

Article

Land Use Change Drives Divergent Assembly and Disassembly Mechanisms in Aquatic Eukaryotic vs. Prokaryotic Microbiomes

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Abstract

Urbanization-driven land use transitions, especially impervious surface expansion, profoundly affect aquatic ecosystems, yet the regulatory mechanisms of microbial assembly and disassembly remain unclear. Using environmental DNA metabarcoding, this study examined relationships between landscape patterns, environmental gradients, and microbial communities in the middle-lower Yangtze River. Impervious surfaces (source landscapes) increased from 10% (midstream) to 37% (downstream), disrupting source–sink balance, elevating nitrogen and phenolics, and worsening water quality. Eukaryotes exhibited higher α -diversity and more complex co-occurrence networks, showing greater responsiveness to disturbance. Stochastic processes dominated community assembly (>68%) for both eukaryotic and prokaryotic communities. Dispersal limitation led to higher total β -diversity and nestedness in eukaryotes; by contrast, land use intensified environmental filtering on prokaryotes, resulting in lower β -diversity but higher turnover contribution. Community disassembly simulation reveals a scenario in which impervious surface expansion is associated with non-random species loss, reducing predicted functional richness of both eukaryotic and prokaryotic microbes; the preferential loss of sensitive species is associated with a sharp decline in predicted functional richness, which may have implications for ecosystem stability. Our findings demonstrate that land use change is significantly associated with altered aquatic environments and affects ecosystem functions through biodiversity changes, providing theoretical support for ecosystem conservation.

Keywords: land use change; source–sink landscape theory; community assembly; redundant species hypothesis; community disassembly hypothesis



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1. Introduction

Land use patterns influence the quantity and composition of river pollutants via surface runoff. According to the source–sink landscape theory, artificial source landscapes (e.g., impervious surfaces) have replaced natural sink landscapes (e.g., forests), and increasing land use intensity has led to negative effects on aquatic ecological environments [1]. In the

densely populated, economically dynamic middle-lower Yangtze River, human activities have altered land use types [2,3], which changed river nutrient input and further affected aquatic environments and microbial community structure [4,5]. Clarifying the relationships between land use, aquatic environments, and microbial communities here is thus crucial for river management and ecosystem stability.

Aquatic microbes, key to material cycling and energy flow in aquatic ecosystems, play a core role in maintaining ecosystem balance [6,7]. The diversity of microbes is influenced by various biotic and abiotic factors, and human activities significantly affect microbial diversity and community assembly [8,9]. Previous studies discussed the relative contributions of neutral theory and niche theory to prokaryotic community assembly in the Yangtze River basin, finding that increased land use intensity degrades water quality and strengthens environmental filtering's impact on their assembly [4]. Eukaryotic and prokaryotic microbes, differing in biological traits, show distinct ecological differences in community composition, functions, and environmental interactions. However, existing studies in the Yangtze River basin have largely focused separately on either prokaryotic communities [4] or eukaryotic communities [7,10], with limited integrated comparative analysis of their community assembly and functional responses to anthropogenic disturbances. A more comprehensive evaluation of how human activities differentially affect these two domains of microbial life is therefore needed [11].

The redundant species hypothesis holds that ecosystems need a certain number of species to maintain normal functions; once the number reaches saturation, remaining species are redundant for ecosystem functions [12]. Previous studies showed that microbial communities usually have high functional redundancy across multiple functions [13]—due to similar functions, redundant species can compensate for lost species' functions, mitigating the impact of species loss on functional diversity and ecosystem functions to maintain or restore them [14,15]. However, in real ecological processes, environmental stress may cause the preferential loss of species with unique (non-redundant) functions. The community disassembly hypothesis suggests that a species' sensitivity to environmental stress determines the order of species loss when the community is stressed [16,17], and this has been validated in other taxa (e.g., mammals, birds, and fish) [18,19]. Amid rapid urbanization, microbial functional diversity (closely linked to ecosystem services) may change significantly, yet studies on how land use-induced microbial community disassembly affects ecosystem functions remain scarce [20].

To address the aforementioned research gaps, this study takes the mainstream of the middle and lower reaches of the Yangtze River as the study area, integrating landscape data, water environment data, and environmental DNA metabarcoding data, aiming to achieve the following objectives: (1) to elucidate the impacts of land use changes on water environment indicators; (2) to identify the key ecological mechanisms dominating community assembly of eukaryotes/prokaryotes in highly urbanized areas; (3) to elucidate the mechanisms underlying the disassembly of eukaryotic/prokaryotic communities in highly urbanized areas. The results of this study are expected to provide scientific support for the protection and sustainable management of river ecosystems in the Yangtze River basin.

2. Materials and Methods

2.1. Study Area

The Yangtze River, originating from the Tanggula Mountains on the Qinghai-Tibet Plateau, is Asia's longest and the world's third-longest river; its mainstream (≈ 6300 km) flows through 11 Chinese provinces. Geographically and hydrologically, the reach from Yichang (Hubei) to its estuary is defined as the middle-lower Yangtze River. In recent decades, anthropogenic activities have driven significant land use changes here, leading

to large nutrient inputs into the mainstream and worsening land use-induced negative impacts on aquatic ecosystems. Thus, the middle-lower Yangtze's mainstream—with distinct land use gradients and notable ecological responses—serves as an ideal study area to explore land use transformation impacts on aquatic ecology [4,21].

2.2. Sampling and Laboratory Analysis

A total of 14 sampling sites were set along the mainstream from the midstream to downstream reaches (Figure 1A). The GPS coordinates of all sites are listed in Table S1. The land use gradients adopted in this study represent the proportional coverage of seven land use categories (cropland, forest, shrub, grassland, water bodies, barren land, and impervious surfaces) within each delineated sub-watershed (see Section 2.4 for details). The dry season was selected to minimize precipitation-driven dilution effects and to capture water quality signals most directly influenced by anthropogenic land use inputs under stable low-flow conditions. During the dry season (April 2023), one discrete 1.5 L grab water sample was collected at each site and stored in clean PET bottles, immediately transported to a nearby laboratory, and stored at $-4\text{ }^{\circ}\text{C}$ to $0\text{ }^{\circ}\text{C}$. All water samples were then filtered through a $0.22\text{ }\mu\text{m}$ polycarbonate membrane (Millipore, Billerica, MA, USA). The filters were frozen at $-80\text{ }^{\circ}\text{C}$ until DNA extraction.

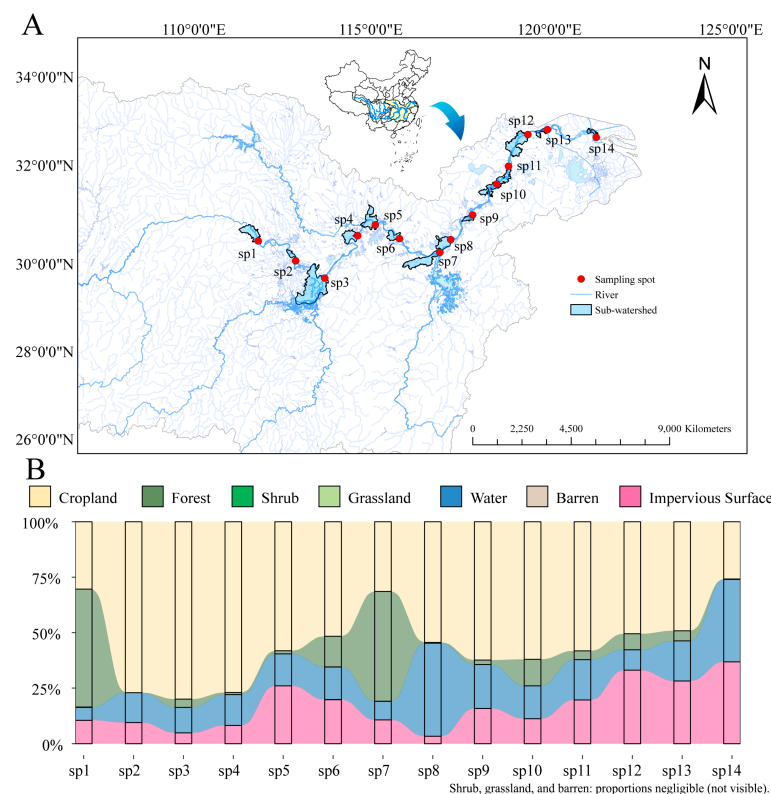


Figure 1. Sampling sites and land use in the study area. (A) Sampling site distribution and sub-watershed boundaries in the middle-lower Yangtze River. (B) Land use type proportions in each site's sub-watershed.

