicated that neither the overall model nor individual environmental variables were statistically significant (Figure 7; Table 2).

**Table 2.** Permutation test results for redundancy analysis (RDA).

| Test/Variable     | Df | Variance | Explained Variation (%) | F-Value | p-Value |
|-------------------|----|----------|-------------------------|---------|---------|
| Overall RDA model | 6  | 0.1985   | 37.65                   | 0.805   | 0.807   |
| pH                | 1  | 0.0373   | 7.07                    | 0.908   | 0.526   |
| Temperature       | 1  | 0.0181   | 3.43                    | 0.440   | 0.892   |
| Salinity          | 1  | 0.0183   | 3.46                    | 0.444   | 0.876   |
| TDS               | 1  | 0.0378   | 7.17                    | 0.920   | 0.497   |
| Pb                | 1  | 0.0553   | 10.49                   | 1.347   | 0.213   |
| Cu                | 1  | 0.0317   | 6.02                    | 0.772   | 0.635   |
| Residual          | 8  | 0.3287   | 62.35                   | —       | —       |

The first two RDA axes explained 42.6% and 22.1% of the constrained variation, respectively. These percentages describe the ordination geometry of the constrained space, not statistically significant environmental effects. Therefore, the directions of vectors for salinity, TDS, pH, temperature, Pb, and Cu are interpreted only as a preliminary visual pattern.

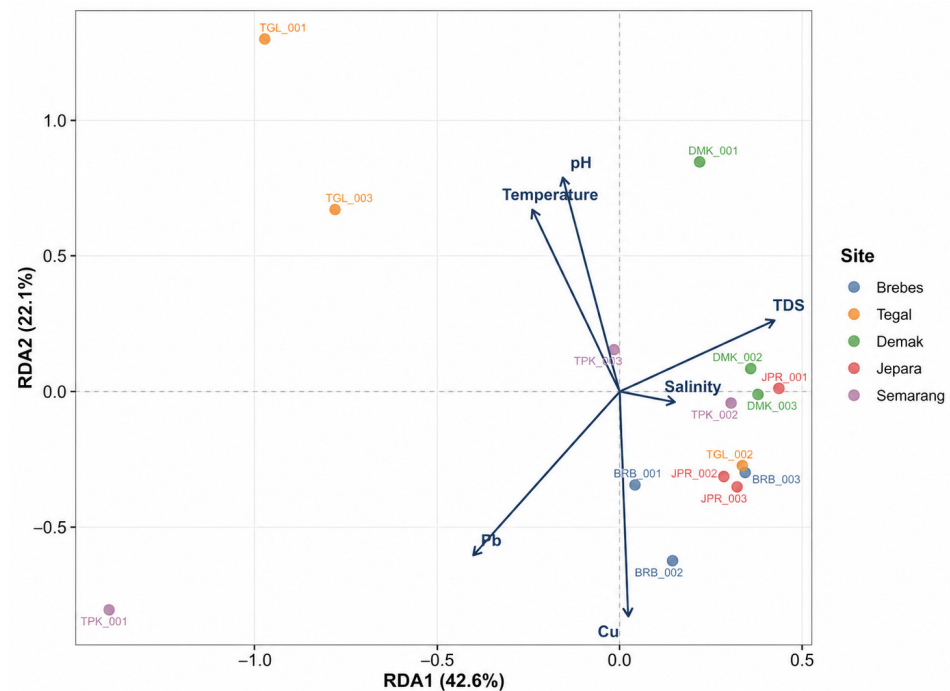


**Figure 7.** Exploratory redundancy analysis (RDA) of yeast community structure and measured environmental variables. The overall model and individual predictors were not statistically significant; therefore, the biplot is shown only to visualize non-inferential gradients and should not be interpreted as evidence of significant environmental control. Arrows indicate environmental vectors representing the direction of association between measured environmental factors and yeast community distribution.

Visual tendencies in the RDA plot suggested that some samples aligned with salinity, TDS, Pb, or Cu gradients, but these tendencies were not supported by permutation tests. Consequently, no measured environmental variable was identified as a significant driver of yeast community composition in this dataset.

Permutation tests confirmed that the measured variables did not significantly explain yeast community composition (Table 2). The RDA results, therefore, support a cautious interpretation centered on limited explanatory power rather than significant environmental control.

Exploratory correlation analysis between dominant yeast genera and environmental variables revealed genus-specific tendencies across mangrove ecosystems (Supplementary Figure S9). Because the RDA model was not significant, these correlations are interpreted as hypothesis-generating patterns rather than confirmatory evidence of environmental control.

Several genera showed associations with physicochemical gradients associated with nutrient and salinity conditions. *Malasseziaceae\_gen\_Incertae\_sedis* and *Naganishia* were positively correlated with TDS, whereas *Candida* and *Starmerella* exhibited moderate positive correlations with pH and temperature. However, many dominant genera showed relatively weak to moderate correlations across multiple variables, suggesting that yeast microbiome dynamics may be influenced by a combination of interacting environmental factors rather than a single dominant driver. Environmental variables with zero variance across samples, such as Cd, were excluded from the correlation heatmap because Spearman correlation coefficients cannot be computed for constant variables.

Overall, the environmental analyses did not identify statistically significant physicochemical drivers of yeast community composition. Salinity, TDS, Pb, and Cu remain ecologically relevant gradients for future testing, but the present dataset supports only cautious, exploratory interpretation.

### 3.7. Partitioning of Environmental Contributions to Yeast Community Variation

Variation Partitioning Analysis (VPA) was re-analyzed in RStudio to describe the relative contribution of sediment properties and trace element concentrations to variation in yeast communities across mangrove sediment ecosystems (Figure 8). The revised visualization reports adjusted  $R^2$  percentages for the sediment-property, shared, trace-element, and unexplained fractions. Because the corresponding RDA model was not significant, the VPA results are interpreted only descriptively and not as evidence of significant environmental effects.

