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The community patterns and assembly mechanism varied in eukaryotic microorganisms with different cell sizes

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Abstract

Cell size is a fundamental determinant of eukaryotic microorganisms' ecology, influencing community dynamics, physiological processes, and ecological interactions. However, it is still unclear whether eukaryotic microorganisms of different sizes have similar structural and assembly mechanisms. In this study, we sorted eukaryotic microorganisms' cells into two sizes classes (cell size: >0.45 μm and 0.1–0.45 μm) and compared their structural and assembly mechanisms in the river. The results showed that there was no significant difference in the number of reads between two groups. The two size classes harbored distinct eukaryotic microorganisms' communities. And large eukaryotic microorganisms' cells had higher diversity compared to the small eukaryotic microorganisms' cells. Assembly mechanisms of the two size classes were significantly different. We conclude that cell size is an important factor that determines eukaryotic microorganisms' structure and assembly mechanisms. These findings underscore the critical role of cell size in determining eukaryotic microorganisms' community and uncover a direct relationship between microbial morphology and ecological traits.

Introduction

River ecosystems, as important carriers of the global water cycle and material flow, support rich biodiversity and complex ecological functions (Barbosa et al., 2022). In river ecosystems, microbial communities (including bacteria, archaea and eukaryotic microorganisms) are key components that drive material cycling, energy flow and ecosystem stability (Wang et al., 2025). Among them, eukaryotic microorganisms (microbial eukaryotes), including protists (such as flagellates, ciliates, amoebae), microalgae (such as diatoms, green algae) and microfungi, play an irreplaceable role in the food web structure, organic matter decomposition and pollutant degradation of river ecosystems (Barbosa et al., 2022; Sleith and Katz, 2022; Wang et al., 2025).

Eukaryotic microorganisms occupy multiple trophic levels in river ecosystems and serve as both primary producers (such as photosynthetic microalgae), consumers (such as predatory protozoa), and decomposers (such as saprophytic fungi), thereby forming complex nutritional interactions within the river food web (An et al., 2025; Xiong et al., 2025). Therefore, studying the community dynamics of eukaryotic microorganisms is crucial for understanding the material cycling and energy flow in river ecosystems.

The heterogeneity of river environments shapes highly diverse eukaryotic microbial communities (Gu et al., 2024; Okamoto et al., 2022; Sun et al., 2023). Water quality parameters and physical factors may select for more adaptable groups. For instance, low-oxygen environments may enrich anaerobic protists, while high-nutrient conditions may promote certain fast-growing algae (Zhang et al., 2012). Predation (such as ciliates feeding on bacteria), competition (such as the competition among different algae for light), and symbiosis (such as the reciprocal relationship between fungi and algae) may further shape the community structure (Xie et al., 2022). Additionally, in sections of rivers with poor connectivity, the diffusion of eukaryotic microorganisms may be limited, leading to the community composition being

more influenced by random factors (Flegontova et al., 2023). Existing studies have shown that the community composition of river eukaryotic microorganisms typically exhibits significant spatial differentiation: the assembly of microbial communities is usually driven by both deterministic processes and random processes (such as diffusion limitation, ecological drift) (Du et al., 2023; Jin et al., 2024).

In recent years, with the rapid development of high-throughput sequencing technologies and bioinformatics analysis methods, researchers have been able to more deeply analyze the composition, diversity of eukaryotic micro-biological communities and their responses to environmental changes(Ma et al., 2020; Wang et al., 2019; Xie et al., 2022). However, at present, most studies still focus on marine or lake ecosystems, while community ecology research on eukaryotic microorganisms in river ecosystems remains rather fragmented. Therefore, systematically exploring the community structure, functional roles and driving factors of eukaryotic microorganisms in rivers not only helps to improve the theory of aquatic microbial ecology, but also provides a scientific basis for the protection, restoration and sustainable management of river ecosystems.

This study aims to systematically explore the community composition, diversity patterns, and driving mechanisms of eukaryotic microorganisms in rivers, with a focus on addressing the following scientific issues: How does the community structure of eukaryotic microorganisms in rivers change? What are the relative contributions of environmental selection, biological interactions and random processes in community assembly? How do human activities (such as pollution and dam construction) affect the community stability and ecological functions of eukaryotic microorganisms? Through the above research, it is expected to reveal the ecological adaptation strategies of eukaryotic microorganisms in rivers and their functional roles in the ecosystem, providing theoretical support for the protection of river biodiversity and water quality management.

Materials and methods

Water samples

Water samples were collected in December 2024 from a tributary near the Danjiangkou Reservoir. Samples were taken from 10 sites along the river. At each site, 5 L of surface water was collected and transported to the laboratory under low-temperature conditions. The sampling locations are shown in **Figure S1**. **Table S1** shows the geographical coordinates and corresponding water parameters for each site.

Filtration

The water sample was sequentially passed through membrane filters with pore sizes of 0.45 μm to separate small-sized eukaryotic microorganisms (UE) and large-sized bacteria eukaryotic microorganisms (LE). The 0.45 μm Durapore® membrane filter (Merck Millipore, USA) was employed to

retain LE, while the 0.1 μm Durapore® membrane filter (Merck Millipore, USA) was used to capture UE from the 0.45 μm filtrate.

DNA Extraction, PCR Amplification and Illumina sequencing

Total environmental DNA was extracted with the Power Water DNA isolation kit (Omega Bio-Tek, USA). The purity and concentration of DNA were detected by agarose gel electrophoresis. Appropriate amount of the sample was taken into the centrifuge tube and diluted to 1 ng/ul with aseptic water. The V4 region of protist 18S rDNA were amplified using the total environmental DNA. And the V4 region of protist 18S rDNA were amplified using a pair of primers: 528F (5'-GCGGTAATTCCAGCTCCAA-3') and 706R (5'-AATCCRAGAATTTCACCTCT-3'). Each DNA sample was individually PCR-amplified in triplicated 25 ul reactions. Cycling conditions were an initial denaturation step at 95°C for 5 min; 35 cycles of 94°C for 30 s, 53°C for 45 s and 72°C for 1 min; and a final 10-min extension at 72°C. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). PCR products were mixed in equidensity ratios. Then, mixture PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany). For 18S rDNA data, sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina HiSeq2500 platform, and the paired-end reads were generated.