In the field, the longitude and latitude of each sampling site were recorded using a portable GPS device (Magellan, Maryland Heights, MO, USA). Dissolved oxygen (DO), water temperature (Temp), and pH were measured in situ using a portable multiparameter water quality analyzer (Xylem Inc., Washington, DC, USA). Other physicochemical parameters and heavy metal concentrations, including total nitrogen (TN), total phosphorus (TP), permanganate index (PI), chemical oxygen demand (COD), biochemical oxygen demand (BOD), ammonia nitrogen (AN), Petroleum, phenols, mercury (Hg), lead (Pb), copper (Cu),

zinc (Zn), fluoride (F), selenium (Se), arsenic (As), cadmium (Cd), hexavalent chromium (Cr), and cyanide (CN), were determined according to “Standard Methods for the Examination of Water and Wastewater” (Editorial Board of the Ministry of Environmental Protection of China, 2002).

2.3. High-Throughput Sequencing and Bioinformatics Analysis

Genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer’s instructions. The 16S rRNA gene was amplified using primers (AGRGTGGATYNTGGCTCAG) and (TASG-GHTACCTTGTTASGACTT) [4], while the 18S rRNA gene was amplified using primers (GGTTGATCCTGCCAGTAG) and (TACGACTTCTCCTTCCTCTA) [10]. PCR reactions were performed in a 20 µL reaction volume containing 10 µL of KOD One PCR Master Mix (Toyobo Co., Ltd., Osaka, Japan), 1 µL of each primer (10 µM), 10 ng of template DNA, and double-distilled water to adjust the volume. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min. Amplicons were excised from a 2% agarose gel, purified using the Monarch DNA Gel Extraction Kit (New England Biolabs, Ipswich, MA, USA) according to the manufacturer’s protocol, and quantified using a microplate reader (Synergy HTX, BioTek Instruments, Inc., Winooski, VT, USA). Negative controls (nuclease-free water) were included in all PCR reactions and were not sequenced as they yielded no visible amplicons. Equimolar amounts of purified amplicons were pooled and sequenced on the PacBio Sequel II platform (Pacific Biosciences, Menlo Park, CA, USA).

Raw reads were demultiplexed based on barcode sequences using lima v1.7.0 (<https://lima.how/>), and circular consensus sequence (CCS) reads were generated from raw PacBio sequencing data using SMRT Link v8 (www.pacb.com/products-and-services/analytical-software/smrt-analysis/, accessed on 20 May 2025). Adapter sequences were removed from CCS reads using Cutadapt v1.17 [22]. CCS reads were then processed using the R package DADA2 v1.22.0 with parameters described previously [23,24]. Amplicon sequence variants (ASVs) that occurred fewer than 10 times across all samples were discarded to remove potential sequencing errors or low-level contaminants [25]. Taxonomic annotation of ASVs was conducted using the “assignTaxonomy” function and “assignSpecies” function. For prokaryotes, ASVs were aligned against the SILVA v138 database; for eukaryotes, ASVs were aligned against the SILVA v132 database (both downloaded on 12 September 2024, from <https://benjjneb.github.io/dada2/training.html>).

2.4. Land Use Data

Land use data with a 30 m resolution for 2023 were obtained from relevant research (<https://doi.org/10.5281/zenodo.12779975>, accessed on 20 May 2025). To accurately assess the land use structure, the watershed was divided into seven main land use types, namely cropland, forest, shrub, grassland, water, barren, and impervious surface, in combination with the land cover characteristics of the watershed. Sub-watersheds were delineated with reference to previous studies [26], and the area of each land use type in the sub-watershed corresponding to each sampling site was extracted using ArcGIS v10.2.

2.5. Data Analysis

Spearman’s correlation tests between environmental factors and land use types were performed using the “cor.test” function in R v4.1.2. Rank-sum tests for different groups were conducted using the “geom_signif” function in the R package “ggsignif” v0.6.4 with the option test = wilcox.test. The relationships between land use and aquatic environment were analyzed using the “geom_smooth” function with method = “gam” based on the

Generalized Additive Model (GAM). Mann–Kendall (MK) trend test was conducted using the “mk.test” function in the R package “trend” v1.1.6.

Prior to analysis, ASV table was rarefied using the “rrarefy” function in the “Vegan” v2.6.2 package to control for sampling intensity [27]. The “diversity” function was used to calculate microbial α diversity indices (the Shannon–Wiener index, Pielou index and Richness). Phylogenetic diversity (PD) indices were calculated using the “pd” function in the “picante” v 1.8.2 package. After screening environmental factors via variance inflation factor (VIF) and excluding collinear indicators with VIF > 10, the “rda” function was employed for redundancy analysis (RDA) to explore the relationships between microbial communities and environmental factors. The co-occurrence network was constructed based on the Spearman’s correlation matrix using the R package “igraph” v1.3.5 and visualized with Gephi v0.9.1 software following the method described in a related study [21].

The Bray–Curtis distance index was used to characterize the β -diversity of microbial communities, where a larger Bray–Curtis distance indicates greater differences (maximum distance = 1) and higher β -diversity. The ecological processes of microbial communities were identified using β -nearest taxon index (β -NTI) and relative contribution of Bray–Curtis (RCBray), which were calculated using the “qpen” function in the R package “iCAMP” v1.5.12 with the options sig.bNTI = 2 and sig.rc = 0.95. Using the “betapart” v1.6 package, β -diversity could be decomposed into two components: turnover and nestedness [28]. Spatial distance matrices between different communities were calculated using latitude and longitude coordinates obtained via global positioning system and the “dism” function in the “geosphere” v1.5 R package [29]. Changes in community diversity were analyzed by creating distance decay curves and environmental decay curves using log-transformed community similarity (1-Bray–Curtis distance index) and log-transformed distances (spatial and environmental). Additionally, linear regression (via the “lm” function in the “stats” v4.1.2 package) was used to investigate the effects of spatial distance matrices and environmental factor matrices on β -diversity in the study area.

Prokaryotes and eukaryotes were functionally annotated using PICRUSt2 v2.4.1. Functional diversity indices of microbial communities were calculated using the “dbFD” function in the “FD” v1.0.12.3 package, including functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), functional dispersion (FDis), and Rao’s quadratic entropy (RaoQ). Functional redundancy (FRed) was measured as the ratio of RaoQ to Shannon index [30]. Rare species occurring in ≤ 3 sampling sites were removed. This threshold was chosen because taxa with very few occurrences yield highly unstable and unreliable generalized linear mixed models (GLMMs) coefficient estimates, and excluding them helps reduce noise in the sensitivity ranking. GLMMs were subsequently used to assess the correlation between each species and the area of impervious surfaces, following the established methodology for quantifying species sensitivity in community disassembly studies [31]. Spatial dependence was accounted for by including sampling site as a random intercept in the GLMMs. Random slopes were considered but could not be implemented due to convergence failures resulting from sparse species occurrence data. The response coefficients of impervious surfaces were extracted from the models, converted to their opposites (to ensure higher values for more sensitive species), and normalized to the 0–1 range to serve as the sensitivity index for each species. In the context of community disassembly, the R programming language was used to simulate the process of species loss in descending order of sensitivity, with FRic of the community calculated after each loss. Meanwhile, FRic during the gradual loss process was simulated under 100 scenarios of random species loss. Differences in functional diversity between the two scenarios were clarified through comparative analysis. The resulting threshold values are simulation-dependent and should be interpreted as relative indicators rather than absolute benchmarks.