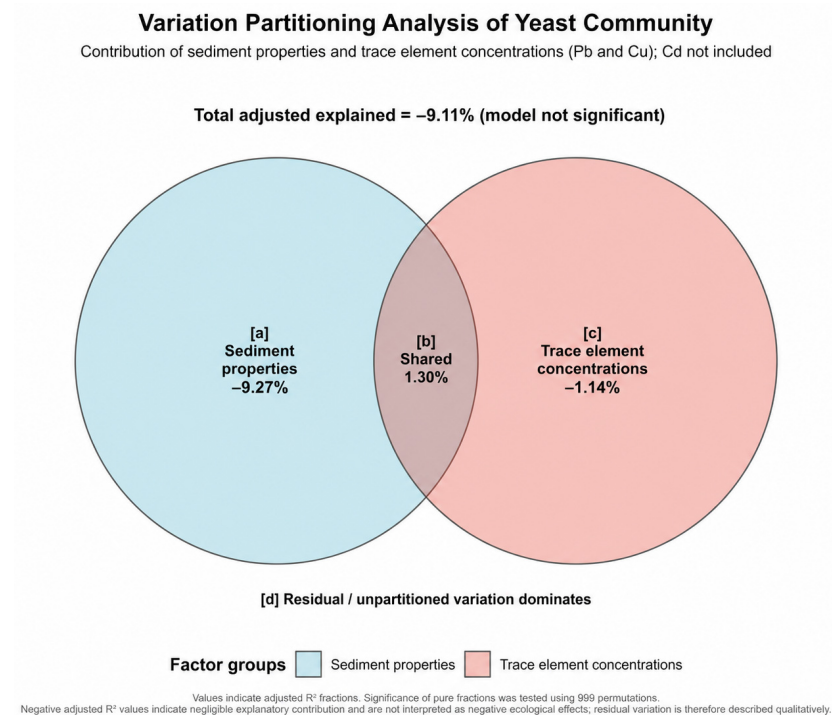


**Figure 8.** Variation Partitioning Analysis (VPA) of environmental contributions to yeast community structure. Sediment properties include pH, temperature, salinity, and TDS, whereas trace element concentrations include Pb and Cu. Values represent adjusted  $R^2$  percentages for the sediment-property fraction, shared fraction, trace-element concentration fraction, and unexplained fraction. Negative adjusted  $R^2$  values indicate negligible explanatory power or fractions not distinguishable from zero. Because RDA permutation tests were not significant, the figure is interpreted descriptively rather than as evidence of significant environmental control.

The revised VPA indicated that the sediment-property fraction was −9.27%, the shared fraction was 1.30%, and the trace-element concentration fraction was −1.14%, yielding a total explained fraction of −9.11%. These negative adjusted  $R^2$  values indicate that, after accounting for model complexity, the measured environmental groups did not provide meaningful explanatory power for variation in the yeast community.

Instead, the VPA results indicate that the measured environmental groups provided negligible explanatory power for yeast community variation after adjustment for model complexity. This pattern suggests that yeast community organization may be influenced by factors not captured in the present model, including unmeasured sediment properties, organic matter quality, oxygen and redox gradients, hydrological exchange, dispersal processes, biotic interactions, or methodological features of total DNA-based metabarcoding.

Overall, the analyzed VPA strengthened the same conclusion as the RDA: the measured variables did not explain meaningful variation in the yeast community and therefore should not be interpreted as significant environmental drivers.

### 3.8. Ecological Network Structure

Ecological co-occurrence network analysis revealed complex interaction patterns among dominant yeast genera within mangrove sediment ecosystems (Figure 9). The network structure consisted of multiple interconnected modules characterized predominantly by positive associations, suggesting potential ecological coexistence, shared environmental preferences, or cooperative interactions among yeast taxa.

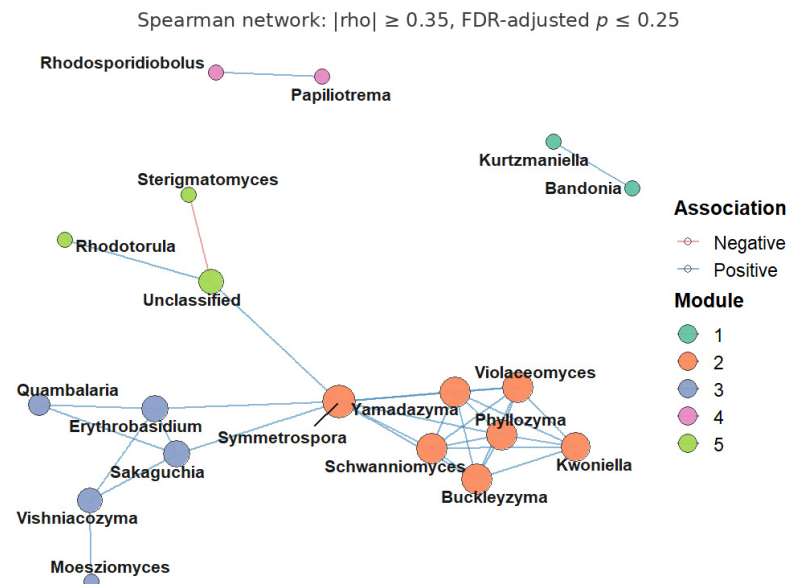


**Figure 9.** High-resolution ecological co-occurrence network of dominant yeast genera across mangrove sediment ecosystems. Nodes represent yeast genera, edges represent significant Spearman correlations after FDR correction, edge color indicates positive or negative associations, and node color indicates network module membership.

The resulting network exhibited moderate modular organization with distinct ecological clusters distributed across several interaction modules (Table 3). Module 2 represented the largest and most interconnected subnetwork, primarily composed of *Yamadazyma*, *Schwanniomyces*, *Buckleyzyma*, *Phyllozyma*, *Kwonella*, and *Violaceomyces*. These genera displayed dense positive associations with one another, forming a tightly connected interaction cluster indicative of potential ecological coordination within mangrove sediments.

Additional modules showed comparatively smaller but ecologically distinct interaction structures. Module 3 included *Erythrobasidium*, *Sakaguchia*, *Vishniacozyma*, *Moesziomyces*, and *Quambalaria*, whereas Module 1 was primarily represented by *Bandonia* and *Kurtzmaniella*. Module 4 formed a separate cluster consisting of *Rhodosporidiobolus* and *Papiliotrema*. In contrast, Module 5 contained *Rhodotorula*, *Sterigmatomyces*, and unclassified taxa exhibiting comparatively fewer but distinct ecological interactions.

Most detected associations within the network were positive, whereas only a limited number of negative correlations were observed (Figure 9). Negative interactions were mainly associated with *Sterigmatomyces* and unclassified taxa, suggesting potential niche differentiation patterns among specific yeast groups. The predominance of positive associations across the network indicates that coexistence and shared habitat adaptation may play important roles in structuring mangrove-associated yeast communities.

**Table 3.** Global topological properties of the yeast ecological network.

| Network Topology Metric       | Value | Definition/Ecological Meaning                                                |
|-------------------------------|-------|------------------------------------------------------------------------------|
| Number of nodes               | 19    | Total number of yeast genera retained in the co-occurrence network.          |
| Number of edges               | 33    | Total number of significant associations among yeast genera.                 |
| Positive edges                | 32    | Number of positive co-occurrence associations.                               |
| Negative edges                | 1     | Number of negative co-exclusion associations.                                |
| Network density               | 0.193 | Proportion of observed associations relative to all possible associations.   |
| Average degree                | 3.474 | Mean number of connections per node.                                         |
| Average path length           | NA    | Average shortest-path distance among connected nodes.                        |
| Diameter                      | NA    | Longest shortest-path distance within the network.                           |
| Global clustering coefficient | 0.750 | Overall tendency of connected genera to form clustered triplets.             |
| Modularity (Louvain)          | 0.429 | Strength of community/module structure detected using the Louvain algorithm. |

Module-specific visualization further demonstrated differences in interaction density and network cohesion among ecological clusters (Supplementary Figure S10). The central interaction cluster comprising *Symmetrospora*, *Schwanniomyces*, *Buckleyzyma*, and *Phyllozyma* exhibited comparatively high connectivity and strong positive associations, suggesting potential importance for maintaining network stability and ecological integration. Peripheral modules exhibited lower connectivity and reduced interaction density, indicating more specialized or weakly connected ecological relationships.

