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eDNA Metabarcoding Reveals Taxonomic Heterogeneity and Functional Guild Structure of Fungal Communities in Mangrove Sediments

Lila Kusuma Rahayu ¹, Budi Warsito ^{1,2}, Muhammad Danie Al Malik ³ and Tri Retnaningsih Soeprbowati ^{1,4,5,*} 

¹ Doctoral Program of Environmental Science, Universitas Diponegoro, Semarang 50275, Central Java, Indonesia

² Department of Mathematics, Faculty of Science and Mathematics, Universitas Diponegoro, Semarang 50275, Central Java, Indonesia

³ Diponegoro Biodiversity Project Laboratory, Integrated Laboratory Unit, Universitas Diponegoro, Semarang 50275, Central Java, Indonesia

⁴ Cluster for Paleolimnology (CPalim), Universitas Diponegoro, Semarang 50275, Central Java, Indonesia

⁵ Department of Biology, Faculty of Science and Mathematics, Universitas Diponegoro, Semarang 50275, Central Java, Indonesia

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

Abstract

Mangrove ecosystems are ecologically important coastal habitats, yet the organization of fungal communities in mangrove sediments remains insufficiently understood. This study investigated the taxonomic composition, community structure, and functional guild distribution of fungal communities in mangrove sediments from five sites in Central Java, Indonesia, using ITS1-based environmental DNA metabarcoding. Sediment samples were analyzed through alpha and beta diversity metrics, taxonomic profiling, shared-versus-unique taxa assessment, and FUNGuild-based functional annotation. Alpha and beta diversity showed no significant differences among sites, indicating weak broad-scale spatial structuring. In contrast, taxonomic profiling revealed pronounced heterogeneity at finer taxonomic levels, with communities dominated by Ascomycota and Basidiomycota and composed of both a conserved core microbiome and numerous site-specific taxa. Genera such as *Malassezia*, *Aspergillus*, and *Cladosporium* were consistently detected across sites. Functional guild analysis showed that fungal communities were dominated by pathotrophic and undefined guilds, whereas saprotrophic fungi formed a secondary but ecologically important component and symbiotrophs remained minor. Overall, these findings indicate that mangrove sediment fungal communities are taxonomically dynamic but functionally more conserved across sites, supporting partial taxonomic–functional decoupling and suggesting a role for functional redundancy in maintaining ecosystem stability.

Keywords: eDNA metabarcoding; functional redundancy; fungal communities; mangrove sediments; taxonomic heterogeneity



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1. Introduction

Mangrove ecosystems rank among the most productive coastal environments worldwide, serving as hotspots of carbon sequestration at the interface between terrestrial and marine realms [1]. Though occupying just 0.5% of global coastal areas, they deliver critical ecosystem services including shoreline stabilization, hydrological regulation, nutrient cycling, water purification, fisheries support, and long-term blue carbon storage with

exceptional below-ground accumulation [2]. Mangrove sediments function as primary reservoirs of organic matter and hubs of biogeochemical activity, underpinning global carbon cycling [3,4]. Despite limited plant diversity, these sediments sustain highly complex microbial communities vital to ecosystem functioning [5].

Microbial communities play a central role in regulating biogeochemical cycles, including carbon mineralization, nitrogen transformation, and phosphorus turnover [6]. Large-scale initiatives such as the Earth Microbiome Project have demonstrated that microbial diversity is strongly structured by environmental gradients and spatial heterogeneity at a global scale [7,8]. However, most studies have predominantly focused on bacterial and archaeal communities, resulting in a significant underrepresentation of fungal diversity in global microbial ecology [9].

Fungi constitute a key functional component of sedimentary ecosystems, particularly through their role as primary decomposers of complex organic substrates such as lignocellulose [10]. Filamentous fungi contribute to nutrient recycling, organic matter turnover, and ecological interactions within sediment matrices, thereby influencing ecosystem stability and resilience [11]. Nevertheless, the diversity, structure, and ecological roles of fungal communities in mangrove sediments remain insufficiently explored [12], particularly in comparison to bacterial counterparts [13].

Previous studies investigating mangrove microbial communities have largely emphasized bacterial composition and functional processes. For instance, Gomez-Acata et al. [14] demonstrated that microbial community structure varies significantly across mangrove ecological types, highlighting the influence of environmental conditions on microbial distribution; however, their study primarily focused on bacterial and archaeal assemblages, with limited attention to fungal communities. Similarly, Wei et al. [15] examined microbial community structure and function in regenerated and natural mangrove forests using biochemical and PLFA-based approaches, revealing differences in microbial activity but lacking high-resolution taxonomic and functional characterization of fungi.

The advent of high-throughput sequencing has enabled more detailed investigation of fungal communities through environmental DNA (eDNA) metabarcoding, particularly using internal transcribed spacer (ITS) markers. This approach allows for comprehensive characterization of fungal diversity, including unculturable taxa. However, translating taxonomic information into ecological function remains a major challenge. Nguyen et al. [16] developed the FUNGuild framework to assign fungal taxa into ecological guilds, providing a valuable tool for functional interpretation of amplicon data. Nevertheless, a large proportion of fungal taxa remain unclassified due to incomplete ecological knowledge, limiting the resolution of functional inference.

In addition, emerging ecological theory suggests that microbial community composition does not always directly correspond to functional potential. Louca et al. [17] demonstrated that microbial ecosystems can exhibit functional redundancy, in which distinct taxa perform similar ecological roles, leading to a decoupling between taxonomic composition and ecosystem function. While this concept has been extensively explored in bacterial systems, its application to fungal communities, particularly in mangrove sediments, remains largely unexplored.

Environmental gradients and spatial heterogeneity are also key drivers of microbial community assembly. Studies in sedimentary ecosystems have shown that fungal communities are influenced by environmental factors such as nutrient availability, salinity, and heavy metal concentrations. For example, Fang et al. [18] reported that environmental filtering plays a dominant role in structuring fungal communities in river sediments. However, comparable studies in mangrove ecosystems, particularly those integrating both taxonomic and functional perspectives, are still limited. Collectively, these studies highlight several

critical knowledge gaps: (i) limited application of eDNA metabarcoding to characterize fungal communities in mangrove sediments; (ii) insufficient integration of taxonomic composition and functional guild structure; (iii) lack of understanding of spatial heterogeneity in fungal community assembly; and (iv) limited evidence regarding functional redundancy in fungal ecosystems.

To address these gaps, this study employs an eDNA metabarcoding approach targeting the ITS region to investigate fungal communities in mangrove sediments. By integrating diversity analysis, taxonomic profiling, shared-taxa assessment, and FUNGuild-based functional interpretation, this work aims to elucidate spatial patterns of fungal diversity and clarify the relationship between community structure and ecological function. Specifically, this study addresses the following questions: (i) How does fungal taxonomic composition vary across different mangrove sediment environments? (ii) What are the dominant functional guilds associated with these fungal communities? (iii) How does spatial heterogeneity influence fungal community structure and assembly? (iv) Is there evidence of functional redundancy between taxonomic composition and functional guild distribution?

Importantly, this study provides one of the few integrative eDNA-based assessments of mangrove sediment fungal communities that simultaneously resolves broad-scale diversity patterns, finer-scale taxonomic heterogeneity, core-versus-site-specific taxa, and guild-level functional structure. The findings demonstrate that mangrove fungal communities can remain weakly structured at broad spatial scales while still exhibiting pronounced ecological heterogeneity at finer taxonomic and functional levels, thereby clarifying how ecosystem-level functional stability may be maintained despite taxonomic turnover. Overall, this study advances fungal ecology in mangrove ecosystems and contributes to a more integrative understanding of microbial community structure, function, and stability in tropical coastal sediments.