3. Results

3.1. Land Use Change and Its Impact on Water Environment

To explore landscape pattern differentiation in the middle-lower Yangtze River, 14 sampling sections were set up (Figure 1A), and land use type proportions in each section's sub-watershed were analyzed. Dominant types included cropland, forest, water, and impervious surfaces (Figure 1B): cropland proportion declined midstream to downstream (midstream, e.g., sp3: 80%; downstream, e.g., sp14: 26%); forest proportion fluctuated (over 50% in midstream sp1, sp7) then decreased downstream; notably, impervious surface (source landscape) proportion rose significantly midstream to downstream (~10% to 37%), indicating stronger human activity along the gradient.

Spatial variations of water environment indices midstream to downstream were analyzed via the MK trend test (Figure 2A). The 14 sampling sites were arranged as a sequential upstream–downstream gradient suitable for Mann–Kendall spatial trend testing. Preceding analyses confirmed non-significant first-order serial autocorrelation among sites, complying with the independence assumption of the standard Mann–Kendall test. Water quality indices fluctuated variably, with TN and phenolic pollutants showing a significant upward trend (MK test $p < 0.01$), indicating higher nitrogen load and phenolic organic pollutant concentrations along the longitudinal gradient. Correlation analysis between water quality indicators and land use types (Figure 2B) revealed that impervious surfaces displayed significant positive correlations with TN and phenolic pollutants; such covariation was further observed in GAM outputs (Figure S1). These statistical patterns align with the longitudinal trends identified via the Mann–Kendall test, which may correspond to the observed covariation pattern of impervious surface hardening amplifies pollutant input into rivers. Moreover, shrub land (sink landscape) showed negative correlations with TN, while grassland (sink landscape) correlated negatively with phenols and Cr, consistent with the statistical pattern of vegetation cover's water purification and regulation capacity.

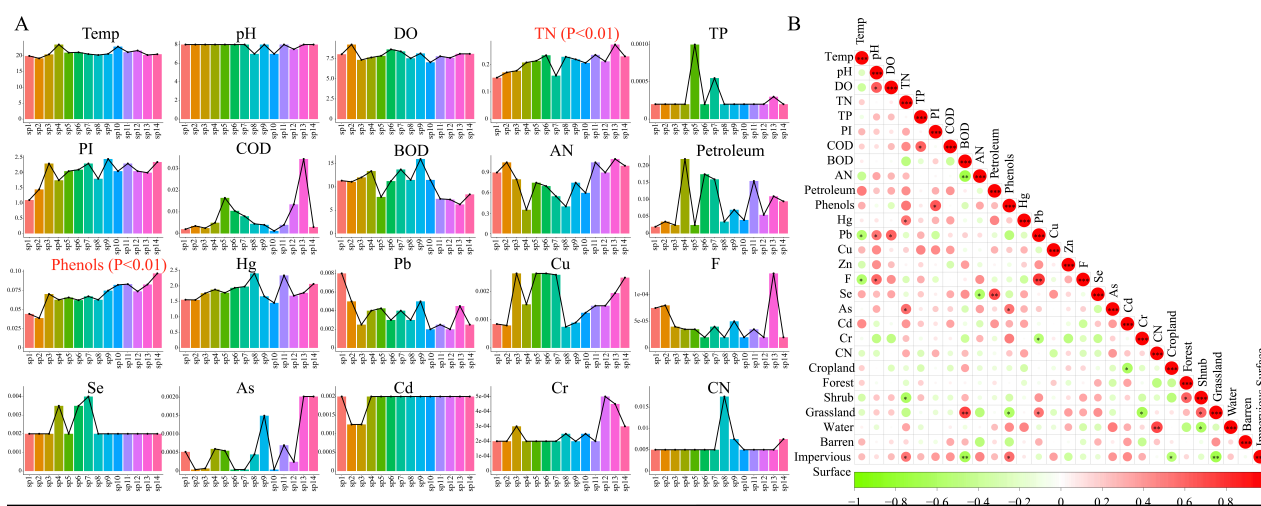


Figure 2. Water environment indices and their associations with landscape types. (A) The bar plot shows water quality index distribution across sites; black solid lines indicate midstream–downstream concentration variations. Indices with significant MK test results are labeled red ($p < 0.01$). (B) Heatmap of water quality–landscape correlations (red = positive, green = negative). Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.2. Community Composition of Prokaryotes and Eukaryotes

Prokaryotic community composition at the phylum level is shown in a chord diagram (Figure 3), with dominant phyla including Actinobacteriota, Proteobacteria, and Cyanobacteria. A Sankey diagram shows prokaryotic species-level spatial succession: the CL500-29

marine group is the dominant taxon, while the hgcI clade, *Cyanobium* PCC-6307, and *Terrimonas* sp. are enriched downstream. For eukaryotes, the dominant phyla are Ciliophora, Bacillariophyta, and Cryptophyta; at the species level, *Tintinnidium* sp. dominates midstream communities, while downstream communities shift to diatoms (e.g., *Cyclotella choctawhatcheeana*, *C. meneghiniana*, and *Actinocyclus* sp.).