Topological analysis identified several taxa with elevated node connectivity and centrality values, suggesting potential keystone-like ecological roles within mangrove yeast communities (Supplementary Table S8). *Symmetrospora* occupied a central bridging position linking multiple modules and exhibited relatively high degree and betweenness centrality values, indicating potential importance in maintaining network connectivity. Similarly, *Schwanniomyces*, *Buckleyzyma*, and *Phyllozyma* exhibited high node connectivity and strong interaction density, suggesting their contributions to overall network organization and community cohesion.

Overall, ecological network analysis demonstrated that modular, predominantly positive interaction networks structure mangrove sediment yeast communities. The presence of highly connected taxa and distinct ecological modules further suggests that specific yeast genera may contribute to network connectivity and ecological organization within heterogeneous mangrove sediment ecosystems.

## 4. Discussion

### 4.1. Mangrove Sediments as Selective Reservoirs of Yeast Microbiomes

Mangrove sediments from the northern coast of Central Java supported structured yeast assemblages dominated by *Malassezia* and related *Malasseziomycetes* lineages. This pattern indicates that lipid-utilizing, stress-tolerant, and marine-associated yeasts may represent an important recurrent component of the tropical mangrove sediment mycobiome [45–47]. However, dominance should be interpreted cautiously because to-

tal DNA-based ITS metabarcoding can detect active, dormant, and relic DNA signals, particularly in sediment matrices [32].

The dominance of *Malassezia* and related Malasseziomycetes in mangrove sediments may reflect their ability to persist under saline, organic-rich, and periodically disturbed sediment conditions [46]. Mangrove sediments contain heterogeneous carbon sources derived from litter decomposition, root exudates, tidal inputs, and anthropogenic organic residues [1,5,6]. These substrates may favor yeast groups with lipid utilization, oxidative metabolism, and stress-tolerance traits. Recent evidence also indicates that *Malassezia* can be detected in environmental sequencing datasets, including coastal and sediment-associated habitats, although its ecological status should be interpreted with caution, as some signals may reflect external biological inputs or DNA persistence in sediments. Therefore, the high relative abundance of *Malassezia*-associated taxa in this study should be interpreted as a dominant taxonomic signal in the sediment yeast dataset rather than direct evidence of active ecological function [46,47].

This finding also strengthens the need to separate yeast-associated ASVs from broader fungal metabarcoding datasets. Mangrove sediment fungal communities contain mixed ecological forms, including filamentous fungi, yeast-like fungi, and ambiguously annotated taxa [8,48]. Without careful taxonomic curation, yeast-specific patterns may be obscured by dominant non-yeast fungal groups or by marker-related biases in ITS metabarcoding. The filtering approach used in this study allowed the analysis to focus on yeast community structure while remaining cautious about taxonomic resolution and functional inference from amplicon data [45,49]. This is important because yeast ecology in mangrove sediments remains less explored than bacterial, archaeal, or total fungal diversity, and a yeast-focused workflow can provide a clearer baseline for future culture-dependent, functional, and genome-informed studies [47].

#### 4.2. Predominance of Basidiomycetous Yeasts and Functional Implications

A key outcome of this study is the predominance of basidiomycetous yeasts over ascomycetous yeasts in several mangrove sediments. This pattern is ecologically relevant because many basidiomycetous yeasts are primarily oxidative and often associated with complex carbon sources, lipid-rich substrates, and stress-prone aquatic or saline habitats [13,16,47]. In contrast, many ascomycetous yeasts include stronger fermentative representatives, such as *Candida*, *Meyerozyma*, *Wickerhamomyces*, *Yamadazyma*, and *Schwanniomyces*, which may respond to simpler organic substrates and localized nutrient pulses [15,17,18,47].

This basidiomycete-skewed composition has functional implications for mangrove sediments. Oxidative basidiomycetous yeasts may contribute to the transformation of organic matter through extracellular enzymes, pigment production, lipid metabolism, and tolerance to salinity or trace-metal stress. These traits may help explain why genera such as *Rhodotorula*, *Cystobasidium*, *Buckleyzyma*, *Phyllozyma*, *Kwoniella*, and *Symmetrospora* appeared as important components of the community and network structure [15,16,47,50].

#### 4.3. Shared and Site-Specific Taxa Indicate Partial Microbiome Connectivity

The coexistence of shared and site-specific yeast genera suggests partial connectivity among mangrove ecosystems. Shared genera may reflect dispersal through tidal water movement and sediment exchange, whereas site-specific genera may reflect localized filtering by salinity, organic matter, oxygen availability, sediment chemistry, or anthropogenic inputs [51,52]. This core-satellite pattern supports a regional yeast microbiome that is connected but not homogenized [53].

The relatively high number of site-specific yeast taxa in Brebes, Demak, and Semarang suggests that local sediment conditions may generate microhabitat-level selectivity within each mangrove ecosystem [54]. Such site specificity may be related to differences in organic substrate availability, sediment texture, oxygen limitation, salinity fluctuation, redox status, and anthropogenic inputs. These conditions can create small-scale ecological niches that favor particular yeast lineages while limiting others [23,29,52,53]. Therefore, the site-specific taxa observed in this study may represent locally adapted yeast assemblages rather than random taxonomic variation. This interpretation is consistent with the concept that mangrove microbiomes are shaped not only by regional connectivity but also by fine-scale environmental heterogeneity within sediment habitats [54].

The coexistence of shared and site-restricted yeast taxa also has implications for interpreting yeast beta diversity in mangrove sediments. A shared core community may provide basic ecological continuity across sites, whereas restricted taxa may contribute to local functional differentiation and community turnover [53,55]. However, because this study used DNA-based metabarcoding, the ecological status of these taxa cannot be fully resolved. Some taxa may represent active populations, while others may reflect dormant cells, transient propagules, or extracellular DNA preserved in sediments [32]. Future studies that combine culture-dependent isolation, RNA-based profiling, enzyme assays, and expanded sediment chemistry measurements are needed to determine whether site-specific taxa are active ecological specialists with distinct functional roles in mangrove sediment processes [53,56,57].

#### *4.4. Alpha Diversity Patterns Require Cautious Interpretation*

Alpha diversity patterns are now interpreted as descriptive tendencies rather than statistically confirmed site-level differences. Brebes, Demak, Jepara, and Semarang showed descriptively higher observed ASVs than Tegal, but ANOVA did not detect significant differences for observed ASVs, Shannon index, or Simpson index [40]. Therefore, the results suggest possible heterogeneity in richness and evenness, but they do not support strong inference about ecological stability or disturbance based on alpha diversity alone [42].