2. Materials and Methods

2.1. Study Area and Sampling Design

Sediment samples were collected from five mangrove ecosystems located along the northern coast of Central Java, Indonesia, representing both natural and rehabilitated systems: Demak (DMK), Jepara (JPR), Tegal (TGL), Semarang (TPK), and Brebes (BRB), as shown in Supplementary Figure S1. These sites were selected to capture spatial heterogeneity in environmental conditions, including differences in salinity, anthropogenic pressure, and ecosystem status (natural vs. rehabilitated). At each site, three sampling points were established using purposive sampling, resulting in a total of 15 sediment samples. Surface sediments (5–10 cm depth) were collected using a sterile stainless-steel scoop and transferred into sterile containers. All samples were transported on ice and stored at -20°C until DNA extraction.

2.2. Environmental DNA Extraction and ITS Amplification

Environmental DNA (eDNA) was extracted from sediment samples using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's protocol. DNA quality and concentration were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Fungal community profiling was performed using high-throughput sequencing targeting the internal transcribed spacer 1 (ITS1) region, a standard barcode marker for fungal metabarcoding. The ITS1 region was amplified using the forward primer ITS5-1737F (GGAAGTAAAAGTCGTAA-CAAGG) and the reverse primer ITS2-2043R (GCTGCGTTCTTCATCGATGC). Library preparation was conducted from the final PCR products, and sequencing was carried

out by PT Genetika Science Indonesia using an Illumina platform to generate paired-end raw reads [19].

2.3. High-Throughput Sequencing and Data Processing

Sequencing data processing was conducted by PT Genetika Science Indonesia, Jakarta, Indonesia using a standard amplicon-based bioinformatics workflow. Paired-end reads were first processed with Cutadapt to remove adapter and PCR primer sequences. Subsequent quality control and amplicon sequence variant (ASV) inference were performed using DADA2, including denoising, read merging, and chimera removal. The resulting ASV dataset was taxonomically classified against the UNITE database for fungal ITS sequences according to the vendor's standard amplicon-processing workflow. Downstream statistical analyses and visualizations were performed in R (version 4.4.0) [20].

2.4. Diversity Analyses

2.4.1. Alpha Diversity

Alpha diversity was calculated to assess within-sample diversity using the Shannon diversity index, Simpson index, and observed ASVs. To minimize bias associated with differences in sequencing depth among samples, sequencing depth was normalized prior to comparison according to the vendor's workflow. Differences among sites were evaluated using one-way ANOVA followed by Tukey's HSD post hoc test. When assumptions of normality were not met, the Kruskal–Wallis test was applied.

2.4.2. Beta Diversity

Beta diversity was analyzed to evaluate differences in community composition among sites using Bray–Curtis dissimilarity (abundance-based) and Jaccard distance (presence-absence-based). Ordination analyses were performed using non-metric multidimensional scaling (NMDS). Statistical significance of community differences was tested using Permutational Multivariate Analysis of Variance (PERMANOVA) and a homogeneity of dispersion test (BETADISPER) [21]. These analyses were conducted using the vegan package in R (version 4.4.0). *p*-values were obtained using 999 permutations.

2.5. Taxonomic Abundance Analysis and Visualization

Relative abundance of fungal taxa was calculated at multiple taxonomic levels (phylum to genus). The most abundant taxa were selected and visualized using heatmaps (top taxa across samples) and stacked bar plots (relative abundance distribution). All visualizations were generated using ggplot2 in R.

2.6. Functional Guild Prediction

Functional guild annotation was conducted as a downstream analysis based on the taxonomically assigned ASV table using the FUNGuild database, which classifies fungal taxa into ecological guilds according to published literature [16]. Taxa were grouped into major trophic categories, including pathotrophs, saprotrophs, and symbiotrophs. Only assignments with “probable” and “highly probable” confidence rankings were retained for interpretation, and relative abundances of guild categories were compared across sites.

2.7. Analysis of Shared and Unique Taxa

To evaluate the distribution of fungal taxa across sites, shared and unique taxa were analyzed using UpSet plots (for high-resolution intersection analysis). The number of exclusive and shared ASVs was calculated for each site and across all site combinations.

2.8. Statistical Analysis and Visualization

All statistical analyses and visualizations were performed in R (version 4.4.0) using the following packages: *vegan* (diversity and ordination analyses), *ggplot2* (visualization), *dplyr* (data manipulation), and *ComplexUpset* (intersection analysis) [22]. A significance level of $p < 0.05$ was applied for all statistical tests.

3. Results

3.1. Sequencing Output and Data Quality

High-throughput sequencing of fungal ITS amplicons generated a total of 986,137 high-quality reads across 15 sediment samples collected from five mangrove sites. Sequencing depth per site showed moderate variation, with mean reads ranging from $57,512 \pm 12,382$ (Jepara) to $71,318 \pm 7904$ (Semarang), and an overall average of $65,742 \pm 10,246$ reads per sample (Table 1), while detailed per-sample read counts are provided in Supplementary Table S1. Individual samples ranged from 43,354 to 84,132 reads, indicating sufficient sequencing coverage for downstream ecological analyses. Across sites, Demak and Semarang exhibited the highest sequencing outputs, whereas Jepara displayed comparatively lower read counts, primarily due to one replicate with reduced sequencing depth. Nevertheless, all samples exceeded commonly accepted thresholds for ITS-based fungal community profiling, ensuring robust representation of community diversity.

Table 1. Summary statistics of ITS sequencing depth across mangrove sediment samples.

Site	n	Total Reads	Mean Reads $\pm$ SD	Min–Max Reads
Pandansari, Brebes	3	202,988	$67,663 \pm 8175$	59,108–75,395
Muara Tuntang, Demak	3	213,314	$71,105 \pm 11,882$	60,862–84,132
Mlonggo, Jepara	3	172,537	$57,512 \pm 12,382$	43,354–66,314
Tapak, Semarang	3	213,955	$71,318 \pm 7904$	65,042–80,195
Batamsari, Tegal	3	183,343	$61,114 \pm 9055$	52,359–70,441
Grand Total	15	986,137	$65,742 \pm 10,246$	43,354–84,132

The study area comprised five mangrove sediment sites distributed along the northern coast of Central Java, Indonesia, namely Pandansari Mangrove in Brebes, Muara Tuntang Mangrove in Demak, Pantai Selayar Mangrove in Jepara, Batamsari Mangrove in Tegal, and Tapak Mangrove in Semarang (Figure 1). These sites represent natural and rehabilitated mangrove systems and provide a spatial framework for evaluating variation in sequencing output, fungal community composition, and FUNGuild-derived trophic structure across coastal sediment environments.

Rarefaction analysis demonstrated that sequencing depth was adequate to capture fungal diversity across all samples (Supplementary Figure S1). Most curves approached clear asymptotic plateaus, particularly in high-depth samples, indicating that additional sequencing would yield minimal increases in observed ASVs. Although a few low-depth samples showed slightly delayed saturation, their trajectories consistently trended toward stabilization, supporting sufficient sampling completeness.

The distribution of sequencing reads across samples is provided in Supplementary Figure S2, which illustrates a relatively balanced sequencing effort among replicates within each site. This consistency minimizes potential biases associated with uneven sequencing depth and strengthens the reliability of comparative analyses across mangrove ecosystems. Collectively, the high sequencing depth, saturation of rarefaction curves, and relatively balanced read distribution

among samples confirm the robustness of the dataset and its suitability for downstream analyses of fungal diversity, taxonomic composition, and functional guild structure.