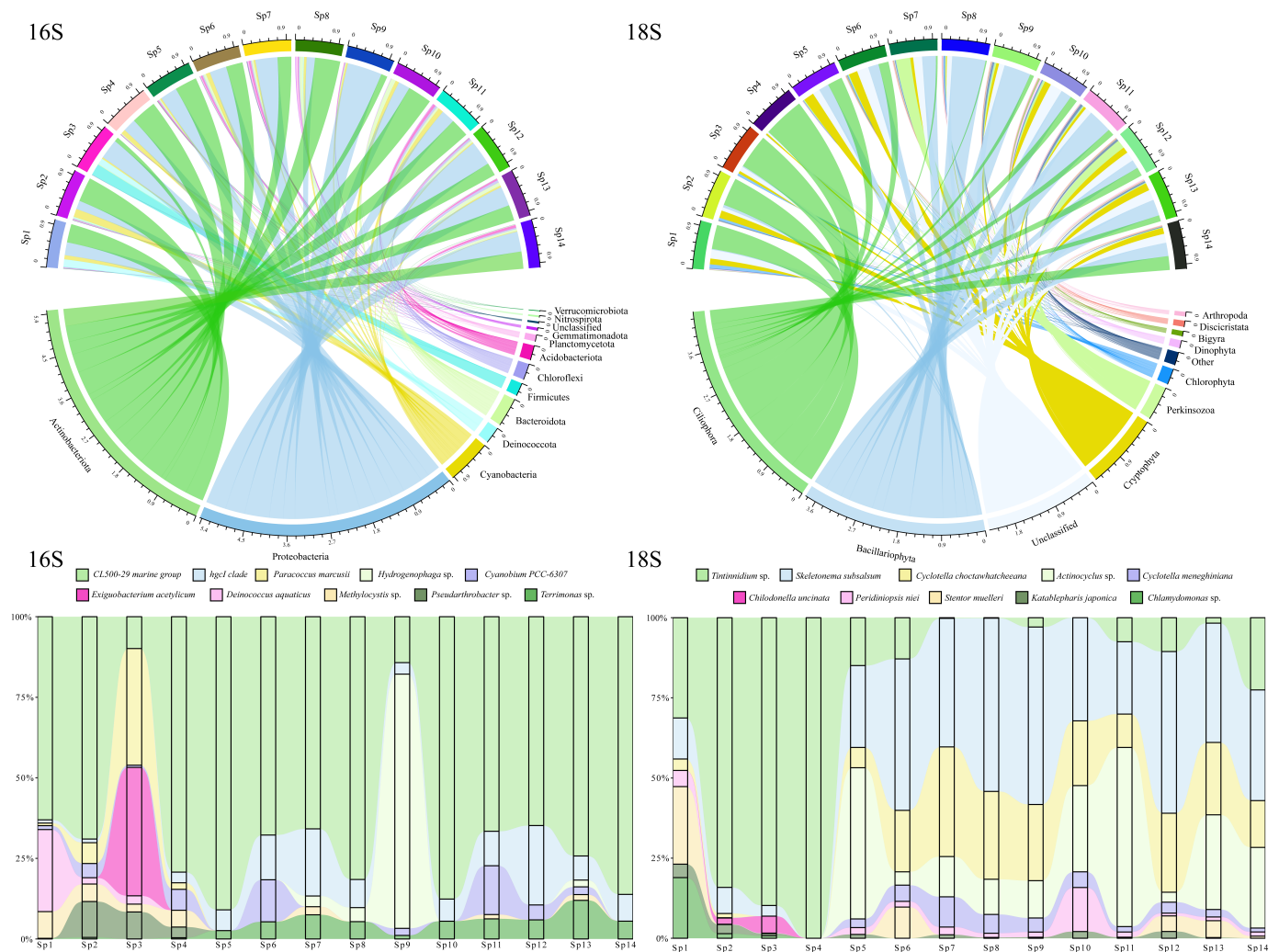


Figure 3. Prokaryotic (16S rRNA) and eukaryotic (18S rRNA) community compositions in the middle-lower Yangtze mainstem. Top row: Phylum-level composition; inner rings show phylum relative abundances, with band width proportional to abundance across sites. Bottom row: Species-level composition (top 10 by relative abundance only).

Comparison of their diversity (Figure S2) shows eukaryotic communities have significantly higher Shannon diversity, richness, and PD than prokaryotes (no significant difference in Pielou evenness), suggesting eukaryotes have greater species richness and phylogenetic complexity, with no essential difference in species distribution evenness between the two. Spatially, diversity indices of both fluctuate sharply, but the MK test shows no significant statistical trends (Figure S2).

Co-occurrence network topological features (Figure 4) varied between eukaryotic and prokaryotic groups. The eukaryotic network contained more nodes (507 vs. 158) and substantially more edges (124,961 vs. 12,204); however, larger networks inherently produce greater edge counts and higher average degree values as a size-related artifact, so these raw differences were not interpreted as biological signals. The eukaryotic network exhibited a

shorter average path length (0.00415) compared with the prokaryotic network (0.00457), which may suggest more direct co-occurrence relationships among eukaryotic taxa.

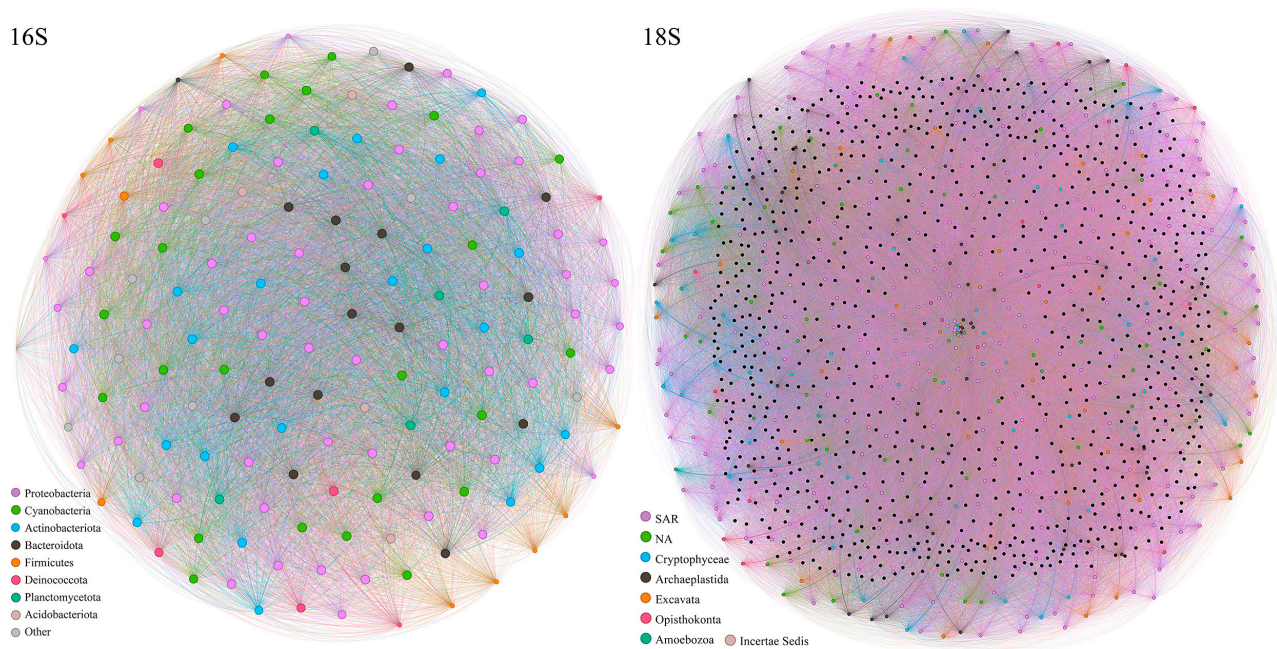


Figure 4. Co-occurrence networks of prokaryotes (16S rRNA) and eukaryotes (18S rRNA).

Environmental factors influencing prokaryotic and eukaryotic community compositions differ significantly. RDA shows PI mainly drives prokaryotic community composition, while eukaryotic composition is significantly affected by multiple factors (TN, PI, phenols, and Pb; Figure S3).

3.3. Community Assembly Mechanisms of Prokaryotes and Eukaryotes

β diversity analysis (Bray–Curtis distance, Figure 5A) showed that eukaryotic community β diversity was significantly higher than that of prokaryotes. Further β NTI analysis revealed that both communities were dominated by stochastic processes (β NTI mostly $-2 \sim 2$), but prokaryotes had significantly higher β NTI values. Specifically, 24% of prokaryotic pairwise comparisons exceeded $|\beta\text{NTI}| > 2$, indicating deterministic assembly in those pairs, whereas only 2% of eukaryotic pairwise comparisons exceeded this threshold, confirming the stronger influence of stochastic processes in eukaryotic community assembly. This indicates prokaryotes are more strongly driven by deterministic processes (in addition to stochastic ones), while eukaryotes are less affected by deterministic processes and have a higher proportion of stochastic processes in assembly.

β diversity decomposition (Figure 5B) showed that both prokaryotic and eukaryotic β diversity were mainly driven by turnover (species replacement). However, eukaryotes exhibited nestedness-dominated distribution (community species as subsets of others) and adapted to gradient habitats via “species subset gain/loss”; this feature is absent in prokaryotes, further highlighting differences in their assembly mechanisms.

Distance decay curve analysis (Figure 5C) showed that prokaryotic communities decayed significantly with both geographic distance (slope = -0.17 , $p < 0.05$) and environmental distance (slope = -0.20 , $p < 0.05$), which means geographic isolation and environmental heterogeneity jointly drive their spatial differentiation. In contrast, eukaryotic communities only decayed significantly with geographic distance (slope = -0.42 , $p < 0.05$), with no significant effect of environmental distance (slope = -0.01 , $p > 0.05$). This suggests geographic

distance dominates eukaryotic spatial differentiation, while environmental filtering plays a limited role.