Rarefaction curves approached asymptotic patterns, indicating that sequencing depth was generally adequate for downstream analyses [58,59]. Nevertheless, future studies should increase the number of replicates and combine diversity metrics with functional assays to test whether higher yeast richness is associated with broader metabolic capacity or greater ecosystem resilience [40].

The absence of statistically significant differences in alpha diversity does not necessarily indicate ecological uniformity among mangrove sediments. Instead, it suggests that the observed variation in richness and evenness was not strong enough to be statistically resolved under the current sampling design [40,42]. In sediment microbiome studies, alpha-diversity metrics are sensitive to sequencing depth, replicate number, spatial heterogeneity, and the community's dominance structure. Therefore, the descriptive tendency toward higher richness in some sites should be interpreted as a preliminary signal that requires stronger replication and complementary ecological measurements before being linked to disturbance, stability, or ecosystem function [59].

#### *4.5. Beta Diversity and Environmental Associations Were Mainly Exploratory*

PCoA patterns suggested visual community turnover among sites, but PERMANOVA did not detect significant differences for Bray–Curtis or Jaccard distances. Bray–Curtis was retained because it captures abundance-weighted shifts in dominant taxa, whereas Jaccard was retained because it captures presence–absence turnover, including rare or site-restricted

taxa [60,61]. Together, these metrics provide complementary but non-confirmatory views of community structure.

The RDA and VPA results also require cautious interpretation. Although salinity, TDS, Pb, and Cu were ecologically relevant candidate gradients, neither the overall RDA model nor any individual predictor was significant. The VPA results indicate that the measured environmental groups explained negligible variation in the yeast community after adjustment for model complexity [62–64]. This pattern suggests that yeast community organization may be influenced by factors not captured in the present model, including unmeasured sediment properties, organic matter quality, oxygen and redox gradients, hydrological exchange, dispersal processes, biotic interactions, or methodological features of total DNA-based metabarcoding [65–67].

The contrast between visible ordination patterns and non-significant PERMANOVA results highlights the importance of separating graphical structure from statistical support. Bray–Curtis and Jaccard ordinations remain useful because they reveal different dimensions of community turnover, namely abundance-weighted compositional shifts and presence–absence replacement, but neither metric provided sufficient evidence for statistically confirmed site separation in this dataset [51,61,63]. Similarly, the non-significant RDA and VPA results suggest that salinity, TDS, Pb, and Cu should be treated as candidate gradients rather than confirmed drivers. These findings support a conservative interpretation in which environmental associations are considered hypothesis-generating and require broader temporal sampling, additional sediment chemistry variables, and higher replication to test more robustly [28,29,63,64].

#### 4.6. Unexplained Variation Reflects Ecological and Methodological Complexity

The large unexplained fraction may reflect unmeasured sediment organic matter quality, sulfide concentration, porewater chemistry, redox gradients, microscale oxygen heterogeneity, hydrological exchange, dispersal limitation, ecological drift, and species interactions [21,67,68]. It may also reflect the composite nature of sediment DNA. Total DNA-based metabarcoding can include DNA from active, dormant, dead, or extracellular sources, potentially weakening correlations between contemporary environmental measurements and active yeast populations [32,69].

Future work should integrate DNA and RNA-based profiling, viability-sensitive approaches such as PMA treatment, culture-dependent functional screening, enzyme assays, and expanded environmental measurements [56,57]. These approaches would help distinguish active yeast responses from dormant or relic DNA signals and would clarify the functional roles of basidiomycetous and ascomycetous yeasts in mangrove sediments [70,71].

The large unexplained component should therefore be viewed not only as a statistical residual, but also as an indication that yeast community organization in mangrove sediments may operate across spatial and temporal scales not captured by the measured variables [68,69]. Mangrove sediments are highly heterogeneous microhabitats where tidal fluctuation, oxygen depletion, organic matter decomposition, and redox transitions can vary over short distances and time intervals [49]. Such microscale variability may decouple single-time environmental measurements from DNA-based community profiles. In addition, biotic interactions, priority effects, dispersal history, and stochastic assembly may independently contribute to community patterns, regardless of the measured sediment properties [21,67,68]. Because total DNA may include active, dormant, dead, and extracellular fractions, future studies should distinguish viable and active yeast populations from preserved DNA signals to clarify whether the detected taxa are responding to contemporary environmental gradients [32,69,72].

#### 4.7. Network Organization Suggests Modular Yeast Associations

The co-occurrence network showed predominantly positive and modular associations. Such patterns may indicate shared habitat preferences, niche overlap, or potential metabolic complementarity, but they should not be interpreted as direct biological interactions without experimental validation [70]. The presence of highly connected taxa, including *Symmetrospora*, *Schwanniomyces*, *Buckleyzyma*, and *Yamadazyma*, suggests possible topological importance within the sediment yeast network [73].

The modular structure of the yeast network provides an additional perspective on community organization beyond taxonomic composition and diversity indices. Modules may represent groups of taxa that share similar habitat preferences, respond to comparable sediment conditions, or possess complementary ecological strategies, although these possibilities cannot be confirmed from correlation-based networks alone [74,75]. The occurrence of highly connected taxa within several modules is useful for identifying candidate taxa for future functional validation, particularly basidiomycetous yeasts with oxidative metabolism, stress tolerance, and potential extracellular enzyme activity [49,76]. Thus, the network analysis should be interpreted as a tool for prioritizing hypotheses about yeast community organization rather than as direct evidence of species interactions or keystone function [73,75].

## 5. Conclusions

This study provides a yeast-focused characterization of mangrove sediment microbiomes from the northern coast of Central Java, Indonesia, using ITS metabarcoding, curated yeast ASV filtering, diversity analysis, environmental association testing, variation partitioning, and ecological network inference. The results showed that mangrove sediments harbor distinct yeast assemblages dominated by *Malassezia* and related *Malasseziomycetes* lineages, together with spatial variation in subordinate taxa and the coexistence of shared and site-specific yeast genera.

Alpha- and beta-diversity analyses revealed descriptive patterns of richness, evenness, and community turnover among mangrove sediments, but these patterns were not statistically confirmed by ANOVA or PERMANOVA. Environmental analyses further showed that salinity, TDS, Pb, and Cu were ecologically relevant candidate gradients, but RDA and VPA did not identify significant environmental drivers. These findings emphasize that measured sediment properties and trace element concentrations alone were insufficient to explain variation in the yeast community, which may also reflect unmeasured microhabitat conditions, biotic interactions, dispersal processes, stochastic assembly, and methodological features of total DNA-based metabarcoding.