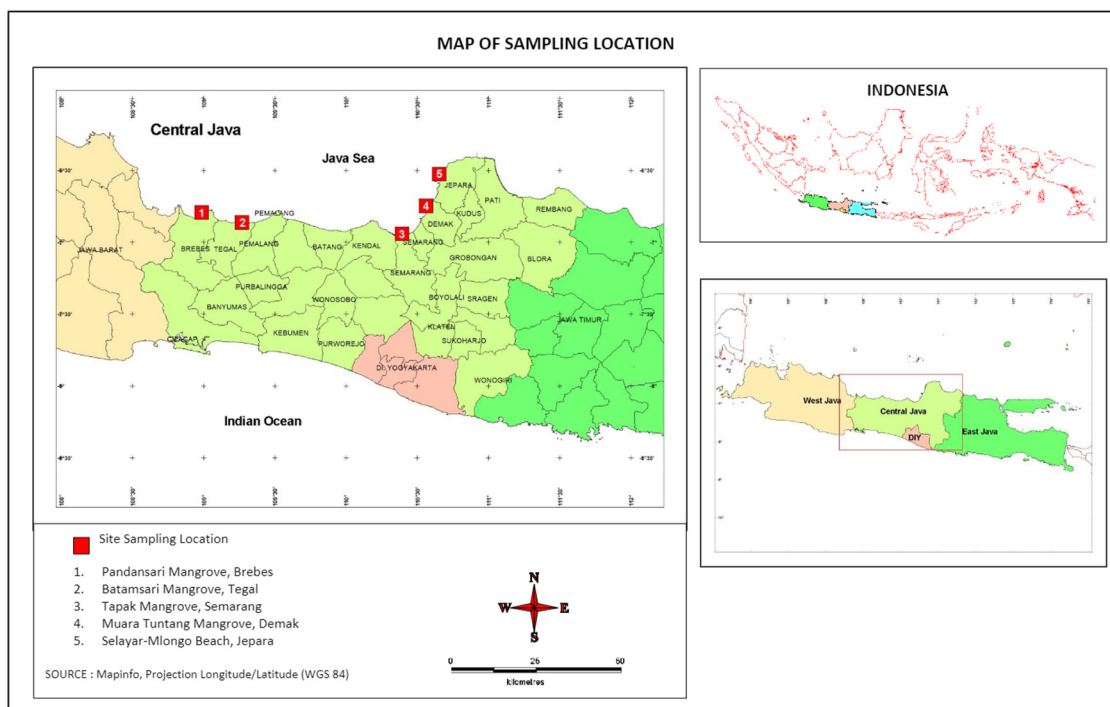


Figure 1. Sampling locations of mangrove sediment sites along the northern coast of Central Java, Indonesia. The pink box in the inset map indicates the Central Java region that is enlarged and shown in detail in the main map.

3.2. Alpha Diversity of Fungal Communities

Alpha diversity analysis revealed moderate variability in fungal richness and diversity across mangrove sediment samples, with no statistically significant differences among sites. The number of observed ASVs ranged widely across samples, reflecting heterogeneity in fungal richness; however, this variation was not significantly associated with sampling location (one-way ANOVA, $F = 1.882$, $p = 0.19$; Table 2).

Table 2. One-way ANOVA results for Observed ASVs, Shannon diversity, and Simpson diversity across mangrove sediment sites.

Metric	Df	Sum Sq (SS)	Mean Sq (MS)	F Value	Pr (>F)
Observed (Location)	4	130,844	32,711	1.882	0.19
Observed (Residual)	10	173,812	17,381	-	-
Shannon (Location)	4	1.658	0.4146	0.382	0.817
Shannon (Residual)	10	10.849	1.0849	-	-
Simpson (Location)	4	0.03168	0.00792	0.899	0.5
Simpson (Residual)	10	0.08805	0.008805	-	-

Similarly, diversity indices based on species evenness showed relatively consistent patterns across sites. The Shannon diversity index exhibited comparable values among all mangrove locations ($F = 0.382$, $p = 0.817$), indicating similar community complexity and distribution of taxa. The Simpson index further supported this pattern ($F = 0.899$, $p = 0.50$), suggesting that dominant taxa were proportionally similar across sites despite environmental differences.

The boxplot visualization of alpha diversity metrics (Figure 2) illustrates overlapping distributions across sites for Observed ASVs, Shannon, and Simpson indices, reinforcing

the statistical results. Although minor fluctuations in median values were observed, particularly in samples from Demak and Semarang, these differences did not translate into significant ecological divergence at the alpha diversity level.

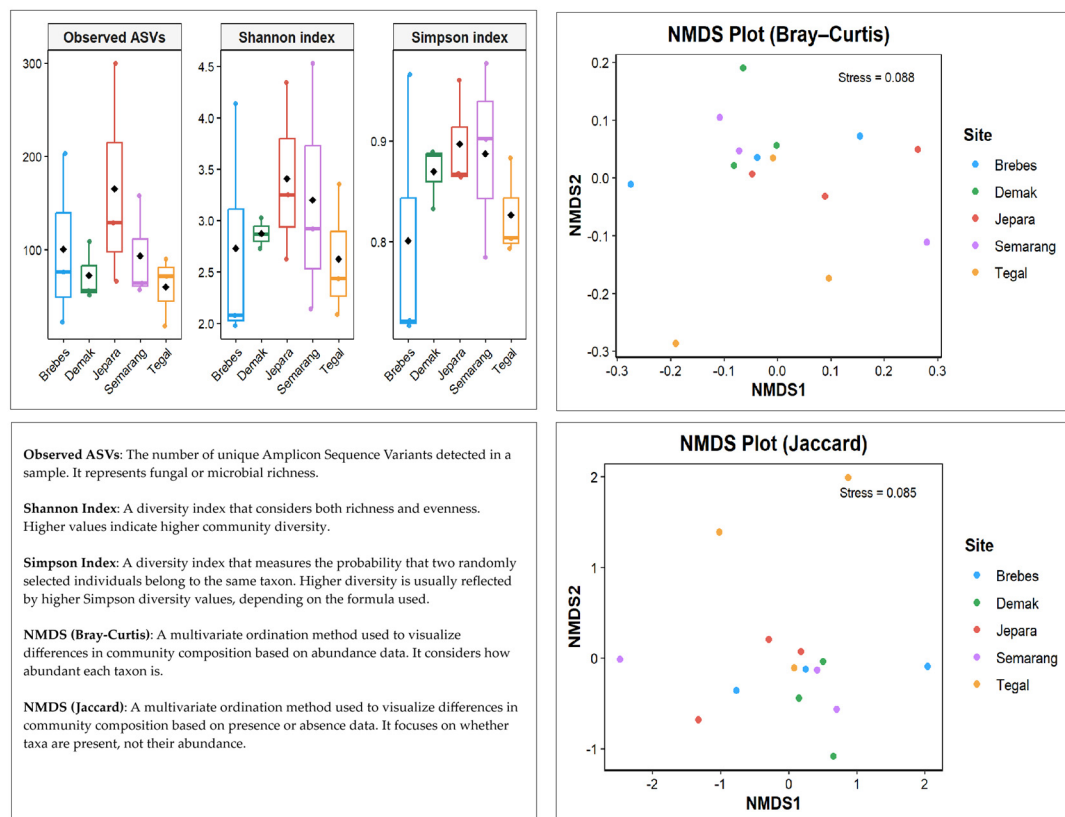


Figure 2. Alpha and beta diversity patterns of fungal communities across mangrove sediment sites.

Post hoc pairwise comparisons based on Tukey's HSD test, presented in Supplementary Table S2, confirmed that no significant differences were detected among mangrove sites for Observed ASVs, Shannon diversity, or Simpson diversity (all adjusted $p > 0.05$), indicating that local environmental variability did not strongly influence within-sample fungal diversity.

Overall, these findings indicate that fungal communities in mangrove sediments maintain relatively stable levels of richness and evenness across sites, despite local variation in read depth and environmental context. This stability suggests that spatial differentiation in these communities is expressed more strongly through compositional turnover at finer taxonomic resolution than through broad differences in within-sample diversity.

3.3. Beta Diversity and Community Structure

Beta diversity analysis based on Bray–Curtis and Jaccard dissimilarities revealed weak spatial structuring of fungal communities among mangrove sediment sites, with substantial compositional overlap across locations.

Non-metric multidimensional scaling (NMDS) ordination showed moderate clustering of samples, with partial overlap among sites (Figure 2). The Bray–Curtis-based NMDS exhibited a low stress value (0.088), indicating a reliable two-dimensional representation of compositional dissimilarity. Similarly, the Jaccard-based NMDS yielded a stress value of 0.085, confirming robustness in presence–absence-based community comparisons.