Data Processing

Purified amplicons were combined in equimolar concentrations and subjected to paired-end sequencing on the Illumina PE300 platform (Illumina, San Diego, USA) following the standard procedures provided by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The resulting raw FASTQ files were de-multiplexed with an in-house Perl script and subsequently quality-filtered with fastp (v0.19.6) (Chen et al., 2018). Subsequently, the optimized sequences were grouped into operational taxonomic units (OTUs) using UPARSE 7.1 at a 97% sequence similarity threshold (Edgar, 2013). For each OTU, the most dominant sequence was designated as the representative sequence. The OTU table was further curated by manually removing chloroplast-related sequences across all samples.

Statistical analysis

Statistical analyses were primarily conducted using R software (v4.3.2) (Liu et al., 2024). For network analysis, Spearman’s rank coefficients (q) between all 97%-cutoff OTUs in each sample were calculated pairwise using R package Hmisc (Harrell, 2008). Subsequently, those significant (FDR-adjusted P-value < 0.01) and robust ($q \geq 0.6$) correlations between OTUs were exported using R package igrap. Network visualization was conducted using Gephi. Principal component analysis (PCA) was utilized to visualize the community composition of eukaryotic microorganisms, employing the vegan and ggplot2 packages

(Hu et al., 2023). Additional bioinformatic analyses of the water microbiota were performed using the Majorbio Cloud platform (https://cloud.majorbio.com).

The null model analysis was used to calculate the β nearest taxonomic unit index (βNTI). The deterministic and stochastic processes were classified into five ecological processes based on the Rap-Crick index (RC Bray) values of βNTI and Bray-Curtis. the fraction with βNTI < − 2 is considered as the percentages of homogeneous selection, whereas those with βNRI > 2 as the percentages of heterogeneous selection. The fraction of pairwise comparisons with RC < − 0.95 and |βNRI|<2 is treated as the percentages of homogenizing dispersal, while those with RC > 0.95 and |βNRI|<2 as dispersal limitation. The remains with |βNRI|<2 and |RC|<0.95 represent the percentages of drift (Hu et al., 2023).

Based on the intra-module connectivity (Zi) and inter-module connectivity (Pi) of each node, all nodes were categorized into four categories: network hubs (Zi > 2.5; Pi > 0.62), module hubs (Zi > 2.5; Pi ≤ 0.62), connectors (Zi ≤ 2.5; Pi > 0.62) and peripherals (Zi ≤ 2.5; Pi ≤ 0.62). Among them, network hubs, module hubs, and connectors were considered putative keystone species (Zhang et al., 2024). The neutral model was employed to assess the contribution of neutral processes to community assembly (Woodcock et al., n.d.). This model characterizes the relationship between species abundance and frequency, with a neutral-based assembly process predicted within the model and a niche-based assembly process implied outside the model. Based on the detected ratio of OTUs among all samples, microbial OTUs were assigned into three groups: persistent (detected in > 80% of samples), intermittent (detected in 20–80% of samples), and transient (detected in < 20% of samples) (Liu et al., 2024). Then, frequency and abundance distribution of these OTU groups among SE and LE were counted and compared (Liu et al., 2024). According to the niche breadth value, microbial OTUs were categorized into environmental specialists (< 1.5) or generalists (> 3) (Liu et al., 2024).

Results

Composition of eukaryotic microbial communities

A total of 561,608 reads in LE group and 530,198 reads in SE were identified. Further analysis showed that there was no significant difference between two groups (**Figure S2**). The NMDS plot showed that the pronounced differences in microeukaryotic community composition (at the OTU level) with different cell size (Fig. 1A), which is statistically confirmed by ANOSIM (R = 0.602, *P* = 0.001, **Table S2**). The total number of OTUs in the two groups is 192, and the number of unique OTUs is 101 (LE) and 46 (SE), respectively (Fig. 1B). A total of 339 OTUs were identified across all groups corresponding to 25 phyla (LE: 24, SE: 22), 59 classes (LE: 54, SE: 56), 86 orders (LE: 77, SE: 81), 116 families (LE: 101, SE: 108) and 146 genera (LE: 128, SE: 129) (**Figure S3**). Further analysis of the community composition of the two groups of eukaryotic microorganisms revealed that 21 (76.03%) phyla and 111 (76.03%) genera were shared, 3 (12%) phyla and 17 (11.64%) genera were unique to the LE group, and 1 (4%) phylum and 18 (12.33%) genera were specific to the SE group (**Figure S3**).

As the total number of OTUs increases, the community similarity of the two groups of eukaryotic microorganisms shows a significant upward trend (Fig. 1C). Additionally, the Shannon index and the community dissimilarity between two groups were significantly different (Fig. 1D and 1F). Beta diversity partitioning analysis uncovered that turnover, rather than nestedness, played a more dominant role in community succession in LE and SE communities (Fig. 1E).

At the phylum level, the dominant eukaryotic microbial communities were SAR (LE: 25.62%-82.44%, SE: 2.12%-56.08%), Cryptophyccae (LE: 0.13%-61.10%, SE: 0.33%-17.44%), Ascomycota (LE: 0%-0.04%, SE: 0.19%-13.49%), respectively (Fig. 2A).