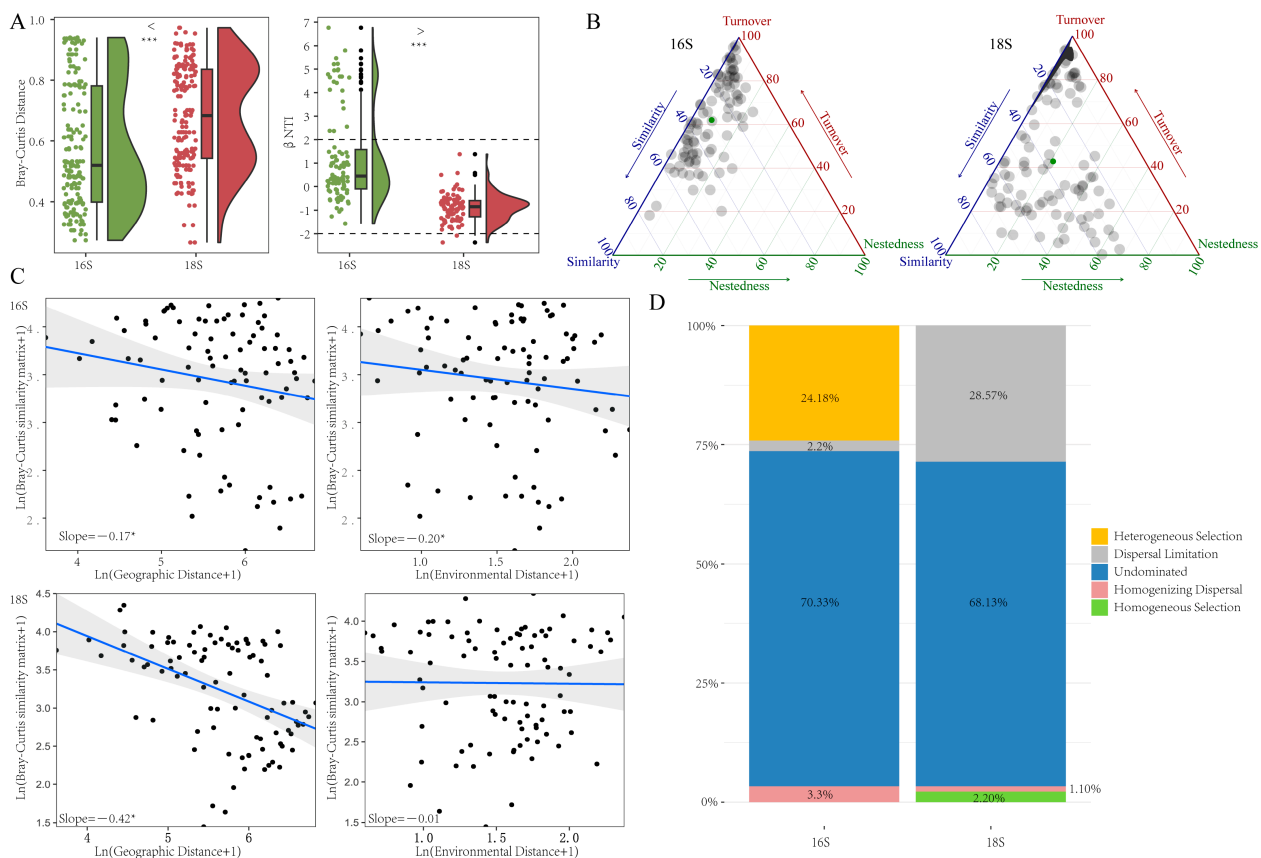


Figure 5. Prokaryotic (16S rRNA) and eukaryotic (18S rRNA) community assembly in the middle-lower Yangtze mainstem. (A) β -diversity (Bray–Curtis distance) and β NTI differences (***) $p < 0.001$; boxplots show central tendency/dispersion; left scatter points and right half-violin plots aid data visualization. (B) β -diversity partitioning (turnover, nestedness, and similarity) via ternary plot (point distribution based on contribution ratios to total β -diversity). (C) Linear regression-fitted decay curves of community β -diversity with geographic/environmental distance (shaded areas = confidence intervals). (D) Proportional differences of five ecological processes between the two groups.

Ecological process decomposition (Figure 5D) revealed prokaryotic assembly was dominated by undominated stochastic processes (70.33%), followed by heterogeneous selection (24.18%), and other processes with minimal contributions. For eukaryotes, undominated stochastic processes remained dominant (68.13%), but dispersal limitation surged to 28.57% (other processes combined <5%). The notably enhanced dispersal limitation in eukaryotes means they have limited dispersal capacity, and geographic isolation imposes stronger constraints on their assembly; this is consistent with prior spatial decay analysis, where eukaryotic community similarity shows a stronger dependence on geographic distance.

3.4. Community Disassembly Mechanisms of Prokaryotes and Eukaryotes

Prokaryotic predicted functional composition in the middle-lower Yangtze River includes multiple coexisting metabolic pathways (e.g., aerobic respiration I, gondoate biosynthesis, L-isoleucine biosynthesis II, etc.). While prokaryotic community composition shows significant spatial variations (Figure 3), their predicted functional composition is highly conservative (Figure 6A). Correlation analysis between prokaryotic predicted functional diversity and land use (Figure 6B) showed FRic was significantly positively correlated with grassland and negatively with impervious surfaces, further validated by

GAM regression (Figure S4). Spatial variations of predicted functional diversity indices (Figure S5) revealed FRic and FRed both had a significant decreasing trend from midstream to downstream, though FRed at Sp3 and Sp9 was significantly higher than at other sites.