Despite slight visual separation, particularly along NMDS axes for samples from Tegal and Jepara, overall clustering patterns suggested considerable overlap among fungal

communities across all locations. This indicates that while site-specific tendencies exist, fungal assemblages are not strongly segregated at the spatial scale examined.

Permutational multivariate analysis of variance (PERMANOVA) further supported these observations, revealing no significant differences in community composition among sites for either Bray–Curtis ($R^2 = 0.284$, $p = 0.473$) or Jaccard distances ($R^2 = 0.298$, $p = 0.151$). These results indicate that site identity explained only a limited proportion of variation in fungal community structure.

To assess whether differences in dispersion influenced PERMANOVA outcomes, permutation tests for homogeneity of multivariate dispersions (PERMDISP) were conducted. The results showed no significant differences in dispersion among groups for both Bray–Curtis ($p = 0.944$) and Jaccard ($p = 0.956$), confirming that the non-significant PERMANOVA results were not confounded by heterogeneity in within-group variability.

Collectively, these findings suggest that fungal communities in mangrove sediments exhibit considerable compositional overlap and relatively weak broad-scale spatial differentiation. However, this does not preclude the presence of ecological heterogeneity at finer taxonomic levels or in the distribution of shared and site-specific taxa, which became more evident in subsequent analyses.

3.4. Taxonomic Profiling of Fungal Communities

Taxonomic profiling based on ITS amplicon sequencing revealed a diverse fungal community structure across mangrove sediment samples, spanning multiple taxonomic levels from phylum to genus.

At the phylum level, fungal communities were overwhelmingly dominated by Ascomycota and Basidiomycota, which together accounted for the majority of relative abundance across all sites (Figure 3). Basidiomycota exhibited consistently high abundance in several samples, particularly at the Brebes and Tegal sites, whereas Ascomycota showed more heterogeneous distribution across locations. Minor contributions from Mucoromycota, Rozellomycota, Chytridiomycota, and Mortierellomycota were detected but remained low in relative abundance, indicating a taxonomically uneven community structure.

At finer taxonomic resolution, class-level composition, as shown in Supplementary Figure S3, revealed that *Malasseziomycetes*, *Eurotiomycetes*, and *Sordariomycetes* were among the most prevalent fungal classes across mangrove sediment samples, with clear spatial variation in their relative abundances. *Malasseziomycetes*, in particular, displayed consistently high abundance in several samples, underscoring their probable ecological importance in mangrove sediment environments. These patterns were further refined at the family level (Supplementary Figure S4), where *Malasseziaceae* and *Aspergillaceae* emerged as dominant taxa across sites. Their uneven distribution among samples suggests localized enrichment and possible niche specialization within particular mangrove sediment microhabitats.

At the genus level, *Malassezia*, *Aspergillus*, and *Cladosporium* emerged as the most abundant genera (Figure 3). *Malassezia* showed particularly high relative abundance in several samples, suggesting a widespread ecological role, while *Aspergillus* and *Cladosporium* exhibited more variable distributions, reflecting potential responses to microenvironmental differences among sites.

Order-level profiling further supported these taxonomic patterns (Supplementary Figure S5), showing that *Malasseziales*, *Eurotiales*, and *Capnodiales* contributed substantially to fungal community composition across mangrove sediment samples. In addition to the dominance of these major orders, the occurrence of several moderately abundant orders suggests that the fungal assemblage is structurally complex and taxonomically diverse at finer phylogenetic resolution.

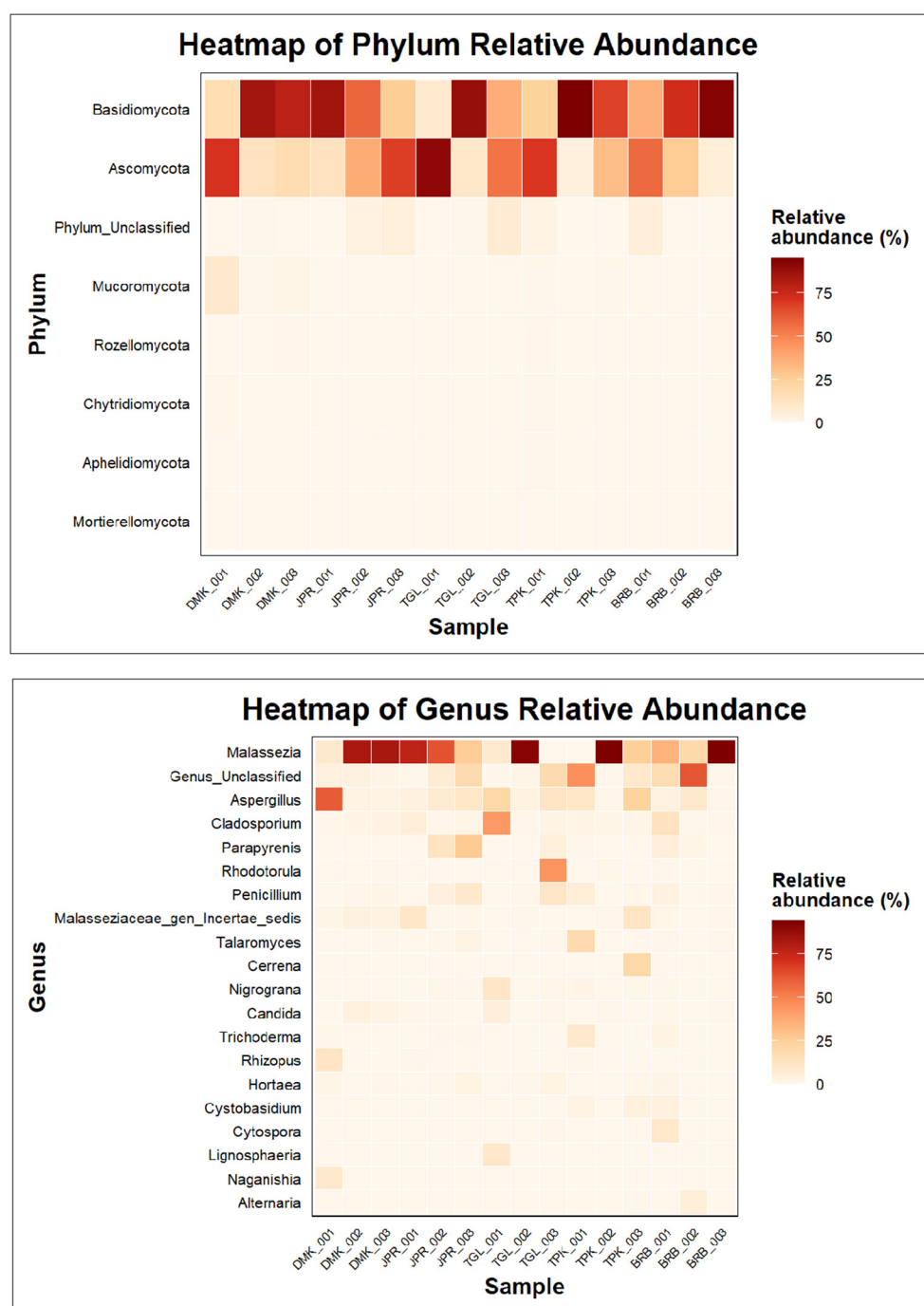


Figure 3. Relative abundance patterns of dominant fungal taxa across mangrove sediment samples at the phylum and genus levels.

Overall, taxonomic profiling demonstrated that although fungal communities were broadly dominated by a limited number of major taxa, substantial heterogeneity emerged at finer taxonomic levels. This pattern indicates that broad community structure is relatively conserved across sites, whereas localized ecological variation is more clearly expressed in the relative abundance of classes, orders, families, and genera.

3.5. Shared and Unique Taxa Across Mangrove Sites

The distribution of fungal taxa across mangrove sites revealed pronounced spatial heterogeneity, characterized by both site-specific assemblages and a conserved core community.