The LEfSe analysis results revealed the classification units with significant differences between the two groups. The evolutionary branch tree (Figure B) shows that the enriched groups in the LE group are mainly composed of the Neocallimastigomycota phylum and its subordinate classification units, while the SE group is mainly characterized by the Ascomycota phylum and its included Sordariomycetes, Eurotiales, etc. groups. The differences between the two groups are reflected at multiple classification levels such as phylum, class, order, family, and genus (Fig. 2B). further analysis showed that the relative abundance of Spirotrichea was significantly higher in LE group than that in SE group. Whereas the relative abundance of Cryptophyceae, Malasseziales, Eurotiales, Capnodiales, Hypocreales, Saccharomycetales, Dothideales, Agaricales, Pleosporales were significantly higher in SE group than that in LE group (Fig. 2C). The stability of the abundance of the dominant species in both groups was relatively high, but there were differences in the persistence patterns of the species. At the abundance level, the proportion of persistent species in the LE group and the SE group was 88.38% and 88.62% respectively, indicating that the dominant species in both groups were mainly stable groups. At the species quantity level, the intermittent species in the LE group accounted for the highest proportion, reaching 65.63%, while the persistent species accounted for only 18.01%; while in the SE group, the proportion of persistent species was 26.01%, significantly higher than that of the LE group, suggesting that the core species diversity of the SE group was higher and the stability of the community structure was better (Fig. 2D).

The assembly process of eukaryotic microbial communities

In the present study, a significant difference of beta deviation index was found between LE and SE group (Fig. 3A). Niche width analysis showed that the SE group had a narrower niche width than the LE group (Fig. 3B). A significant difference in niche overlap among species was also observed between the LE and SE group (Fig. 3C). Additionally, all species were subsequently categorized into generalist and specialist groups (Figs. 3D). The proportions of generalist, neutralists and specialist species in the LE group were 87.85%, 6.16% and 5.99%, respectively, whereas in the SE group, these proportions were 93.37%, 3.52% and 3.11%, respectively (Fig. 3E).

A null model was used to explore the community assembly mechanisms of the LE and SE group (Fig. 4A). The results revealed that homogeneous selection (63.08%) was the dominant process,

followed by drift (22.65%) and dispersal limitation (9.16%) in the LE group. In contrast, drift (66.36%) was the dominant process in the SE group, followed by dispersal limitation (18.11%) and homogeneous selection (12.69%). All the relative importance of different ecological processes in the two groups were significantly different (Fig. 4B). Overall, stochastic processes primarily shaped the LE and SE group (Fig. 4C). The neutral community model (NCM) analysis further showed that species dispersal was higher in the LE group (m = 0.0755; Fig. 4D) than in the SE group (m = 0.0036; Fig. 4E).

The co-occurrence pattern of eukaryotic microbial communities

In the present study, LE group network contains 149 nodes (species) and 943 edges (associations), among which the proportion of positive correlations is 74% and the proportion of negative correlations is 26%. The SE group network consists of 147 nodes and 708 edges, with a positive correlation ratio of 82% and a negative correlation ratio of 18% (Figs. 5A and 5B). Compared with the LE group, the SE group had fewer network edges and a higher proportion of positive correlations, suggesting that the interspecies associations in the LE group were more complex and the proportion of negative interactions was higher. In the SE group, positive interactions among species were dominant, and the network connections were sparser.

The statistical test results of key topological parameters showed that there were significant differences in the structural characteristics of the two networks ($P < 0.05$) (Figs. 5): the node connectivity (Degree), degree centrality (Degree Centrality), and closeness centrality (Closeness Centrality) of the LE group were significantly higher than those of the SE group, indicating that the majority of species in the LE group had higher connectivity and more direct connections within the network; while the betweenness centrality of the SE group was significantly higher than that of the LE group, suggesting that a few species in the SE group had stronger "bridging" roles and the core nodes of the network were more concentrated.

Based on Zi-Pi analysis, the key species in the two groups were identified (Figs. 5C and 5D): the LE group identified 3 network hub species and 1 module hub species, while the majority of the remaining species were peripheral species; the SE group identified 7 connector species and 1 network hub species, with no module hub species, and the proportion of peripheral species was higher. This result indicates that there are more global hub species in the LE group, which have a stronger role in maintaining the overall network structure; while the key species in the SE group are mainly connectors between modules, the network structure is more dependent on weak connections between modules, and the stability of the community may be more susceptible to changes in key nodes.

Drivers of eukaryotic microbial communities

In the present study, we found that the community of SE group was significantly correlated with the environmental factors including TC, TN and TP (Fig. 6A). And no environmental factors were significantly correlated with the community of LE group (Fig. 6A). At the genus level, the relative abundance of the

taxa such as *strombidium* in LE group and *Malassezia* in SE group were significantly corrected with environmental factors including TC, TN and TP (Fig. 6B and 6C). Further analysis showed that the higher contribution of environmental factors to the distribution of LE community were T, pH, TN, TP (Fig. 6D); Whereas higher contribution of environmental factors to the distribution of SE community were TOC, DO, pH, TC (Fig. 6E).

Discussion

The cell size of eukaryotic microorganisms (such as algae, protozoa, and fungi) is a core characterization of their fundamental life history strategies (Zhang et al., 2024), which is directly related to energy acquisition and metabolic rate (Andersen et al., 2016; Blanchard et al., 2017), environmental adaptability and interspecific interaction (Brose et al., 2017), and thereby shapes community structure through multi-dimensional mechanisms. Previous study reported that eukaryotic plankton of different particle size ranges have different species compositions, ecological functions and community assembly mechanisms (Worden et al., 2015). Several studies have characterized the distribution of eukaryotic microorganisms of different sizes in water bodies (De Vargas et al., 2015). For example, the microbial eukaryotic plankton communities within different sizes (0.2–3 and 3–200 μm) had significant differences in their community composition (Ma et al., 2022), which was found that the small and the large size fractions of the eukaryotic microorganism's community differed markedly in taxonomic structure in the present study. This size-dependent effect is commonly reported in the bacteria in freshwater and marine ecosystems (Hu et al., 2023; Liu et al., 2025, 2016). Additionally, in this study, we also found that a greater OTU overlap between LE and SE groups, indicating that different groups of eukaryotic microorganisms can also undergo dynamic transformations. Similar phenomena were reported in the previous study that the variability in community dissimilarity between bacterial size fractions suggests a dynamic exchange between the two communities (Hu et al., 2023).