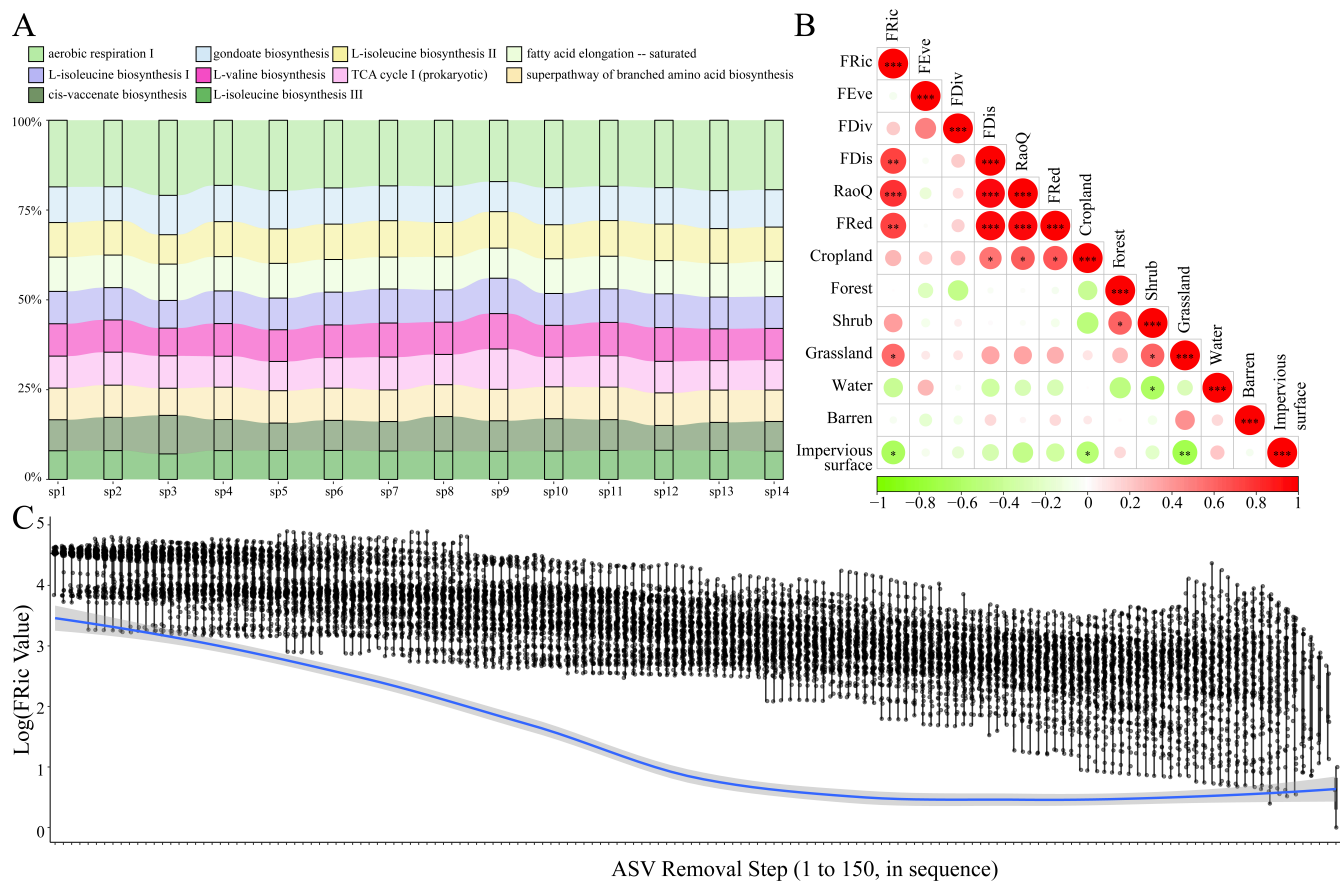


Figure 6. Prokaryotic predicted functional diversity and community disassembly in the middle-lower Yangtze mainstem. **(A)** A Sankey diagram shows prokaryotic predicted functional composition across sites (top 10 functions by abundance only). **(B)** A heatmap of prokaryotic predicted functional diversity-land use correlations (red = positive, green = negative). Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **(C)** Prokaryotic predicted functional diversity dynamics under species loss simulations: X-axis = sequential species loss gradient, Y-axis = FRic log values. Boxplots show distributions from 100 random loss simulations; the GAM-fitted curve shows FRic dynamics under urbanization sensitivity-based loss.

GLMMs assessing species urbanization sensitivity showed 56% of prokaryotic ASVs had positive responses and 44% negative to urbanization (Figure S6), indicating most species adapted to high-urbanization environments. Positive-response taxa (urbanization-adapted) mainly included Bacteroidota, Actinobacteriota, and Chloroflexi; negative-response taxa (urbanization-sensitive) included Proteobacteria, Cyanobacteria, and Deinococcota (Figure S7). Based on the community disassembly hypothesis, FRic comparisons between random and sensitivity-based species loss scenarios (Figure 6C) revealed lower mean community FRic under sensitivity-based loss. When species were lost in descending order of urbanization sensitivity, in our simulation, FRic remained high initially and dropped almost to a minimum when ~50% of species were lost, though this threshold is model-derived and depends on the assumptions of the simulation framework.

Eukaryotic predicted functional proportions were conserved across sites (Figure 7A), with predicted functional compositional differences smaller than taxonomic ones; aerobic respiration pathways (aerobic respiration I and II) dominated in abundance. Correlations between eukaryotic predicted functional diversity and land use mirrored prokaryotes

(Figure 7B): FRic was significantly positively correlated with shrub and negatively with impervious surfaces. GLMMs showed 43% of eukaryotic ASVs had positive urbanization responses (e.g., Cryptophyta, Figures S8 and S9) and 57% negative (e.g., SAR group, Figures S8 and S9). Additionally, FRic comparisons between random and sensitivity-based species loss (Figure 7C) showed lower mean community FRic under sensitivity-gradient loss. Similar to prokaryotes, in our simulation, FRic was high initially when species were lost in descending order of urbanization sensitivity and dropped almost to a minimum when ~50% of species were lost, though this threshold is model-derived and depends on the assumptions of the simulation framework.

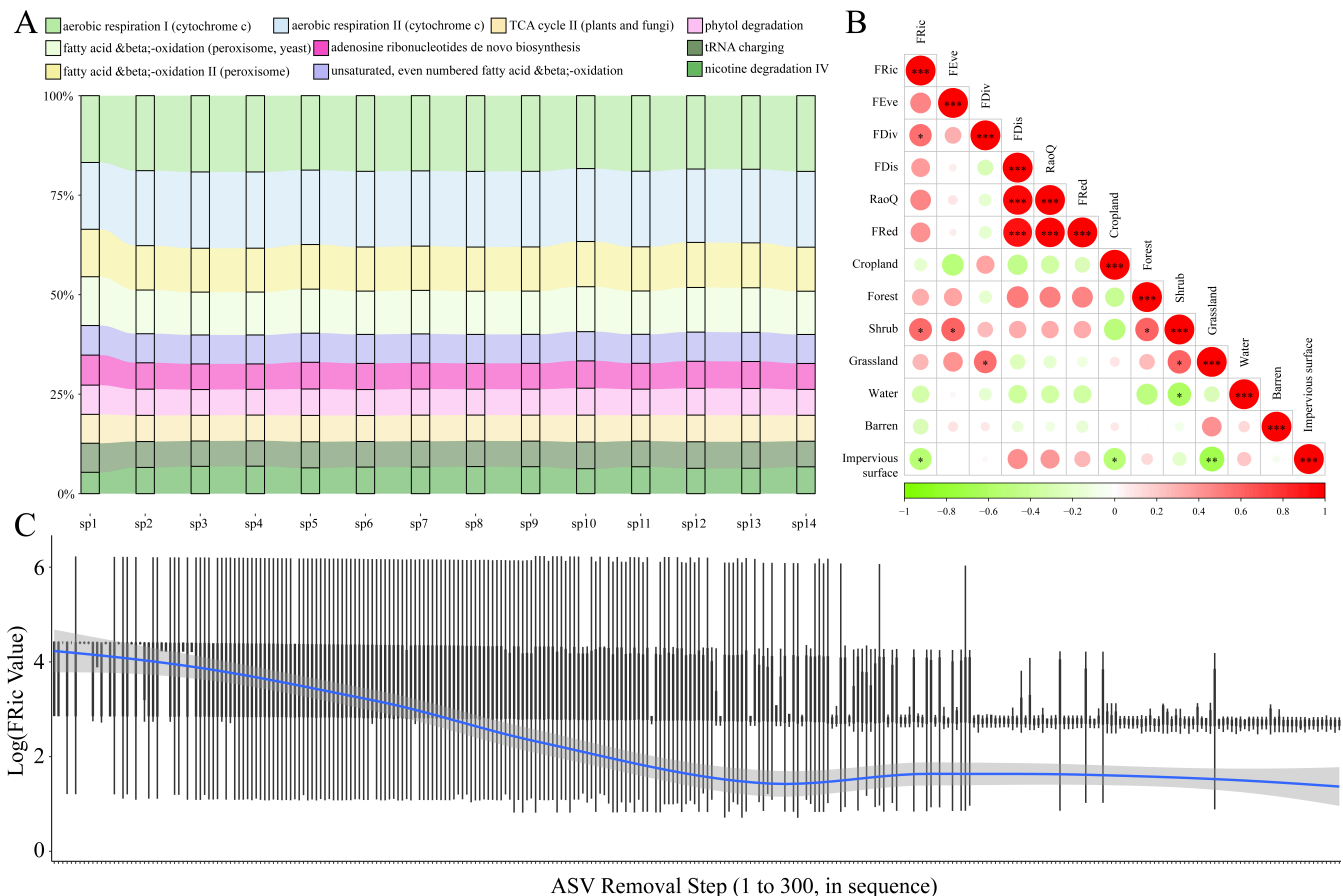


Figure 7. Eukaryotic predicted functional diversity and community disassembly in the middle-lower Yangtze mainstem. **(A)** Eukaryotic predicted functional composition across sites (top 10 functions by abundance only). **(B)** Heatmap of eukaryotic predicted functional diversity–land use correlations (red = positive, green = negative). Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **(C)** Eukaryotic FRic dynamics under sensitivity-based loss (GAM-fitted) and random loss (boxplots).

4. Discussion

4.1. Mechanism of Water Quality Variation Driven by Land Use Conversion

As China's largest river, the Yangtze River is both a key carrier for basin economic and social development and a critical ecological barrier for regional water resource security. Amid recent rapid urbanization and industrialization, however, large areas of forests and grasslands in its middle-lower reaches have been converted to impervious surfaces [4,26,32]. This study showed the proportion of impervious surfaces increased continuously from mid-stream to downstream (Figure 1B). The expansion of this source land cover—closely tied to urbanization—enhances surface runoff intensity, expands pollutant migration paths, accelerates nutrient and pollutant input, and significantly burdens aquatic ecosystems [4,26,33].