The co-occurrence network revealed predominantly positive and modular associations, with several highly connected taxa, including *Symmetrospora*, *Schwanniomyces*, *Buckleyzyma*, and *Yamadazyma*, suggesting potential roles in yeast community connectivity. The main novelty of this study lies in integrating yeast-specific metabarcoding curation, cautious environmental interpretation, variation partitioning, and network analysis to provide a baseline framework for understanding the yeast microbiomes of mangrove sediments. This framework supports future studies combining DNA- and RNA-based profiling, culture-dependent isolation, enzyme assays, and genome-informed analyses to clarify the active ecological and functional roles of yeasts in mangrove sediment ecosystems.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/applmicrobiol6070080/s1>, Figure S1: Sampling site map and geographic coordinates of mangrove ecosystems in Central Java, Indonesia; Figure S2: Individual environmental parameter distributions across mangrove sites; Figure S3: Relative abundance of yeast

communities at the class level; Figure S4: Relative abundance of yeast communities at the order level; Figure S5: Relative abundance of yeast communities at the family level; Figure S6: Venn diagram of shared and unique yeast taxa across mangrove sediment ecosystems; Figure S7: Rarefaction curves for sequencing depth validation; Figure S8: Confidence ellipse visualization of Bray–Curtis PCoA ordination; Figure S9: Correlation heatmap between dominant yeast genera and environmental variables; Figure S10: Module-specific clustering and interaction patterns within the yeast ecological network; Table S1: Environmental parameter measurements and summary statistics across mangrove sediment samples; Table S2: Summary statistics of ITS sequencing depth across mangrove sediment samples; Table S3: ITS sequencing output and filtered yeast ASV summary; Table S4: Complete taxonomic annotation of yeast-associated ASVs; Table S5: Presence–absence matrix of dominant yeast taxa across mangrove ecosystems; Table S6: One-way ANOVA and Tukey HSD comparisons for alpha diversity indices; Table S7: Percentage contribution of environmental variable groups to yeast community variation; Table S8: Topological properties of highly connected yeast taxa in mangrove sediment networks.

**Author Contributions:** Conceptualization, L.K.R., T.R.S. and B.W.; methodology, L.K.R., T.R.S. and B.W.; software, M.D.A.M.; validation, T.R.S. and B.W.; formal analysis, L.K.R. and M.D.A.M.; investigation, L.K.R.; Resources, L.K.R.; data curation, L.K.R. and M.D.A.M.; writing—original draft preparation, L.K.R.; writing—review and editing, L.K.R., T.R.S. and B.W.; visualization, L.K.R.; supervision, T.R.S. and B.W. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

**Acknowledgments:** The authors would like to thank the Diponegoro Biodiversity Project Laboratory, Integrated Laboratory Unit, Diponegoro University, for providing laboratory facilities and technical support during sample processing and sequencing preparation. The authors also thank all parties who assisted with field sampling in the mangrove ecosystems of Central Java, Indonesia.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|           |                                                    |
|-----------|----------------------------------------------------|
| DNA       | Deoxyribonucleic Acid                              |
| ASV       | Amplicon Sequence Variant                          |
| ITS       | Internal Transcribed Spacer                        |
| QIIME2    | Quantitative Insights Into Microbial Ecology 2     |
| DADA2     | Divisive Amplicon Denoising Algorithm 2            |
| TDS       | Total Dissolved Solids                             |
| Pb        | Plumbum, Lead                                      |
| Cu        | Cuprum, Copper                                     |
| Cd        | Cadmium                                            |
| PMA       | Propidium monoazide                                |
| ANOVA     | Analysis of Variance                               |
| PERMANOVA | Permutational Multivariate Analysis of Variance    |
| PERMIDISP | Permutational Analysis of Multivariate Dispersions |
| USEPA     | United States Environmental Protection Agency      |
| APHA      | American Public Health Association                 |

|     |                                 |
|-----|---------------------------------|
| RDA | Redundancy Analysis             |
| Df  | Degree of freedom               |
| VPA | Variation partitioning analysis |