The diversity in SE group was significantly higher than LE group, which was reported in the previous study (Blais et al., 2024). However, it contrasts to the bacteria, the diversity of bacteria with larger cell size was significantly higher than the bacteria with small cell size. Such patterns are likely to vary greatly among eukaryotic microorganisms and prokaryotic microorganisms, given that size fractions in eukaryotes represent mostly taxonomic differences in cell size and associated environmental preferences. The results indicated that in the smaller cell size groups, there are more diverse species of eukaryotic microorganisms, future research will need to pay closer attention to this.

In the present study, we found that the ecological niche width of LE was significantly greater than that of SE, indicating higher metabolic plasticity in the LE community (Malard et al., 2022). Based on the ecological niche breadth and distribution, eukaryotes communities were classified into generalists and specialists: generalists occur across diverse habitats and possess broad ecological niches, whereas specialists are restricted to specific habitats due to their narrow environmental tolerance (Li et al., 2023). In the present study, the SE community exhibited a higher proportion of generalists and a lower proportion of specialists compared with LE in terms of relative abundance, indicating that the degree of

ecological variability in the LE community during the succession stage was greater than that of SE community.

The community construction mechanism is one of the cores of ecological research (Nemergut et al., 2013). In this study, we found that homogeneous selection (63.08%) was the dominant process in the LE group and drift (66.36%) was the dominant process in the SE group (Fig. 4B). All the relative importance of different ecological processes in the two groups were significantly different (Fig. 4B). Similar phenomena have also been observed in bacterial community assembly. For example, the assembly processes of small-sized and large-sized bacterial communities differ in marine ecosystems (Hu et al., 2023). A study based on the distance-decay model also confirmed the role of cell size (Astorga et al., 2012), and dispersal limitation may gradually become more powerful in explaining community variation with the increase in cell size (Zhang et al., 2018), which was in line with our study (Fig. 4B). And larger plankton may undergo more environmental filtration and have lower plasticization, while smaller plankton have a stronger diffusion capacity (Li et al., 2023).

These results indicated that cell size exerts a significant impact on microbial community assembly. Further analysis showed that the assembly process of LE and SE communities were dominated by stochastic processes (Fig. 4C). Despite limited research on the assembly of eukaryotic microbial communities with different cell sizes, the results in the previous study have supported the predominant role of stochastic processes in shaping eukaryotic community assembly (Chen et al., 2019; Mo et al., 2021).

Eukaryotic plankton are indicator species of environmental changes in the environment and are crucial for maintaining the stability of aquatic ecosystems. In this study, we found that the SE group had a stronger correlation with TC, TN, and TP than other environmental factors, such T, pH, DO and TOC, which may be attributed to the fact that these environmental factors could directly influence the growth and reproduction of LE communities by regulating their substrate utilization and metabolic processes (Ji et al., 2025). Those results indicated that nitrogen and phosphorus nutrients are the key environmental factors driving the differentiation of microbial community structure in water environments. The results were consistent with the research laws of microbial ecology in most freshwater and landscape water bodies (Chen et al., 2019). Total nitrogen and total phosphorus, as core nutrient indicators of water bodies, directly regulate the available resource levels of microorganisms (Hu et al., 2023). Through environmental screening, they change the growth rates, niche adaptability and competitiveness of different groups, thereby leading to directional succession of community composition.

Conclusion

This study systematically analyzed the composition differences and construction mechanisms of eukaryotic microbial communities of different particle sizes, and clarified that particle size is a key trait regulating community structure and ecological processes. The research showed that the communities of different cell sizes exhibit significant differentiation in terms of α diversity and community composition.

The large-sized eukaryotic microorganisms have lower diversity and broader ecological niches than small-sized group. The assembly process of large-sized and small-sized eukaryotic microorganisms' communities were dominated by stochastic processes. As the cell size decreases, the contributions of drift and dispersal limitation increase significantly, while the contributions of homogeneous selection and heterogeneous selection decrease significantly. In conclusion, the size differentiation that drives the ecological niche differentiation and construction process of eukaryotic microorganisms is an important dimension for understanding the structure of micro-food webs, biogeochemical cycles, and community stability. The study provides a unified framework for the assembly mechanism of different-sized eukaryotic microbial communities, and also offers theoretical support from the size perspective for resource protection and microbial community regulation.

Declarations

Author Contribution

W.H.: Conceptualization, experiment operation, data curation, formal analysis, writing-original draft, visualization,funding acquisition. Y.Q.: data validation. Z.Z., X. Z.: figure plotting. X.L., K. Z.: result interpretation. P.Y.: Supervision, Z. Y., H. H.: manuscript revision.All authors have read and agreed to the published version of the manuscript.