The significant increase in TN concentration from midstream to downstream of the Yangtze River (Figure 2B) further confirms this trend. Downstream regions, with high population density, large domestic sewage discharge, concentrated industrial activities, and substantial industrial wastewater discharge, are important sources of nitrogen input [34].

In addition to nitrogen, phenolic pollutants also showed a significant spatial upward trend (Figure 2B). Phenolic compounds are widely used in industries and easily enter aquatic environments via insufficiently treated industrial or domestic wastewater. Studies confirm phenolic substances mainly come from anthropogenic activities, with much higher detected concentrations in urbanized areas than other land use regions [35,36]. Notably, phenolic compounds pose potential risks to human health, especially endocrine disruption, reproductive toxicity, and adverse early pregnancy outcomes [37].

4.2. Heterogeneity of Aquatic Microbial Community Structure

Results showed prokaryotic communities in the middle-lower Yangtze River were dominated by Proteobacteria, Actinobacteriota, and Cyanobacteria at the phylum level, likely due to their strong ecological adaptability in river ecosystems [38,39]. At the species level, the CL500-29 marine group was widely distributed in high land use intensity areas [4], while the hgcI clade, Cyanobium PCC-6307, and *Terrimonas* sp. were significantly enriched downstream. Given their common occurrence in eutrophic waters [40–42], this reflects nutrient load (increasing along the river) strongly shaping microbial community structure, consistent with the environmental filtering hypothesis that environmental gradients control community spatial distribution [43,44].

For eukaryotic communities, *Tintinnidium* sp. (a dominant Ciliophora taxon) is a key zooplankton component. It acts as a critical link between micro food webs and classical food chains [45,46]. Additionally, downstream-dominant Bacillariophyta and Cryptophyta are widely distributed, likely due to broad tolerance to multiple environmental factors, forming wide niches and strong competitiveness [47]. The results also show that the α/β diversity of eukaryotes in the mainstream is significantly higher than that of prokaryotes, a finding that may be highly context-dependent. Eukaryotes possess more complex cellular structures, sexual reproduction-driven lineage differentiation, and broader niche differentiation—reflecting intrinsic differences in evolutionary paths and ecological strategies between the two groups, leading to distinct diversity levels [48]. Some studies have shown that the phylogenetic diversity of eukaryotic microorganisms in freshwater far exceeds that of metazoans and terrestrial plants, and that the diversity of eukaryotic plankton in terms of phylogenetic lineages, ecotypes, and functional groups is much higher than that of prokaryotes [49]. Moreover, in response to immediate environmental fluctuations, the α diversity of eukaryotic communities increases continuously over time, meaning that eukaryotic communities can accumulate species diversity continuously on a temporal scale, whereas prokaryotic diversity is more susceptible to constraints from environmental conditions and ecological competition, tending to decline [50]. Furthermore, many protozoa within eukaryotic communities acquire nutrition by grazing on bacteria, and this top-down regulatory mechanism prevents the excessive dominance of competitively superior species in prokaryotic communities, thereby creating favorable conditions for species coexistence and diversity maintenance in eukaryotic communities [51].

The observed topological differences between eukaryotic and prokaryotic co-occurrence networks suggest distinct patterns of species associations. Eukaryotic networks exhibited shorter average path lengths, which may indicate more direct co-occurrence relationships among taxa. However, these patterns should be interpreted cautiously, as they are based on correlation and may be influenced by network size and other methodological factors.

Direct evidence for functional differences in network organization would require additional experimental approaches [52,53].

This study found that prokaryotic community composition is mainly driven by PI, which denotes reductive organic pollution in water [39]. Variations in organic matter concentration can stimulate microbial metabolic activity, affect community structure/function, and influence ecosystem stability [54]. Eukaryotic communities are significantly affected by multiple factors: TN and PI elevate water nutrient levels [55]; phenolic substances induce “toxic responses” in eukaryotes, leading to mortality [35]; and Pb (persistent in the environment) causes toxic metal pollution even at low concentrations and may enter food chains, severely endangering organisms [56].

4.3. Differences in Assembly Mechanisms of Aquatic Microbial Communities

β NTI analysis showed that prokaryotic and eukaryotic communities are dominated by stochastic processes (β NTI mostly $-2 \sim 2$), consistent with prior findings [4]. Microbes, with their small size, high abundance, and low settling rates, have stronger passive dispersal capacity [57,58], making microbial communities more prone to stochastic regulation [59]. Both groups exhibited significant geographic distance decay, with community similarity decreasing and compositional differences increasing with spatial distance (high β -diversity), suggesting stochastic processes mainly regulate community structure [60]. However, stochastic processes affect eukaryotes more strongly than prokaryotes (Figure 5A: some prokaryotic β NTI >2 , while eukaryotic β NTI is mostly $-2 \sim 2$). This may be due to prokaryotes’ stronger dispersal capacity, whereas eukaryotes’ dispersal varies with propagule size and abundance, making them more susceptible to dispersal limitation [61]. Additionally, prokaryotes had significantly higher β NTI and significant environmental distance decay (Figure 5C), with heterogeneous selection accounting for 24.18% in ecological process decomposition. This indicates their community structure is also regulated by deterministic processes, possibly due to high impervious surface proportions in the middle-lower Yangtze River; environmental filtering promotes assembly of specific species during environment–organism interactions, increasing prokaryotic compositional differences [4,62].

β -diversity decomposition effectively reveals microbial community distribution patterns and underlying assembly mechanisms. This study found that total β -diversity of both prokaryotes and eukaryotes was dominated by species turnover (Figure 5B). Turnover represents species replacement along spatial/environmental gradients, driven by environmental filtering or geographic isolation [63,64]. For example, species sorting along gradients restricts taxa to suitable habitats [63], or geographic isolation limits dispersal of once-continuous species, influencing β -diversity [65]. Notably, some eukaryotic sites showed a nestedness-dominated distribution. Eukaryotes are mainly affected by dispersal limitation (Figure 5C,D), restricting species exchange between communities. This limited dispersal makes composition more prone to local processes like stochastic extinction, leading to high total β -diversity and nestedness [65]. In contrast, prokaryotes are less affected by dispersal limitation (Figure 5A,D), facilitating cross-geographic species exchange. Frequent migration reduces isolation-induced “species absence” or “compositional divergence,” lowering inter-community β -diversity (Figure 5A) [66–68]. Additionally, highly dispersive prokaryotes adapt to diverse habitats, enhancing turnover via “species replacement” after overcoming geographic barriers (Figure 5B) [69].

4.4. Mechanisms of Aquatic Microbial Community Disassembly

Microbial community structure is highly sensitive to environmental changes, and such responses may affect macroscale ecosystem functional stability via biological functional

diversity [70]. Functional diversity—referring to microbes' multiple ecosystem functions—is a key link between biodiversity and ecosystem functions [70]. This study shows that microbial taxonomic compositions varied significantly across samples (Figure 3), but their predicted functional compositions were more conserved (Figures 6 and 7), with core metabolic functions stable despite species turnover. This aligns with prior findings that environmental selection shapes microbial functional composition, leading to high functional conservatism amid taxonomic divergence, a phenomenon also seen in aquatic, soil, and gut microbiota [20]. Thus, studying microbial functional diversity helps elucidate microbe–environment interactions and response mechanisms [52,70,71].

Correlation analysis between eukaryotic/prokaryotic predicted functional diversity and land use showed that FRic was significantly negative with impervious surfaces. This is because impervious surfaces (human-dominated source landscape) accumulate pollutants, harming aquatic environments [26]. Such environmental filtering excludes urban-intolerant species, homogenizing functional traits and reducing functional richness [72,73]. Notably, the significant negative correlation between impervious surfaces and grassland ($p < 0.01$), combined with the midstream–downstream land use gradient (Figure 1B), reveals a human-driven trajectory: converting sink (grassland) to source (impervious surface) landscapes may be a core driver of functional diversity loss, supported by the distinct midstream–downstream predicted functional diversity decline (Figures S5 and S10).