The UpSet analysis (Figure 4) demonstrated that a large proportion of ASVs were uniquely associated with individual sites. Among these, Tegal exhibited the highest number of unique taxa, followed by Jepara and Brebes, whereas Demak and Semarang displayed comparatively lower levels of exclusivity. This pattern suggests that local environmental conditions strongly influence the assembly of fungal communities.

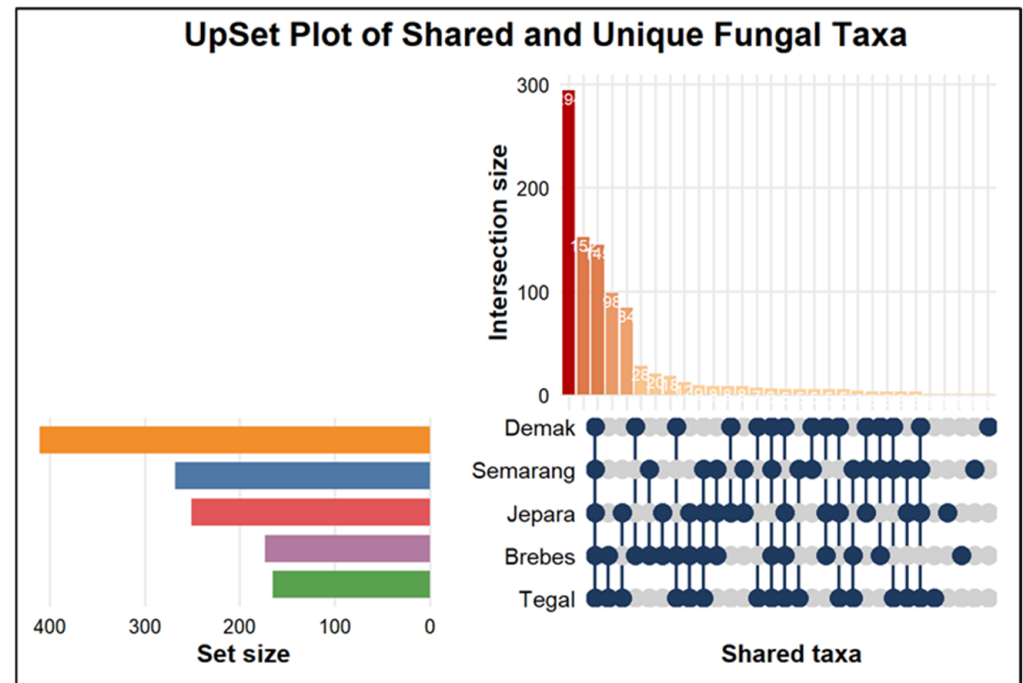


Figure 4. UpSet plot showing shared and unique fungal taxa across mangrove sediment sites. Horizontal colored bars indicate the total set size for each site: orange = Demak, blue = Semarang, red = Jepara, purple = Brebes, and green = Tegal. Dark navy dots and connecting lines indicate the sites included in each shared-taxa intersection, while light gray dots indicate sites not included in that intersection. Vertical bars represent the size of each taxon intersection.

In addition to the high degree of uniqueness, several taxa were shared among multiple sites. The largest intersection corresponded to taxa present across all five mangrove ecosystems, representing a core fungal community adapted to the general physicochemical conditions of mangrove sediments. However, the size of this core assemblage was relatively small compared to the total observed diversity, indicating that most taxa are not ubiquitously distributed.

The Venn diagram (Supplementary Figure S6) further supported these findings by visually summarizing both unique and shared fungal taxa among mangrove sites. While the UpSet plot provided a more resolved quantification of higher-order intersections, the Venn diagram emphasized the predominance of site-specific taxa and the relatively small fraction of taxa shared across all locations. Taxonomic characterization of the shared assemblage (Supplementary Table S3) revealed that the core community was dominated by representatives of Basidiomycota and Ascomycota, particularly the genera *Malassezia*, *Aspergillus*, *Penicillium*, and *Cladosporium*. The repeated occurrence of these taxa across spatially distinct sites suggests a high degree of ecological versatility and tolerance to fluctuating environmental conditions, indicating their probable role in sustaining community persistence and ecosystem-level functional stability in mangrove sediments.

Collectively, these results indicate that fungal communities in mangrove sediments exhibit a dual ecological organization consisting of: (i) a stable core microbiome shared across all sites, and (ii) a large pool of site-specific taxa reflecting local environmental

filtering and habitat heterogeneity. This combination supports the interpretation that spatial heterogeneity is expressed primarily through taxonomic turnover and site specificity rather than through big differences in overall diversity metrics.

3.6. Functional Guild Composition of Fungal Communities

Functional guild annotation revealed that the confidently assigned fungal communities were consistently dominated by the predicted pathotroph–saprotroph mode across all mangrove sediment sites, as shown in Figure 5 and Table S4. This category accounted for 66.59% of retained FUNGuild-assigned reads overall, comprising 200 ASVs and 514,924 reads. At the site level, pathotroph–saprotroph remained the dominant trophic mode, with mean relative abundance ranging from $51.00 \pm 37.84\%$ in Tegal to $76.98 \pm 14.01\%$ in Jepara.

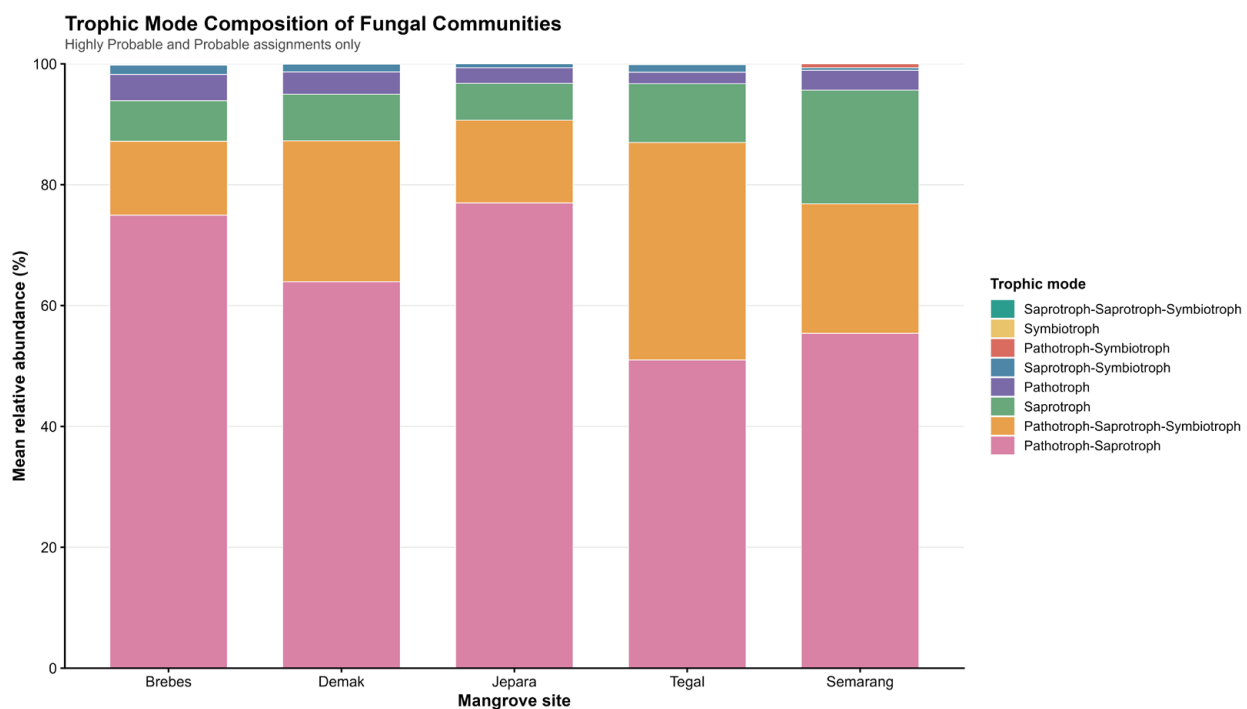


Figure 5. Trophic mode composition of fungal communities across mangrove sediment sites based on FUNGuild annotation.