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Figures

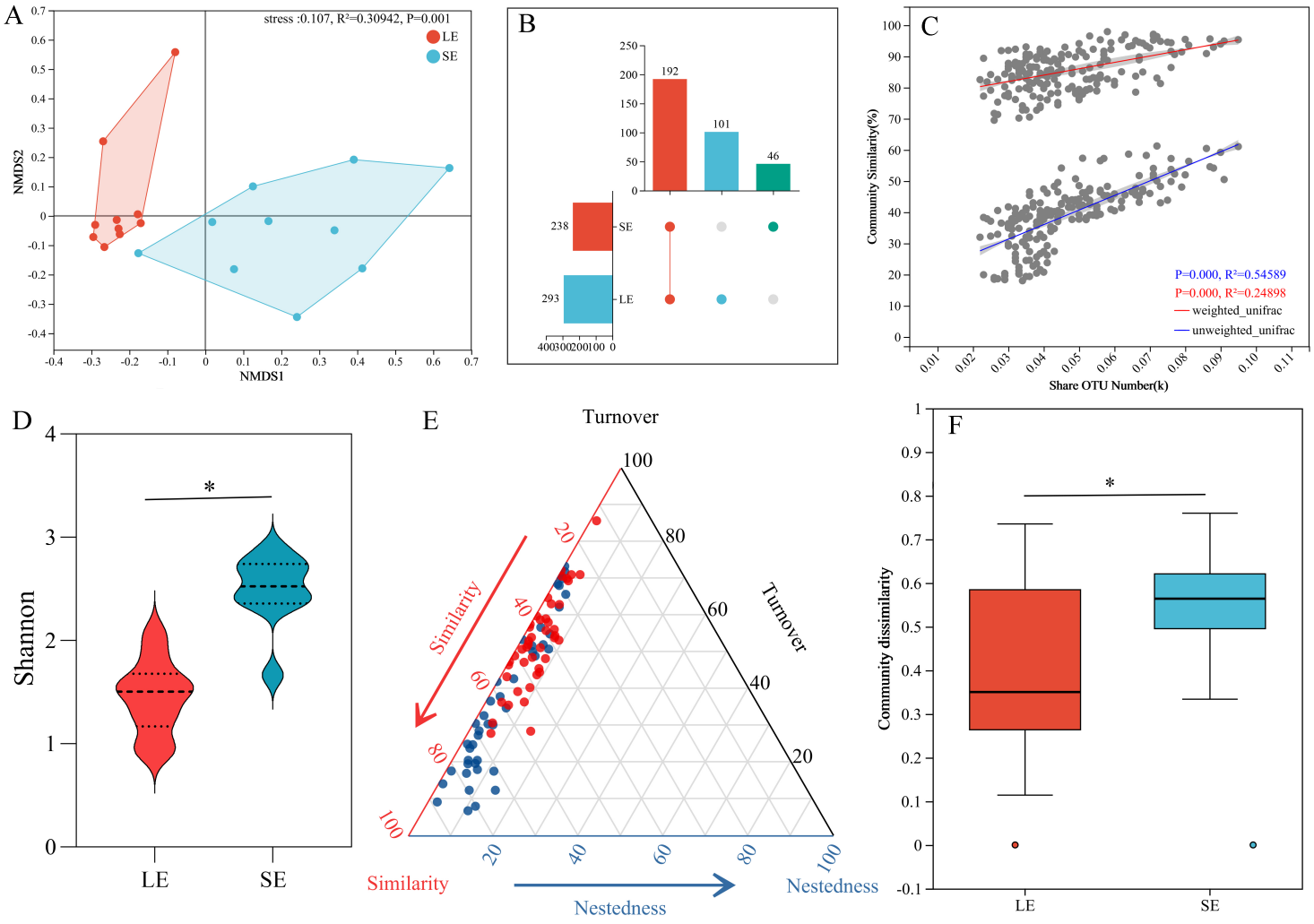


Figure 1

Principal component analysis showing the distribution pattern of bacterial community (A); The number of unique and common species in UB and LB (B); Trend analysis of similarity in common species communities based on the Bray-Curtis distance matrix (C); Analysis of Shannon index differences between different groups (D); Demonstrate the main contributions of the similarity of beta diversity communities in different groups and the two components of decomposition (turnover and nnesyedness) to the changes in community ecological processes (E); Analysis of community dissimilarity differences between different groups (F);