Notably, prokaryotic communities at Sp3 and Sp9 exhibited higher FRed values compared with those at other sites. This pattern was associated with the higher abundance of specific taxa (*Paracoccus marcusii* and *Exiguobacterium acetylicum* at Sp3; *Hydrogenophaga* sp. at Sp9) and corresponding metabolic pathways at these locations (Figures 3 and 6A). This observation is consistent with the idea that higher species abundance may enhance functional redundancy, but we caution that this interpretation is based on only two sites and lacks direct statistical testing of the redundancy–stability relationship. Further studies with more extensive sampling are needed to validate this pattern [14,15].

The environmental filtering hypothesis posits that environmental stress selects species by functional traits, excluding intolerant ones and their traits [74,75]. Prior studies note that urbanization may filter organisms by removing specific traits [76–78]. Our results show that aquatic microbial predicted functional diversity was lower when species were lost along urbanization sensitivity gradients than randomly (Figures 6C and 7C). This means urbanization-sensitive species are not random redundant taxa but carry unique functions—community function relies heavily on them. Non-random elimination of sensitive species by urbanization thus worsens predicted functional diversity loss [79]. Though the initial slow predicted functional diversity decline suggests functional redundancy buffers early species loss [14,15], in our simulation, FRic dropped sharply to a minimum when ~50% of prokaryotic species were lost (Figure 6C). This stage primarily involved losses of photosynthesis-related functions in Cyanobacteria, significantly impacting material cycling and ecosystem stability [80]. For eukaryotes, species loss also caused a sharp FRic decline, with ~50% loss severely harming ecosystem functions (Figure 7C). This threshold marks critical points where microbially mediated ecosystem services may degrade significantly and potentially irreversibly.

4.5. Limitations

Several limitations of this study should be acknowledged. First, this study is an observational cross-sectional investigation based on a single dry-season sampling campaign at a limited number of sites along the middle-lower Yangtze River mainstream. As such, causal relationships cannot be inferred from our data. The observed patterns are only correlative and consistent with the community disassembly hypothesis, but do not establish

causal links between land use change, environmental filtering, and microbial functional loss. Future controlled experiments and long-term monitoring are needed to validate the underlying mechanisms.

Second, the community disassembly simulations conducted in this study are based on species sensitivity rankings and represent simplified deterministic scenarios. In real ecosystems, species are unlikely to disappear in a strict sequential order due to the complexity of environmental interactions, stochastic processes, and species co-occurrence dynamics. Therefore, our simulation results should be viewed as hypothetical scenarios that generate testable predictions, rather than quantitative forecasts of actual ecosystem collapse. Furthermore, the simulation-derived threshold of approximately 50% species loss is condition-dependent, being specific to the sensitivity ranking and functional prediction framework used in this study. Alternative analytical approaches or parameter settings might yield different numerical thresholds. Nevertheless, the qualitative pattern that non-random species loss leads to accelerated functional decline is likely to remain robust.

Third, rare taxa were defined using a conventional threshold of occurrence in ≤ 3 sites. This subjective cutoff may influence the exact numerical values of species sensitivity rankings. Additionally, functional annotations were derived from PICRUSt2 predictions, which represent inferred metabolic potential rather than directly measured metabolic activities. This introduces inherent uncertainty that may propagate through the analytical workflow.

Fourth, due to logistical constraints during the field campaign, replicate samples were not collected at each site, limiting our ability to quantify within-site community variability. Although we included sampling site as a random intercept in generalized linear mixed models (GLMMs) to account for non-independence of observations, spatial autocorrelation among sites was not fully modeled. Moreover, our sensitivity analysis relied solely on GLMM coefficients as the primary metric, without exploring alternative sensitivity indices or propagating coefficient uncertainties into the disassembly simulations.

Finally, comparisons of network topological features between eukaryotic and prokaryotic communities may be influenced by differences in network size and sequencing depth. In addition, as a single time-point spatial survey, this study does not capture seasonal or interannual temporal dynamics, which may be important for understanding microbial community succession. Future studies with expanded spatiotemporal sampling, replicate sampling, in situ functional measurements, and multi-dimensional analytical approaches are needed to further validate and extend the generality and reliability of our findings.

5. Conclusions

This study reveals patterns consistent with a link between urbanization expansion and changes in aquatic microbial community structure and function in the middle-lower Yangtze River. From midstream to downstream, elevated impervious surfaces raised water TN and phenol concentrations and altered eukaryotic community structure. In highly urbanized downstream areas, both eukaryotic and prokaryotic communities were dominated by nutrient-tolerant taxa; eukaryotes exhibited more complex co-occurrence network structures and higher connectivity, enabling more efficient responses to environmental changes. Eukaryotic and prokaryotic community assembly was dominated by stochastic processes. However, prokaryotes experienced stronger environmental filtering, with lower total β -diversity and higher turnover contribution. Eukaryotes, due to dispersal limitation, had higher total β -diversity and nestedness components. While the presence of redundant taxa can buffer ecosystem stability by elevating functional redundancy under mild environmental disturbance, persistent expansion of impervious surfaces triggers non-random species loss across both eukaryotic and prokaryotic assemblages, where highly sensitive taxa are selectively eliminated. Under such severe urbanization pressure, the buffering

effect of functional redundancy is overwhelmed, suggesting potential threats to ecosystem stability under continued urbanization pressure.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/d18080455/s1>. Table S1: Geographic coordinates of monitoring stations. Figure S1: Relationships between impervious surface area and TN (upper panel), phenols (lower panel) in the middle and lower reaches of the Yangtze River, analyzed using GAM. Figure S2: α -diversity of eukaryotes and prokaryotes in the middle and lower reaches of the Yangtze River. 16S represents prokaryotes, and 18S represents eukaryotes. The first row of violin plots shows differences in diversity indices between the two groups ($*** p < 0.001$, $** p < 0.01$, NS not significant). The second and third rows of bar plots display prokaryotic and eukaryotic diversity indices across individual sites from the middle to lower reaches, respectively, with black lines connecting values across sites. Figure S3: Responses of prokaryotic and eukaryotic community characteristics to environmental factors in the mainstream of the middle-lower Yangtze River (based on RDA) are illustrated as follows: red dots represent species; blue arrows represent environmental variables, with arrow length and direction reflecting explanatory power and association direction, respectively. Figure S4: Relationship between impervious surface area and prokaryotic FRic in the middle and lower reaches of the Yangtze River, analyzed using GAM. Figure S5: Bar plots showing prokaryotic functional diversity indices across individual sites from the middle to lower reaches of the Yangtze River, with black lines connecting values across sites. Figure S6: Sensitivity of prokaryotic species to urbanization assessed by GLMM. Red dots indicate negative response coefficients of species to urbanization, and blue dots indicate positive coefficients. Figure S7: Circular visualization of phylum-level prokaryotic species with different response types to urbanization. The inner ring shows the relative abundance of different phyla; the width of the ribbon corresponding to each phylum is proportional to its relative abundance across sites (blue indicates negatively responsive species, red indicates positively responsive species). Figure S8: Sensitivity of eukaryotic species to urbanization assessed by GLMM. Red dots indicate negative response coefficients of species to urbanization, and blue dots indicate positive coefficients. Figure S9: Circular visualization of phylum-level eukaryotic species with different response types to urbanization. The inner ring shows the relative abundance of different phyla; the width of the ribbon corresponding to each phylum is proportional to its relative abundance across sites (blue indicates negatively responsive species, red indicates positively responsive species). Figure S10: Bar plots showing eukaryotic functional diversity indices across individual sites from the middle to lower reaches of the Yangtze River, with black lines connecting values across sites.

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