The second most abundant category was pathotroph–saprotroph–symbiotroph, representing 19.99% of retained reads overall. This multifunctional trophic mode showed its highest relative contribution in Tegal ($35.98 \pm 30.66\%$), followed by Demak ($23.30 \pm 27.43\%$) and Semarang ($21.43 \pm 17.33\%$). Saprotrrophs formed a secondary but ecologically relevant component, accounting for 9.14% of retained reads overall and reaching the highest site-level abundance in Semarang ($18.80 \pm 16.01\%$). Other trophic modes, including pathotroph, saprotroph–symbiotroph, pathotroph–symbiotroph, symbiotroph, and saprotroph–saprotroph–symbiotroph, contributed only minor fractions of the confidently assigned community. Collectively, these results indicate that the predicted functional structure of mangrove sediment fungal communities was primarily organized around mixed pathotrophic–saprotrophic strategies, with site-specific shifts in multifunctional and saprotrophic components.

3.7. Integration of Taxonomic and Functional Patterns

Integration of taxonomic composition and functional guild structure revealed a complex relationship between fungal community structure and ecological function across mangrove sediment sites.

Despite the absence of statistically significant differences in alpha diversity and beta diversity among sites (Sections 3.2 and 3.3), taxonomic profiling demonstrated clear variation in the relative abundance of dominant taxa, particularly at finer taxonomic levels (Section 3.4). Genera such as *Malassezia*, *Aspergillus*, and *Cladosporium* were consistently detected across sites, forming a core taxonomic framework, while numerous site-specific taxa contributed to overall community heterogeneity.

This pattern was further supported by the analysis of shared and unique taxa (Section 3.5), which identified a relatively small core set of ASVs present across all sites alongside a large proportion of site-specific taxa. These findings indicate that fungal communities exhibit considerable taxonomic variability at finer resolutions, likely shaped by local environmental conditions. In contrast, functional guild analysis revealed a more conserved ecological structure across sites (Section 3.6). Although relative abundances of guilds varied among locations, all mangrove sites were characterized by pathotroph–saprotroph and pathotroph–saprotroph–symbiotroph taxa dominating the overall functional profile.

This apparent discrepancy between taxonomic variability and functional consistency suggests a partial decoupling between community composition and ecological function. In other words, distinct fungal taxa may contribute to similar ecological roles across mangrove environments, reflecting a degree of functional redundancy within fungal communities. Furthermore, the consistent dominance of the pathotroph–saprotroph trophic mode across sites, as shown in Figure 5, supports the interpretation that taxonomically variable fungal communities may retain broadly similar ecological strategies. This pattern suggests partial functional redundancy, whereby different fungal taxa contribute to overlapping trophic roles in mangrove sediments.

Overall, the integration of taxonomic and functional analyses suggests that mangrove fungal communities are shaped by environmental filtering at the taxonomic level, while maintaining ecosystem-level functional stability through redundancy and ecological convergence. This dual pattern reflects the adaptive capacity of fungal communities in dynamic and heterogeneous mangrove environments.

4. Discussion

4.1. Mangrove Fungal Communities: High Diversity but Weak Spatial Structuring

The present study reveals a consistent pattern of relatively high fungal diversity across all mangrove sites, yet accompanied by limited spatial differentiation in community structure. This apparent paradox—high diversity but weak spatial structuring—is increasingly recognised in microbial ecology, particularly in environmentally connected systems such as coastal sediments [23].

Alpha diversity metrics, including Observed ASVs, Shannon, and Simpson indices, showed no statistically significant differences among locations (ANOVA, $p > 0.05$). Although Jepara and Semarang exhibited numerically higher richness and diversity values, these differences were not sufficient to indicate site-specific ecological partitioning. This suggests that fungal communities are broadly comparable in richness and evenness across sites, despite geographic separation [24].

This pattern is further supported by beta diversity analyses. NMDS ordination based on both Bray–Curtis and Jaccard distances demonstrated substantial overlap among samples from different locations, with no clear clustering by site. The low stress values (Bray–Curtis: 0.088; Jaccard: 0.085) confirm that the ordination is reliable, yet the spatial arrangement indicates weak compositional segregation. Consistently, PERMANOVA results showed that location explained only a modest proportion of community variation

(Bray–Curtis $R^2 = 0.28$, $p = 0.473$; Jaccard $R^2 = 0.30$, $p = 0.151$), and these effects were not statistically significant (Table 3).

Importantly, the absence of significant PERMDISP results ($p > 0.94$) confirms that within-group dispersion was homogeneous, indicating that the lack of spatial structure is not an artifact of unequal variability among sites. Instead, this pattern reflects a genuinely weak spatial signal in fungal community assembly.

Table 3. Multivariate statistical analyses of fungal community structure across mangrove sediment sites.

Analysis	Distance Metric	R^2	F	p -Value	Interpretation
PERMANOVA	Bray–Curtis	0.284	-	0.473	Not significant
PERMANOVA	Jaccard	0.298	-	0.151	Not significant
PERMDISP	Bray–Curtis	-	0.199	0.944	Homogeneous dispersion
PERMDISP	Jaccard	-	0.199	0.956	Homogeneous dispersion

Ecologically, these findings suggest that regional-scale environmental filtering may be relatively uniform across the studied mangrove systems, allowing similar fungal assemblages to persist across locations. Mangrove sediments are characterized by shared environmental constraints, including salinity gradients, tidal inundation, and organic-rich substrates, which may act as strong deterministic filters selecting for functionally adaptable fungal taxa. Such conditions can promote the establishment of generalist or cosmopolitan fungal taxa, reducing spatial turnover [25]. Another plausible explanation is the role of high dispersal connectivity. Mangrove ecosystems are hydrologically linked through tidal exchange, facilitating the movement of spores and propagules across sites [26]. This can lead to the homogenization of fungal communities, consistent with the “mass effects” framework in metacommunity theory, in which dispersal overrides local environmental differentiation [27].

Despite the lack of strong spatial structuring, the observed diversity remains ecologically significant. High richness combined with compositional overlap suggests that functional redundancy may be prevalent, potentially enhancing ecosystem resilience to environmental disturbances [28,29]. However, this also implies that taxonomic diversity alone may not fully capture ecological differentiation, highlighting the importance of integrating functional analyses [30].

Taken together, these results indicate that fungal communities in mangrove sediments are diverse but only weakly structured at broad spatial scales, likely due to the combined influence of shared environmental constraints and dispersal-driven homogenization [31,32]. Importantly, the absence of strong alpha- and beta-diversity differentiation does not imply ecological uniformity, because finer-scale taxonomic composition and site-specific taxa still reveal meaningful spatial heterogeneity [23].

4.2. Dominance of Key Taxa and Ecological Adaptation

Taxonomic profiling revealed clear dominance of Basidiomycota and Ascomycota across all mangrove sediment samples, with Basidiomycota reaching higher relative abundance in several sites. This pattern was consistent across replicates and locations, indicating that these two phyla form the core fungal backbone of mangrove sediment ecosystems [33]. Minor phyla such as *Mucoromycota*, *Rozellomycota*, and *Chytridiomycota* were consistently detected but at negligible abundances, suggesting they are a rare component of the biosphere rather than dominant ecological roles [34].

At finer taxonomic resolution, class-level patterns revealed strong predominance of *Malasseziomycetes*, followed by *Eurotiomycetes*, *Sordariomycetes*, and *Dothideomycetes*. This structure was mirrored at the order level, with *Malasseziales* consistently dominating across

sites alongside secondary contributions from *Eurotiales* and *Capnodiales*. At the genus level, *Malassezia* emerged as the most dominant and widespread taxon, accompanied by genera such as *Aspergillus*, *Cladosporium*, and *Rhodotorula*.