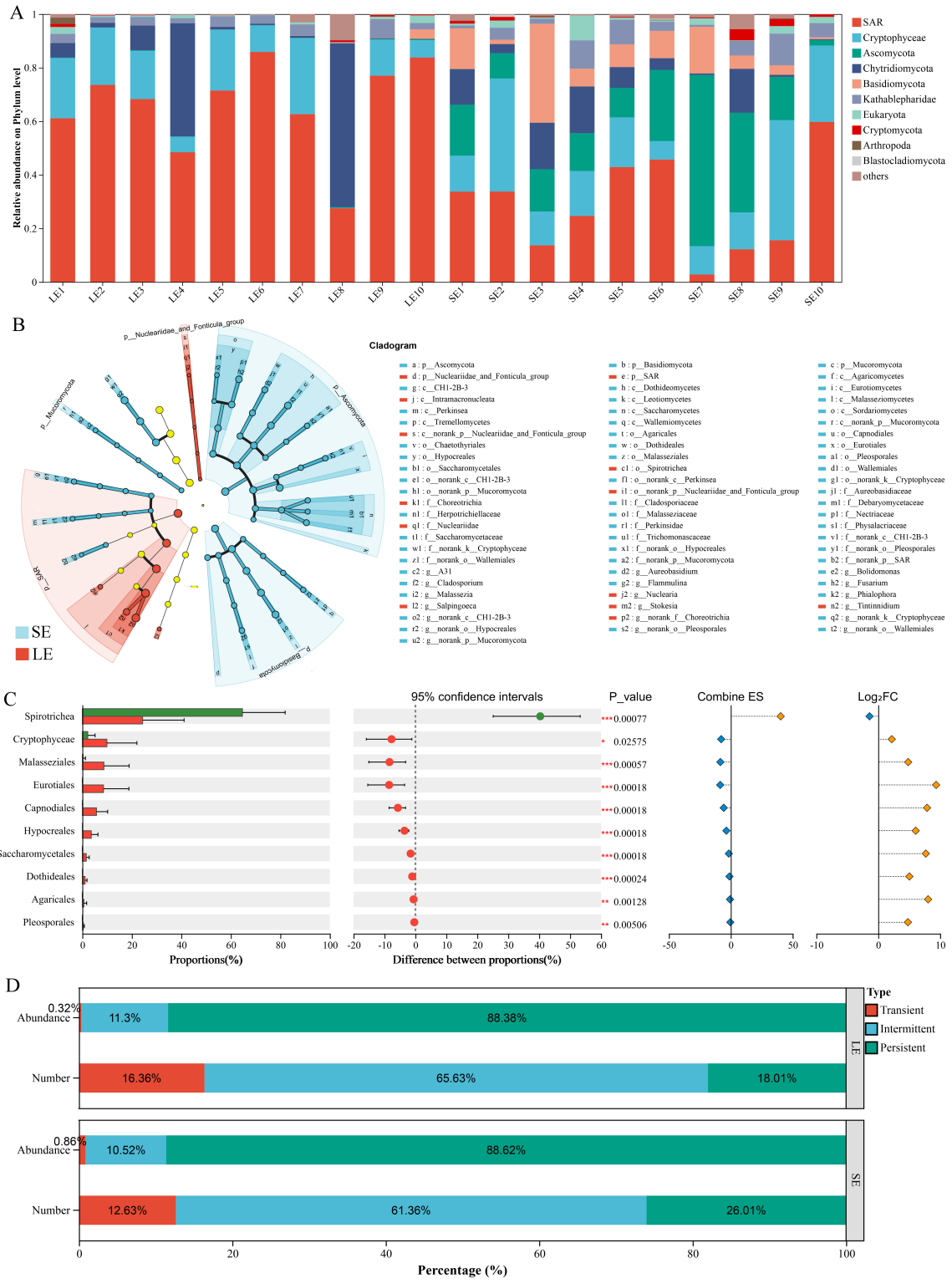


Figure 2

The composition, diverse species and persistent patterns of eukaryotic microbial communities; Relative abundance of eukaryotic microbial communities at the phylum level(A); LEfSe differential species evolutionary tree(B); the bar chart shows the average relative abundance differences of the same species among different groups(C); Persistence pattern of fungal species (abundance and proportion of quantity) (D).

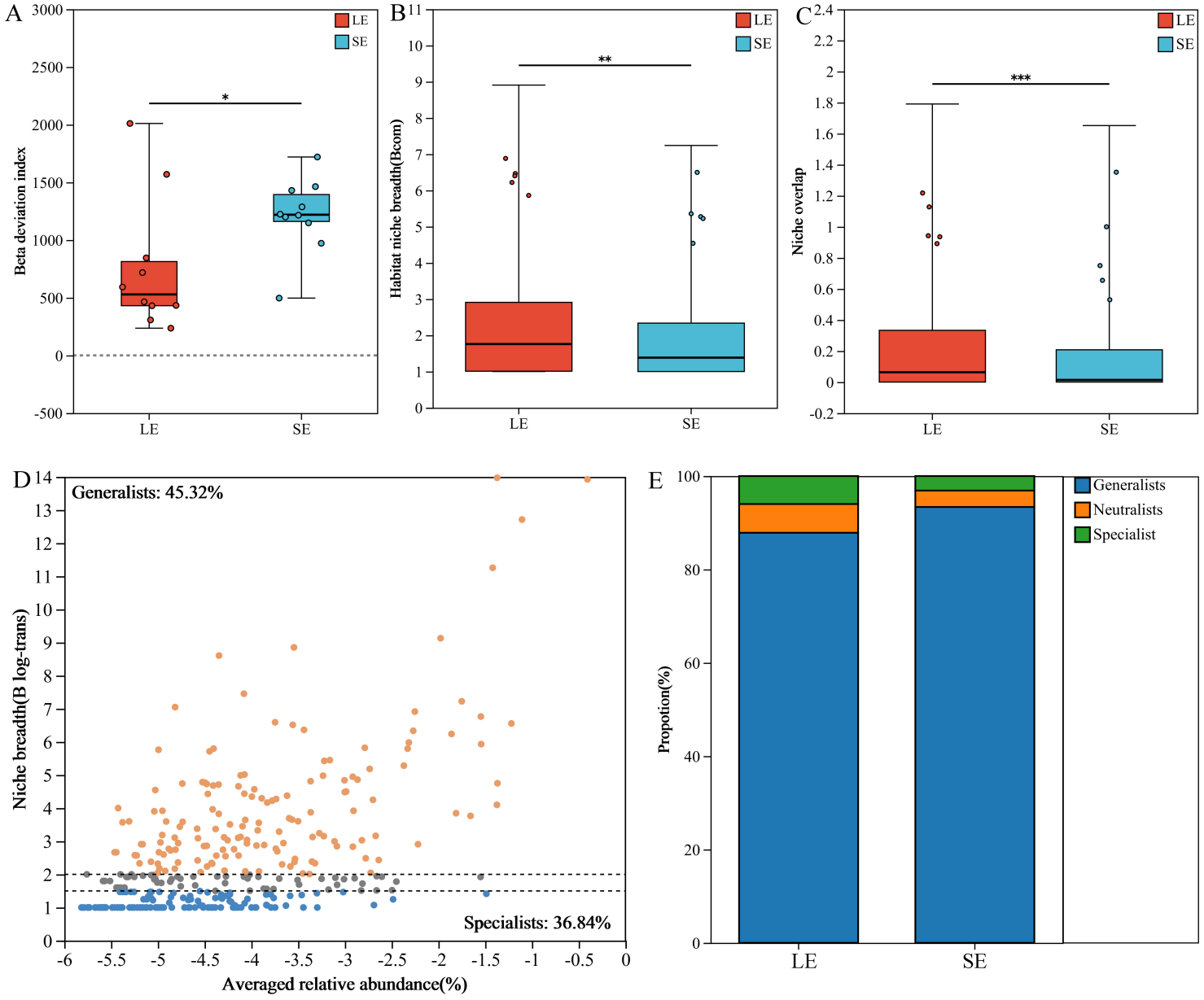


Figure 3

The beta deviation index (A), habitat breath (B) and niche overlap (C) of the bacterial community. The average relative abundance ratio (Abundance) and detected quantity ratio (Number) of the three types (transient, intermediate and persistent) of all group (D), UB and LB (E).