## References

- Jaleel, K.A.; Prakash, P.S.; Manjula, K.T.; Mini, P.V. Ecological and Economic Significance of Mangroves: A Comprehensive Review. *Aquat. Life Sci.* **2026**, *3*, 9–19. [\[CrossRef\]](#)
- Ximenes, A.C.; Cavanaugh, K.C.; Arvor, D.; Murdiyarso, D.; Thomas, N.; Arcoverde, G.F.B.; Bispo, P.C.; Van der Stocken, T. A comparison of global mangrove maps: Assessing spatial and bioclimatic discrepancies at poleward range limits. *Sci. Total Environ.* **2023**, *860*, 160380. [\[CrossRef\]](#) [\[PubMed\]](#)
- Adame, M.F.; Kelleway, J.; Krauss, K.W.; Lovelock, C.E.; Adams, J.B.; Trevathan-Tackett, S.M.; Noe, G.; Jeffrey, L.; Ronan, M.; Zann, M.; et al. All tidal wetlands are blue carbon ecosystems. *BioScience* **2024**, *74*, 253–268. [\[CrossRef\]](#) [\[PubMed\]](#)
- Friess, D.A.; Shribman, Z.I.; Stankovic, M.; Iram, N.; Baustian, M.M.; Lewis, C.J.E. Restoring blue carbon ecosystems. *Camb. Prism. Coast. Futur.* **2024**, *2*, e9. [\[CrossRef\]](#) [\[PubMed\]](#)
- Hagger, V.; Worthington, T.A.; Lovelock, C.E.; Adame, M.F.; Amano, T.; Brown, B.M.; Friess, D.A.; Landis, E.; Mumby, P.J.; Morrison, T.H.; et al. Drivers of global mangrove loss and gain in social-ecological systems. *Nat. Commun.* **2022**, *13*, 6373. [\[CrossRef\]](#) [\[PubMed\]](#)
- Goldberg, L.; Lagomasino, D.; Thomas, N.; Fatoyinbo, T. Global declines in human-driven mangrove loss. *Glob. Change Biol.* **2020**, *26*, 5844–5855. [\[CrossRef\]](#)
- He, M.; Jiang, S.; Li, X.; Yao, L.; Shui, B. Seasonal dynamics of bacterial communities in mangrove sediments of Shupaisha island, Zhejiang Province, China. *Front. Microbiol.* **2025**, *16*, 1526730. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zhang, Y.; Gui, H.; Zhang, S.; Li, C. Diversity and Potential Function of Prokaryotic and Eukaryotic Communities from Different Mangrove Sediments. *Sustainability* **2022**, *14*, 3333. [\[CrossRef\]](#)
- Banchi, E.; Del Negro, P.; Celussi, M.; Malfatti, F. Sediment Features and Human Activities Structure the Surface Microbial Communities of the Venice Lagoon. *Front. Mar. Sci.* **2021**, *8*, 762292. [\[CrossRef\]](#)
- Zhao, H.; Yan, B.; Mo, S.; Nie, S.; Li, Q.; Ou, Q.; Wu, B.; Jiang, G.; Tang, J.; Li, N.; et al. Carbohydrate metabolism genes dominant in a subtropical marine mangrove ecosystem revealed by metagenomics analysis. *J. Microbiol.* **2019**, *57*, 575–586. [\[CrossRef\]](#) [\[PubMed\]](#)
- Padhy, S.R.; Bhattacharyya, P.; Nayak, S.K.; Dash, P.K.; Mohapatra, T. A unique bacterial and archaeal diversity make mangrove a green production system compared to rice in wetland ecology: A metagenomic approach. *Sci. Total Environ.* **2021**, *781*, 146713. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sengupta, S.; Pramanik, A.; Nag, S.; Roy, D.; Bhattacharyya, A.; Basak, P.; Bhattacharyya, M. Microbial diversity and related secondary metabolite gene assortment at an estuarine mangrove ecosystem. *Reg. Stud. Mar. Sci.* **2020**, *34*, 101051. [\[CrossRef\]](#)
- Nimsi, K.A.; Manjusha, K.; Hatha, A.A.M.; Kathiresan, K. Diversity, distribution, and bioprospecting potentials of manglicolous yeasts: A review. *FEMS Microbiol. Ecol.* **2023**, *99*, fiad044. [\[CrossRef\]](#)
- Vidya, P.; Sebastian, C.D. Yeast Diversity in the Mangrove Sediments of North Kerala, India. *Eur. J. Biol.* **2022**, *81*, 50–57. [\[CrossRef\]](#)
- Phale, S. Yeast: Characteristics and Economic Significance. *J. Bioprocess. Biotech.* **2018**, *8*, 8–10.
- Zhu, H.Y.; Han, D.Y.; Guo, L.C.; Li, J.N.; Wei, X.Y.; Zhang, R.P.; Wang, Q.M.; Shang, Y.J.; Luo, L.J.; Wei, Y.H.; et al. Diversity and distribution of yeasts in intertidal zones of China. *Front. Mar. Sci.* **2023**, *10*, 1286511. [\[CrossRef\]](#)
- de Oliveira, J.B.M.; Junior, D.C.; Parente, C.E.T.; Frases, S. Fungi in Mangrove: Ecological Importance, Climate Change Impacts, and the Role in Environmental Remediation. *Microorganisms* **2025**, *13*, 878. [\[CrossRef\]](#)
- Jia, S.L.; Chi, Z.; Liu, G.L.; Hu, Z.; Chi, Z.M. Fungi in mangrove ecosystems and their potential applications. *Crit. Rev. Biotechnol.* **2020**, *40*, 852–864. [\[CrossRef\]](#) [\[PubMed\]](#)
- Hyde, K.D.; Baldrian, P.; Chen, Y.; Chethana, K.W.T.; De Hoog, S.; Doilom, M.; Gomes de Farias, A.R.; Gonçalves, M.F.M.; Gonkhom, D.; Gui, H.; et al. Current trends, limitations and future research in the fungi? *Fungal Divers.* **2024**, *125*, 1–71. [\[CrossRef\]](#)
- Baldrian, P.; Větrovský, T.; Lepinay, C.; Kohout, P. High-throughput sequencing view on the magnitude of global fungal diversity. *Fungal Divers.* **2022**, *114*, 539–547.
- Liu, Y.; Ding, C.; Li, X.; Su, D.; He, J. Biotic interactions contribute more than environmental factors and geographic distance to biogeographic patterns of soil prokaryotic and fungal communities. *Front. Microbiol.* **2023**, *14*, 1134440. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wagg, C.; Schlaeppli, K.; Banerjee, S.; Kuramae, E.E.; van der Heijden, M.G.A. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nat. Commun.* **2019**, *10*, 4841. [\[CrossRef\]](#) [\[PubMed\]](#)
- Bahram, M.; Hildebrand, F.; Forslund, S.K.; Anderson, J.L.; Soudzilovskaia, N.A.; Bodegom, P.M.; Bengtsson-Palme, J.; Anslan, S.; Coelho, L.P.; Harend, H.; et al. Structure and function of the global topsoil microbiome. *Nature* **2018**, *560*, 233–237. [\[CrossRef\]](#) [\[PubMed\]](#)