Although overall community structure showed weak spatial differentiation (Section 4.1), relative abundance patterns still exhibited site-specific variation, indicating subtle ecological filtering at lower taxonomic levels [35].

The dominance of *Malassezia* and other basidiomycetous yeasts suggests adaptation to high-salinity, lipid-rich, and periodically anoxic environments typical of mangrove sediments [13]. Members of *Malasseziomycetes* are known for their lipophilic metabolism and osmotic stress tolerance, which likely provide a competitive advantage under fluctuating tidal conditions. Although *Malassezia* is commonly associated with human and animal skin, it should not be interpreted solely as a human-derived contaminant in this dataset. Recent evidence indicates that *Malassezia* lineages can be repeatedly detected in marine and sediment-associated environments, suggesting a broader ecological distribution than previously recognised [36]. In the present study, *Malassezia* was not restricted to a single sample or site but occurred consistently across spatially independent mangrove sediment samples and was embedded within a coherent taxonomic pattern involving *Malasseziomycetes*, *Malasseziales*, and *Malasseziaceae*. Its high and repeated abundance across sites, together with the sterile sediment sampling procedure and consistent downstream processing, makes sporadic laboratory or handling contamination unlikely as the sole explanation. Instead, its occurrence may reflect the lipid-rich, saline, organic-matter-enriched, and periodically anoxic conditions of mangrove sediments, which could favor lipophilic and stress-tolerant basidiomycetous yeasts. Nevertheless, given recent concerns that some environmental *Malassezia* detections may reflect human inputs, this interpretation should be treated cautiously and validated in future studies using negative controls, source-tracking, and targeted isolation or metagenomic approaches [37].

Similarly, the consistent presence of *Eurotiomycetes* (e.g., *Aspergillus*, *Penicillium*) and *Sordariomycetes* indicates the importance of saprotrophic decomposition processes, particularly in organic-rich mangrove sediments [38]. These taxa are well-recognized for their ability to degrade complex organic matter, including lignocellulosic substrates derived from mangrove litter [39].

Together, these taxa suggest that mangrove fungal communities are structured by physicochemical filtering (salinity, redox conditions, and organic matter), resource specialization, and physiological tolerance to environmental stress [13,39]. The relatively uniform distribution of these dominant taxa across sites reinforces the conclusion that environmental conditions are broadly comparable across the study region, contributing to the weak spatial structuring observed in beta-diversity analyses [40,41]. The observed dominance patterns also imply a functionally structured fungal community despite the absence of strong taxonomic segregation [42,43]. When interpreted alongside FUNGuild-derived trophic mode data, the widespread occurrence of taxa such as *Malassezia*, *Aspergillus*, and *Cladosporium* to a trophic profile dominated by mixed pathotroph–saprotroph strategies, with secondary contributions from pathotroph–saprotroph–symbiotroph and Saprotroph categories. This suggests that mangrove fungal communities are shaped not only by decomposition-related processes, but also by opportunistic, host-associated, and trophically flexible ecological strategies [44]. Despite limited broad-scale differences in community structure among sites, variation in trophic mode proportions suggests that ecological differentiation may be expressed more clearly through the balance of inferred trophic strategies than through taxonomic turnover alone. Together, these findings support the view that mangrove fungal ecosystems are characterized by functional plasticity and partial taxonomic–functional decoupling [44–46]. This functional perspective helps explain the pattern described in

Section 4.1, where high diversity coexists with weak spatial structuring. In such systems, functional redundancy and metabolic flexibility may allow similar taxa to fulfill different ecological roles under different local microenvironmental conditions.

Taken together, these findings indicate that mangrove fungal communities are structured around a limited number of well-adapted clades, particularly Basidiomycota and Ascomycota, while still showing marked variation in relative abundance across sites. Rather than reflecting strong taxonomic segregation, this pattern points to ecological specialization under broadly shared physicochemical constraints, with community differentiation expressed more clearly through finer-scale taxonomic shifts and functional balance than through broad compositional turnover [40]. These observations underscore the value of integrating taxonomic and functional perspectives in eDNA-based fungal ecology, particularly in environmentally dynamic coastal sediments where ecological stability may persist despite taxonomic heterogeneity [47,48].

4.3. Functional Taxonomic Coupling and Ecosystem-Level Implications

The integration of taxonomic composition, diversity patterns, and FUNGuild-derived trophic mode assignment indicates that community structure and ecological function can be only partly aligned in mangrove sediment fungi [44]. While beta-diversity analyses showed weak broad-scale spatial structuring (Section 4.1) and taxonomic profiling revealed the dominance of a limited number of major clades (Section 4.2), the shared-versus-unique taxa analysis provides an additional layer of ecological resolution [48,49]. Although relatively small compared with total richness, this shared fraction likely represents ecologically stable taxa capable of persisting under common mangrove sediment constraints. By contrast, the larger pool of low-frequency and site-specific taxa contributes substantially to the long tail of rare biodiversity and reflects local ecological differentiation [50,51].

The coexistence of a conserved core and a large site-specific fraction further suggests that functional redundancy plays an important stabilizing role in these ecosystems. Dominant taxa such as *Malassezia*, *Aspergillus*, and *Cladosporium* may contribute overlapping ecological functions related to host association, stress tolerance, opportunistic lifestyles, and organic matter turnover [24]. Even where taxonomic composition varies among sites, the persistence of broadly comparable trophic mode categories implies that multiple taxa can support similar ecosystem processes [43]. Such redundancy is likely to be particularly important in mangrove habitats, where tidal inundation, salinity fluctuation, and variable organic matter inputs impose strong and recurrent environmental stress [51–53]. In this context, functional overlap may buffer nutrient cycling and decomposition-related processes against local shifts in taxonomic composition.

An important implication of these findings is that spatial heterogeneity in mangrove fungal communities is expressed more clearly through finer-scale taxonomic turnover and functional balance than through broad compositional separation [37,38]. Although PERMANOVA results indicated no significant site effect on overall community composition, the distributions of dominant taxa, site-specific ASVs, and trophic mode proportions still reveal meaningful ecological variation among locations. FUNGuild-derived profiles suggest site-dependent differences in ecological strategy, even though all sites were consistently dominated by the mixed pathotroph–saprotroph mode, accompanied by variable contributions of pathotroph–saprotroph–symbiotroph and saprotroph categories [53,54]. Accordingly, weak ordination-based spatial structure should not be interpreted as ecological homogeneity; rather, it indicates that functionally comparable fungal communities can be assembled from partly different taxonomic components under similar environmental constraints [31].

Taken together, these results support a framework in which mangrove fungal communities are organized around three interacting components: (i) a conserved set of core taxa

that persist across sites, (ii) a satellite pool of site-specific taxa that contributes to ecological flexibility and local adaptation, and (iii) functional plasticity and redundancy that help sustain ecosystem processes despite taxonomic turnover. This interpretation is consistent with the view that functional profiling can provide a more sensitive indicator of ecological variation than taxonomy alone in environmentally dynamic sediment systems.

4.4. Trophic Mode Structure and Ecosystem Roles

FUNGuild-derived trophic mode analysis provides a critical ecological complement to the taxonomic patterns observed in this study. Across all sites, fungal assemblages were dominated by the mixed pathotroph–saprotroph mode, whereas pathotroph–saprotroph–symbiotroph and saprotroph categories formed secondary but ecologically relevant components. This overall pattern suggests that mangrove sediment fungal communities are not organized around strictly discrete ecological roles, but rather around flexible trophic strategies that combine host-associated, opportunistic, and decomposition-related functions [13].