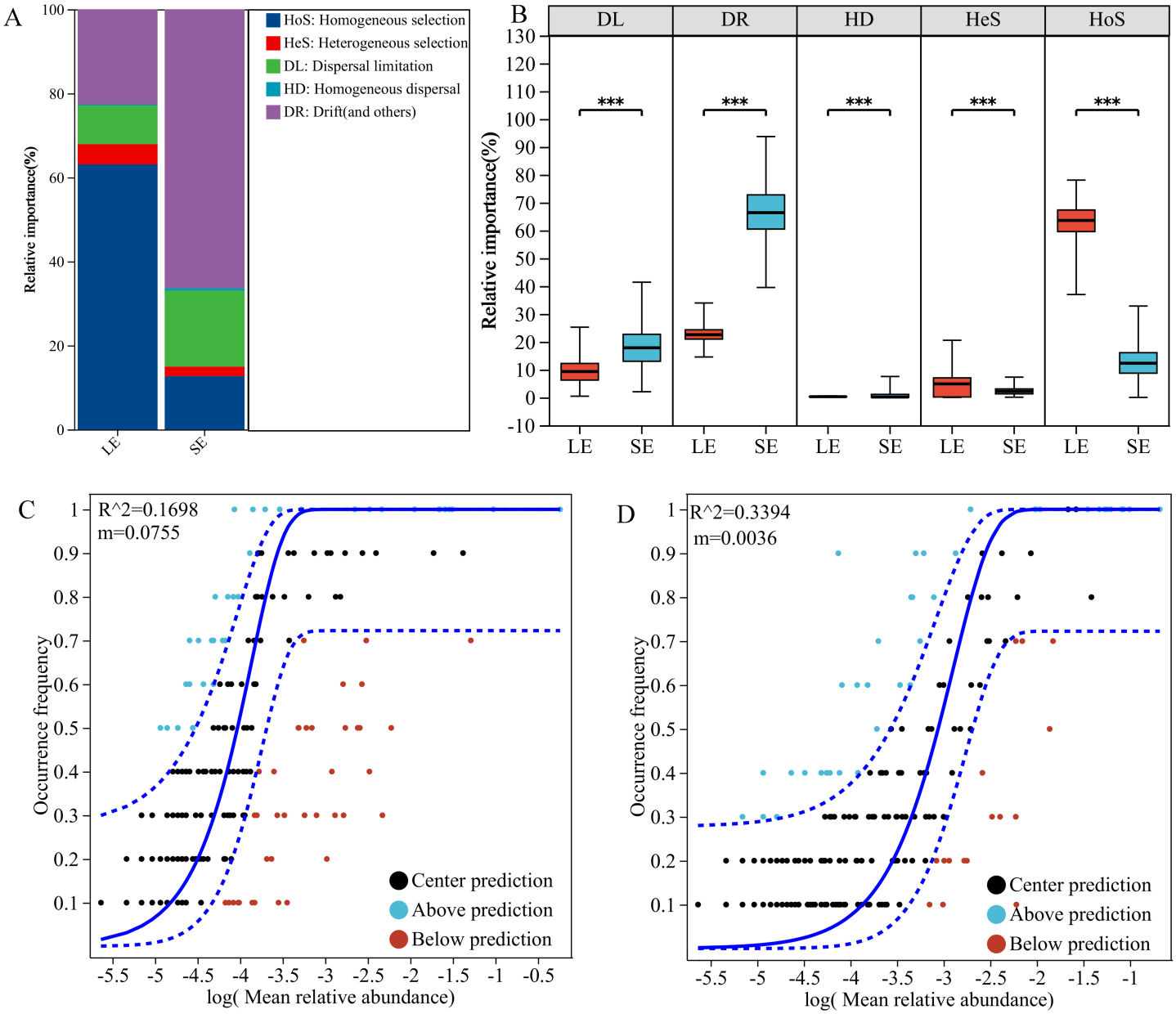


Figure 4

The assembly mechanism of UB and LB communities (A and B). Neutral models evaluating the role of neutral processes in community assembly (C and D).