24. Wang, Z.; Liu, F.; Li, E.; Yuan, Y.; Yang, Y.; Xu, M.; Qiu, R. Network analysis reveals microbe-mediated impacts of aeration on deep sediment layer microbial communities. *Front. Microbiol.* **2022**, *13*, 931585. [[CrossRef](#)] [[PubMed](#)]
25. Pothayi, V.; Kutty, S.N.; Devasia, S.C. Metagenomic Assessment of Fungal Diversity in the Mangrove Sediments from North and Central Kerala, India. *Environ. Ecol.* **2024**, *42*, 871–880. [[CrossRef](#)]
26. Zhuang, W.; Yu, X.; Hu, R.; Luo, Z.; Liu, X.; Zheng, X.; Xiao, F.; Peng, Y.; He, Q.; Tian, Y.; et al. Diversity, function and assembly of mangrove root-associated microbial communities at a continuous fine-scale. *npj Biofilms Microbiomes* **2020**, *6*, 52. [[CrossRef](#)] [[PubMed](#)]
27. Friess, D.A.; Chua, S.C.; Jaafar, Z.; Krauss, K.W.; Yando, E.S. Mangroves and people: Impacts and interactions. *Estuar. Coast. Shelf Sci.* **2021**, *248*, 107155. [[CrossRef](#)]
28. Yi, J.; Lo, L.S.H.; Cheng, J. Dynamics of Microbial Community Structure and Ecological Functions in Estuarine Intertidal Sediments. *Front. Mar. Sci.* **2020**, *7*, 585970. [[CrossRef](#)]
29. Glassman, S.I.; Wang, I.J.; Bruns, T.D. Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales. *Mol. Ecol.* **2017**, *26*, 6960–6973. [[CrossRef](#)] [[PubMed](#)]
30. Gantayat, R.R.; Viswanathan, P.M.; Ramasamy, N.; Sabarathinam, C. Spatial and temporal variations of geochemical processes and toxicity of water, sediments, and suspended solids in Sibuti River Estuary, NW Borneo. *Environ. Sci. Pollut. Res.* **2023**, *30*, 92692–92719. [[CrossRef](#)]
31. Ozbay, G.; Chintapenta, L.K.; Cannon, A.; Hannum, K. Effects of microbial and heavy metal contaminants on environmental/ecological health and revitalization of coastal ecosystems in Delaware Bay. *Front. Environ. Sci.* **2017**, *5*, 26. [[CrossRef](#)]
32. Gerald, N.R.; Díaz-Rúa, R.; Shea, L.A.; Duarte, C.M. Performance of extraction methods for extracellular DNA from sediments across marine habitats. *Environ. DNA* **2020**, *2*, 91–98.
33. Schoch, C.L.; Seifert, K.A.; Huhndorf, S.; Robert, V.; Spouge, J.L.; Levesque, C.A.; Chen, W.; Fungal Barcoding Consortium; Fungal Barcoding Consortium Author List; Bolchacova, E.; et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 6241–6246. [[CrossRef](#)] [[PubMed](#)]
34. Modi, A.; Vai, S.; Caramelli, D.; Lari, M. The Illumina Sequencing Protocol and the NovaSeq 6000 System. In *Bacterial Pangenomics: Methods and Protocols*; Mengoni, A., Bacci, G., Fondi, M., Eds.; Springer: New York, NY, USA, 2021; Volume 2242, pp. 15–42.
35. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* **2011**, *17*, 10–12. [[CrossRef](#)]
36. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.; Holmes, S.P. DADA2: High resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [[CrossRef](#)] [[PubMed](#)]
37. Abarenkov, K.; Nilsson, R.H.; Larsson, K.H.; Taylor, A.F.S.; May, T.W.; Frøslev, T.G.; Pawlowska, J.; Lindahl, B.; Poldmaa, K.; Truong, C.; et al. The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: Sequences, taxa and classifications reconsidered. *Nucleic Acids Res.* **2024**, *52*, D791–D797. [[PubMed](#)]
38. Nilsson, R.H.; Larsson, K.H.; Taylor, A.F.S.; Bengtsson-Palme, J.; Jeppesen, T.S.; Schigel, D.; Kennedy, P.; Picard, K.; Glockner, F.O.; Tedersoo, L.; et al. The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* **2019**, *47*, D259–D264. [[PubMed](#)]
39. McMurdie, P.J.; Holmes, S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **2013**, *8*, e61217. [[CrossRef](#)] [[PubMed](#)]
40. Cassol, I.; Ibañez, M.; Bustamante, J.P. Key features and guidelines for the application of microbial alpha diversity metrics. *Sci. Rep.* **2025**, *15*, 622. [[CrossRef](#)] [[PubMed](#)]
41. Nanda, A.; Mohapatra, B.B.; Mahapatra, A.P.K.; Mahapatra, A.P.K.; Mahapatra, A.P.K. Multiple comparison test by Tukey's honestly significant difference (HSD): Do the confident level control type I error. *Int. J. Stat. Appl. Math.* **2021**, *6*, 59–65. [[CrossRef](#)]
42. Lengyel, A.; Botta-Dukát, Z. A guide to between-community functional dissimilarity measures. *Ecography* **2023**, *2023*, e06718. [[CrossRef](#)]
43. Skidmore, A.K.; Siegenthaler, A.; Wang, T.; Darvishzadeh, R.; Zhu, X.; Chariton, A.; de Groot, G.A. Mapping the relative abundance of soil microbiome biodiversity from eDNA and remote sensing. *Sci. Remote Sens.* **2022**, *6*, 100065. [[CrossRef](#)]
44. Souza, T. Redundancy Analysis (RDA). In *Advanced Statistical Analysis for Soil Scientists*; Springer Nature: Cham, Switzerland, 2025; pp. 57–77.
45. Rahimlou, S.; Amend, A.S.; James, T.Y. Malassezia in environmental studies is derived from human inputs. *MBio* **2025**, *16*, e0114225. [[CrossRef](#)] [[PubMed](#)]
46. Steinbach, R.M.; Baidouri, F.E.; Mitchison-Field, L.M.; Lim, F.Y.; Ekena, J.; Vogt, E.J.; Gladfelter, A.; Theberge, A.B.; Amend, A.S. Malassezia is widespread and has undescribed diversity in the marine environment. *Fungal Ecol.* **2023**, *65*, 101273. [[CrossRef](#)] [[PubMed](#)]
47. Boekhout, T.; Amend, A.S.; Baidouri, E.F.; Gabaldon, T.; Geml, J.; Mittelbach, M.; Robert, V.; Tan, C.S.; Turchetti, B.; Vu, D.; et al. Trends in yeast diversity discovery. *Fungal Divers.* **2022**, *114*, 491–537.
48. Nimsi, K.A.S.; Manjusha, K. Diversity, Distribution and Hydrolytic Enzyme Potential of Yeasts Associated with Mangroves in Kumbalam, Kerala. *MycoAsia* **2025**, *10*, 1–12.