Saprotrophic fungi nevertheless appear to play an important ecological role because mangrove sediments receive substantial inputs of plant-derived organic matter, including lignocellulosic litter and root-associated detritus [54,55]. Taxa such as *Aspergillus* and *Cladosporium* are likely to contribute to decomposition and nutrient recycling, thereby supporting carbon turnover in these organic-rich coastal sediments. This interpretation is consistent with the high detrital load typical of mangrove sediments, including leaf litter and root exudates, which provide abundant substrates for fungal-mediated organic matter processing [13,55]. By contrast, the dominance of the mixed pathotroph–saprotroph mode should not be interpreted as direct evidence of disease-causing activity. Instead, it may reflect facultative or opportunistic lifestyles in which fungi shift between host association and saprotrophic substrate use depending on local physicochemical conditions and resource availability [56,57].

Symbiotrophic fungi categories occurred at very low abundance throughout the dataset, suggesting that obligate mutualistic interactions play a comparatively limited role in sediment-associated fungal communities relative to terrestrial root-dominated systems. Nevertheless, their recurrent detection indicates that plant–fungus interactions may still contribute to nutrient exchange and microhabitat stability at specific sediment–root interfaces [57,58]. Taken together, these findings indicate that mangrove fungal communities are shaped by a combination of opportunistic or host-associated strategies, decomposition-related functions, and a substantial pool of functionally unresolved taxa, reinforcing the view that mangrove sediments represent ecologically distinctive habitats with considerable hidden fungal functional potential [32,59].

4.5. Mechanisms of Community Assembly and Functional Stability

The integration of diversity patterns, taxonomic composition, shared-versus-unique taxa, and FUNGuild-derived trophic mode profiles provides a coherent framework for understanding fungal community assembly in mangrove ecosystems [39,60]. Despite high taxonomic diversity, the absence of significant broad-scale spatial differentiation suggests that community assembly is shaped less by geographic isolation alone than by the combined influence of environmental filtering and dispersal connectivity [61]. Mangrove sediments across the study region share key physicochemical characteristics, including salinity gradients, tidal inundation, and high organic matter content, all of which may act as recurrent selective pressures favoring environmentally tolerant and ecologically flexible fungal taxa [62].

At the same time, the presence of a conserved core microbiome across all sites indicates that dispersal processes promote regional connectivity, allowing key taxa to persist across

spatially separated locations. This interpretation aligns with metacommunity theory, in which mass effects and environmental filtering jointly shape community structure across connected habitats. Under such conditions, broad compositional similarity can coexist with finer-scale taxonomic heterogeneity and localized ecological differentiation [25,62]. The large fraction of site-specific ASVs observed in this study, therefore, does not contradict the weak beta-diversity separation; rather, it suggests that local environmental conditions modulate the rare and low-abundance component of the community while a subset of dominant taxa remains broadly distributed.

The trophic mode profiles further suggest that ecosystem-level functional organization is comparatively conserved despite taxonomic variability. Across sites, fungal communities were consistently dominated by the pathotroph–saprotroph mode, accompanied by variable contributions of pathotroph–saprotroph–symbiotroph and saprotroph categories. This pattern indicates that mangrove sediment fungi are largely organized around mixed trophic strategies rather than strictly discrete ecological roles. Such trophic flexibility is ecologically plausible in mangrove sediments, where fluctuating redox conditions, tidal disturbance, root-derived substrates, and decomposing plant material create heterogeneous microhabitats and variable resource availability [63–65]. Fungi assigned to mixed pathotrophic–saprotrophic categories may therefore reflect opportunistic or facultative lifestyles, including substrate use, host association, and decomposition-related activity, rather than direct evidence of disease-causing function.

The relatively consistent dominance of mixed pathotroph–saprotroph strategies across all sites supports the idea of functional redundancy within mangrove fungal communities. Different taxonomic groups may contribute to overlapping trophic functions, allowing key ecological processes to persist even when local species composition changes [43]. This redundancy is likely to be particularly important in mangrove ecosystems, where environmental stressors and resource pulses are recurrent. In this context, functional stability does not imply identical taxonomic composition among sites, but rather the persistence of comparable trophic strategies across taxonomically heterogeneous communities [66].

Site-specific deviations in trophic mode composition also provide evidence of ecological differentiation. For example, the higher contribution of pathotroph–saprotroph–symbiotroph in Tegal and the relatively stronger saprotrophic component in Semarang suggest that local sediment conditions may influence the balance between multifunctional and decomposition-associated fungal strategies. These differences indicate that trophic-mode profiling can reveal ecological variation that may not be fully captured by alpha diversity, beta diversity, or broad taxonomic composition alone. Nevertheless, because FUNGuild assignments are annotation-based predictions derived from taxonomic identity, these patterns should be interpreted as inferred ecological tendencies rather than direct measurements of metabolic activity, gene expression, or realized ecosystem function [13].

Collectively, this study advances current understanding of fungal ecology in mangrove ecosystems by showing that high taxonomic diversity can coexist with weak broad-scale spatial structuring while inferred trophic organization remains comparatively stable. By integrating diversity analysis, taxonomic profiling, shared-taxa assessment, and FUNGuild-derived trophic mode interpretation, this work supports a conceptual framework in which mangrove fungal communities are organized around a conserved core microbiome embedded within a dynamic pool of site-specific taxa [59]. These findings also have broader implications for understanding microbial contributions to nutrient cycling, carbon turnover, and ecosystem resilience in coastal sediments, further reinforcing the value of integrative eDNA-based approaches for linking biodiversity, function, and ecological stability in mangrove systems.

5. Conclusions

This study provides an integrated understanding of fungal community structure and function in mangrove sediments using an ITS-based eDNA metabarcoding approach. Despite the lack of significant differences in alpha and beta diversity across sites, fungal communities exhibited pronounced variation in taxonomic composition, particularly at finer taxonomic levels. The identification of a core fungal microbiome, dominated by genera such as *Malassezia*, *Aspergillus*, and *Cladosporium*, together with a large pool of site-specific taxa, highlights the combined influence of environmental filtering and dispersal connectivity in shaping community assembly. FUNGuild-derived trophic mode analysis showed that confidently assigned fungal communities were primarily dominated by mixed pathotroph–saprotroph strategies, with additional contributions from pathotroph–saprotroph–symbiotroph and saprotroph categories. This pattern suggests that mangrove sediment fungal communities are organized around flexible trophic strategies associated with host interaction, opportunistic lifestyles, and organic matter utilization. The persistence of comparable trophic modes across sites, despite taxonomic variability, supports the presence of functional redundancy and indicates that key ecological processes may be maintained by different fungal taxa performing overlapping roles. Overall, mangrove fungal communities appear taxonomically dynamic yet functionally resilient, with spatial heterogeneity expressed more clearly through finer-scale taxonomic turnover, shared-versus-unique taxa, and variation in trophic-mode balance than through broad differences in alpha or beta diversity. These findings strengthen the ecological value of integrating eDNA metabarcoding with functional annotation frameworks to explore fungal biodiversity, hidden microbial functionality, and inferred ecosystem roles in tropical coastal sediments.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d18070419/s1>, Figure S1: Rarefaction curves of fungal ITS libraries across mangrove sediment samples; Figure S2: Distribution of sequencing reads across mangrove sediment samples; Figure S3: Class-level taxonomic composition of fungal communities across mangrove sediment samples; Figure S4: Family-level taxonomic composition of fungal communities across mangrove sediment samples; Figure S5: Order-level taxonomic composition of fungal communities across mangrove sediment samples; Figure S6: Venn diagram of shared and unique fungal taxa among mangrove sediment sites; Table S1: Read counts of individual mangrove sediment samples; Table S2: Pairwise Tukey’s HSD comparisons for alpha diversity metrics among mangrove sediment sites; Table S3: Core fungal taxa detected across all mangrove sediment sites; Table S4: Predicted trophic mode composition of fungal communities across mangrove sediment sites.

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Abbreviations

The following abbreviations are used in this manuscript:

eDNA	environmental Deoxyribonucleic Acid
PERMANOVA	permutational multivariate analysis of variance
PERMIDISP	permutation test for homogeneity of multivariate dispersions
ASV	Amplicon Sequences Variance
PLFA	Phospholipid Fatty Acid
FUNGuild	Fungi Functional Guild
ITS	Internal Transcribed Spacer

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