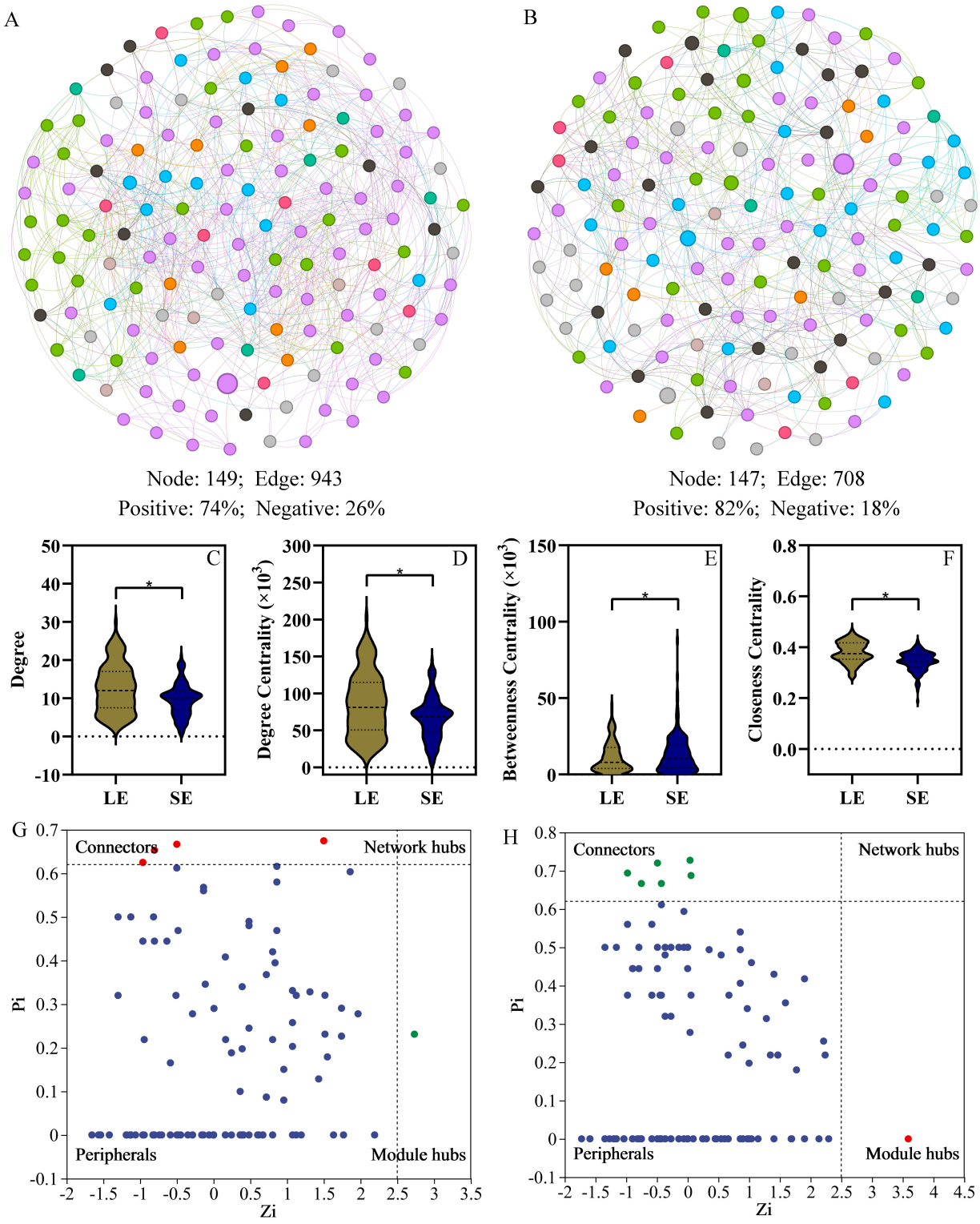


Figure 5

Co-occurrence network analysis of bacterial communities of different cell sizes. Network visualization depicting microbial interactions within different bacterial size fractions (A: LE, B: SE). Comparison of node-level topological properties across bacterial size fractions (C, D, E and F). Zi-Pi plots show the distribution of OTUs based on their topological roles in networks (G: LE, H: SE).

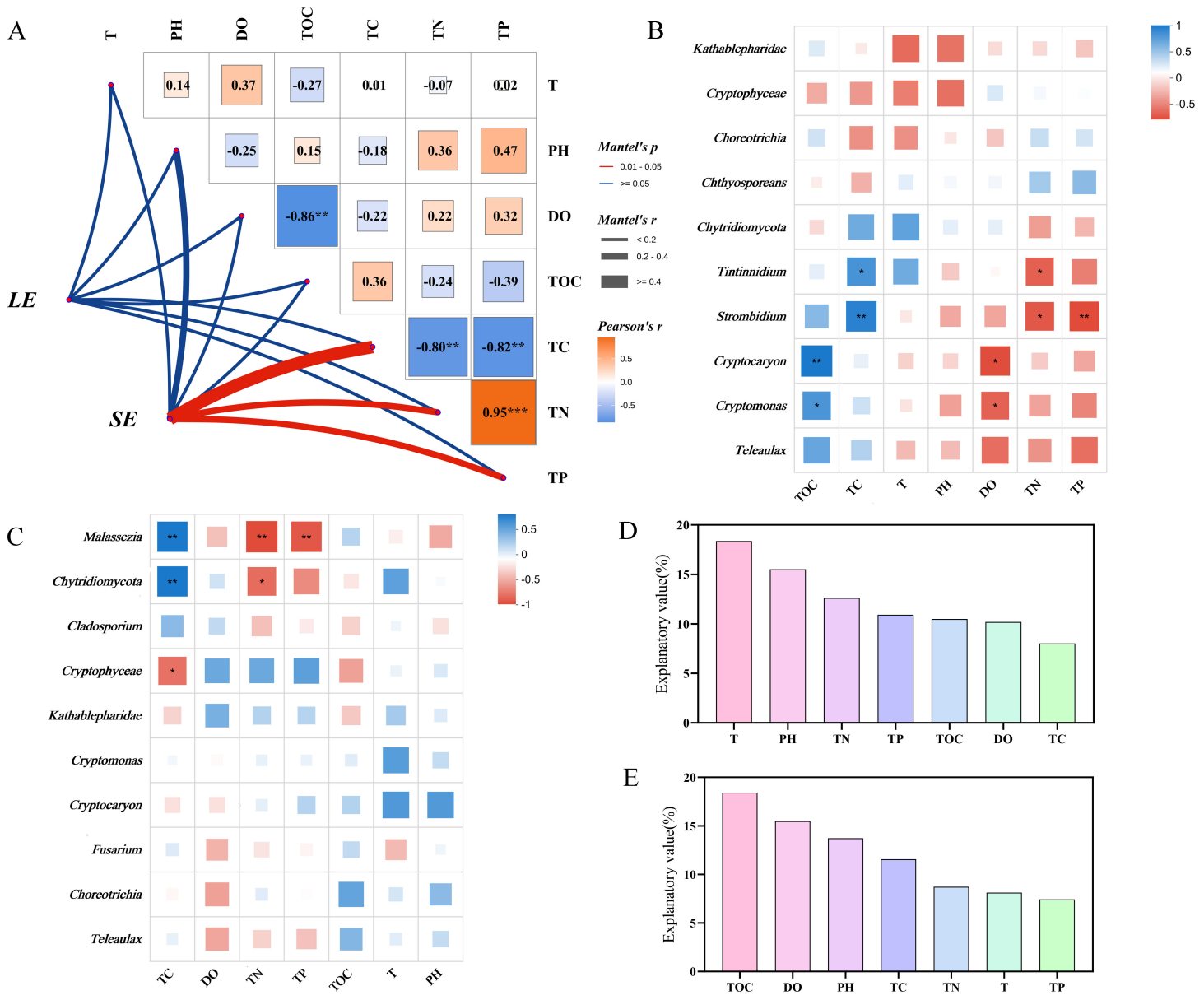


Figure 6

Influence of environmental factors variables on bacterial communities of different cell sizes. Mantel test results illustrate the correlation between environmental factors and microbial community structure (A). Relationships between bacterial taxa and environmental factors (B: LE, C: SE). Contribution of environmental factors to the distribution of bacterial communities of different cell sizes (D: LE, E: SE).

Supplementary Files

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- SupplementaryMaterials.docx