49. Luis, P.; Saint-Genis, G.; Vallon, L.; Bourgeois, C.; Bruto, M.; Marchand, C.; Record, E.; Hugoni, M. Contrasted ecological niches shape fungal and prokaryotic community structure in mangrove sediments. *Environ. Microbiol.* **2019**, *21*, 1407–1424. [[CrossRef](#)] [[PubMed](#)]
50. Renzi, S.; Nenciarini, S.; Bacci, G.; Cavalieri, D. Yeast metagenomics: Analytical challenges in the analysis of the eukaryotic microbiome. *Microbiome Res. Rep.* **2024**, *3*, 2. [[PubMed](#)]
51. Muwawa, E.M.; Makonde, H.M.; Obieze, C.C.; de Oliveira, I.G.; Jefwa, J.M.; Kahindi, J.H.P.; Khasa, D.P. Diversity and assembly patterns of mangrove rhizosphere mycobiome along the Coast of Gazi Bay and Mida Creek in Kenya. *PLoS ONE* **2024**, *19*, e0298237. [[CrossRef](#)] [[PubMed](#)]
52. Yan, Q.; Deng, J.; Wang, F.; Liu, Y.; Liu, K. Community Assembly and Co-occurrence Patterns Underlying the Core and Satellite Bacterial Sub-communities in the Tibetan Lakes. *Front. Microbiol.* **2021**, *12*, 695465. [[CrossRef](#)] [[PubMed](#)]
53. Wainwright, B.J.; Millar, T.; Bowen, L.; Semon, L.; Hickman, K.J.E.; Lee, J.N.; Yeo, Z.Y.; Zahn, G. The core mangrove microbiome reveals shared taxa potentially involved in nutrient cycling and promoting host survival. *Environ. Microbiome* **2023**, *18*, 47. [[CrossRef](#)] [[PubMed](#)]
54. Li, Y.; Ge, Y.; Wang, J.; Shen, C.; Wang, J.; Liu, Y.J. Functional redundancy and specific taxa modulate the contribution of prokaryotic diversity and composition to multifunctionality. *Mol. Ecol.* **2021**, *30*, 2915–2930. [[CrossRef](#)] [[PubMed](#)]
55. Lee, N.L.Y.; Huang, D.; Quek, Z.B.R.; Lee, J.N.; Wainwright, B.J. Mangrove-associated fungal communities are differentiated by geographic location and host structure. *Front. Microbiol.* **2019**, *10*, 2456. [[CrossRef](#)] [[PubMed](#)]
56. Selbmann, L.; Stoppiello, G.A.; Onofri, S.; Stajich, J.E.; Coleine, C. Culture-dependent and amplicon sequencing approaches reveal diversity and distribution of black fungi in Antarctic cryptoendolithic communities. *J. Fungi* **2021**, *7*, 213. [[CrossRef](#)]
57. Sha, S.P.; Suryavanshi, M.V.; Jani, K.; Sharma, A.; Shouche, Y.; Tamang, J.P. Diversity of yeasts and molds by culture-dependent and culture-independent methods for mycobiome surveillance of traditionally prepared dried starters for the production of Indian alcoholic beverages. *Front. Microbiol.* **2018**, *9*, 2237. [[CrossRef](#)] [[PubMed](#)]
58. Schloss, P.D. Rarefaction is currently the best approach to control for uneven sequencing effort in amplicon sequence analyses. *mSphere* **2024**, *9*, e0035423. [[CrossRef](#)] [[PubMed](#)]
59. Cameron, E.S.; Schmidt, P.J.; Tremblay, B.J.M.; Emelko, M.B.; Müller, K.M. Enhancing diversity analysis by repeatedly rarefying next-generation sequencing data describing microbial communities. *Sci. Rep.* **2021**, *11*, 22302. [[CrossRef](#)] [[PubMed](#)]
60. Zhu, S.; Cai, Y.; Li, Y.; Xiong, J.; Lei, Y.; Sun, Y. Effects of temporal and spatial scales on soil yeast communities in the peach orchard. *Front. Microbiol.* **2023**, *14*, 1226142. [[CrossRef](#)] [[PubMed](#)]
61. Tang, Z.Z.; Chen, G.; Alekseyenko, A.V. PERMANOVA-S: Association test for microbial community composition that accommodates confounders and multiple distances. *Bioinformatics* **2016**, *32*, 2618–2625. [[CrossRef](#)] [[PubMed](#)]
62. Zhu, S.; Lei, Y.; Wang, C.; Wei, Y.; Wang, C.; Sun, Y. Patterns of yeast diversity distribution and its drivers in rhizosphere soil of Hami melon orchards in different regions of Xinjiang. *BMC Microbiol.* **2021**, *21*, 170. [[CrossRef](#)] [[PubMed](#)]
63. Shi, X.; Zhou, S.; Xu, L.; Nethmini, R.T.; Zhang, Y.; Huang, L.; Dong, K.; Zhao, H.; Pan, L. Shifts in soil fungal community and trophic modes during mangrove ecosystem restoration. *J. Fungi* **2025**, *11*, 146. [[CrossRef](#)]
64. Zhang, Z.F.; Pan, Y.P.; Liu, Y.; Li, M. High-level diversity of basal fungal lineages and the control of fungal community assembly by stochastic processes in mangrove sediments. *Appl. Environ. Microbiol.* **2021**, *87*, e00928–21. [[CrossRef](#)] [[PubMed](#)]
65. Xun, W.; Liu, Y.; Li, W.; Ren, Y.; Xiong, W.; Xu, Z.; Zhang, N.; Miao, Y.; Shen, Q.; Zhang, R. Specialized metabolic functions of keystone taxa sustain soil microbiome stability. *Microbiome* **2021**, *9*, 35. [[CrossRef](#)] [[PubMed](#)]
66. Rothig, T.; Bravo, H.; Corley, A.; Prigge, T.L.; Chung, A.; Yu, V.; McIlroy, S.E.; Bulling, M.; Sweet, M.; Baker, D.M. Environmental flexibility in *Oulastrea crispata* in a highly urbanized environment: A microbial perspective. *Coral Reefs* **2020**, *39*, 649–662.
67. Liao, J.; Xu, Y.; Zhang, Z.; Zeng, L.; Qiao, Y.; Guo, Z.; Chen, J.; Jia, B.; Shang, C.; Chen, S. Effect of Cu addition on sedimentary bacterial community structure and heavy metal resistance gene abundance in mangrove wetlands. *Front. Mar. Sci.* **2023**, *10*, 1157905. [[CrossRef](#)]
68. Chen, J.; McIlroy, S.E.; Archana, A.; Baker, D.M.; Panagiotou, G. A pollution gradient contributes to the taxonomic, functional, and resistome diversity of microbial communities in marine sediments. *Microbiome* **2019**, *7*, 104. [[CrossRef](#)] [[PubMed](#)]
69. Deng, H.; He, C.; Worden, A.Z.; Gong, J. Employing a triple metabarcoding approach to differentiate active, dormant and dead microeukaryotes in sediments. *Environ. Microbiol.* **2024**, *26*, e16615. [[CrossRef](#)] [[PubMed](#)]
70. Hu, X.; Tlili, A.; Schirmer, K.; Bao, M.; Bürgmann, H. Metal concentration in freshwater sediments is linked to microbial biodiversity and community composition. *Environ. Int.* **2025**, *199*, 109465. [[CrossRef](#)] [[PubMed](#)]
71. Zarate, A.; Molina, V.; Valdés, J.; Icaza, G.; Vega, S.E.; Castillo, A.; Ugalde, J.A.; Dorador, C. Spatial co-occurrence patterns of benthic microbial assemblage in response to trace metals in the Atacama Desert Coastline. *Front. Microbiol.* **2023**, *13*, 1020491. [[CrossRef](#)] [[PubMed](#)]
72. Lin, W.; Liu, X.; Gong, L.; Liu, R.; Ling, M.; Guo, C.; Meng, H.; Luo, Z.; Du, X.; Guo, Y.; et al. Impact of environmental factors on diversity of fungi in sediments from the Shenzhen River Estuary. *Arch. Microbiol.* **2023**, *205*, 96. [[CrossRef](#)] [[PubMed](#)]

73. Ma, B.; Wang, Y.; Ya, S.; Liu, S.; Stirling, E.; Gilbert, J.A.; Faust, K.; Knight, R.; Jansson, J.K.; Cardona, C.; et al. Earth microbial co-occurrence network reveals interconnection pattern across microbiomes. *Microbiome* **2020**, *8*, 82. [[CrossRef](#)] [[PubMed](#)]
74. Pechlivanis, N.; Karakatsoulis, G.; Kyritsis, K.; Tsagiopoulou, M.; Sgardelis, S.; Kappas, I.; Psomopoulos, F. Microbial co-occurrence network demonstrates spatial and climatic trends for global soil diversity. *Sci. Data* **2024**, *11*, 672. [[CrossRef](#)] [[PubMed](#)]
75. Du, H.; Pan, J.; Zou, D.; Huang, Y.; Liu, Y.; Li, M. Microbial active functional modules derived from network analysis and metabolic interactions decipher the complex microbiome assembly in mangrove sediments. *Microbiome* **2022**, *10*, 224. [[CrossRef](#)] [[PubMed](#)]
76. Gao, C.; Xu, L.; Montoya, L.; Madera, M.; Hollingsworth, J.; Chen, L.; Purdom, E.; Singan, V.; Vogel, J.; Hutmacher, R.B.; et al. Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities. *Nat. Commun.* **2022**, *13*, 3867. [[CrossRef](#)] [[PubMed](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.