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RESEARCH ARTICLE

Hydrological variability and connectivity shape floodplain microbial community dynamics

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Abstract

Floodplains are dynamic interfaces between aquatic and terrestrial ecosystems, where ecosystem functioning is strongly influenced by microbial communities. To investigate the composition of free-living and particle-associated prokaryotic and microbial eukaryotic communities, five interconnected study sites were sampled in one of the best-preserved Danube floodplains and subsequently analyzed using 16S and 18S rRNA gene amplicon sequencing. We compared community dynamics across low-water periods and minor to moderate floods and observed flooding to increase microbial diversity and promote gradual community shifts depending on flood intensity, whereas low-water conditions limited microbial exchange and reduced compositional connectivity across floodplain ecosystems. Dispersal effects were particularly pronounced in microbial eukaryotes, including Perkinsea and Fungi, pointing to the importance of hydrological connectivity in structuring micro-eukaryotic communities. Flooding also facilitated community mixing and more balanced interspecific interactions, while low-water periods led to more compartmentalized networks. Core microbial community size increased with flooding intensity, reflecting the influence of ecosystem mixing, allochthonous inputs, and increased nutrient availability in shaping floodplain communities. This study highlights the effects of flooding intensity on both prokaryotic and microbial eukaryotic communities, advancing our understanding of how hydrological variability shapes microbial dynamics in riverine floodplains.

Floodplains are dynamic and complex landscapes that integrate aquatic and terrestrial ecosystems, and provide essential geomorphic, ecological, and hydrological functions that enhance habitat diversity, support biodiversity, and regulate sediment dynamics (Junk et al. 1989; Preiner et al. 2018). Floodplains also provide essential ecosystem services to humans, including flood peak reduction, water quality improvement, carbon sequestration, groundwater recharge, fishery, all of which contribute to the resilience and economic value of riverine systems (Opperman et al. 2013). They host high biodiversity, associated with high spatial and temporal heterogeneity (Junk et al. 1989). However, understanding the mechanisms that influence microbial distribution in floodplains is challenging, since local environmental conditions and habitat connectivity are strongly influenced by hydrological regimes and vary continuously (Chaparro et al. 2018). In general, floodplains are periodically inundated by the lateral overflow of the main river, resulting in the exchange of water, sediment, nutrients, organic matter and aquatic organisms (Ward and Stanford 1995). Floods connect aquatic biotopes with distinct hydrological characteristics, which reduces spatial variability and leads to habitat homogenization (Thomaz

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et al. 2007). However, during low-water periods, floodplain habitats remain isolated from each other and from the main river, and develop specific environmental characteristics and species assemblages (Junk and Wantzen 2004).

Both free-living (FL) and particle-associated (PA) microorganisms are integral components of aquatic microbial communities, and play an essential role in the functioning of floodplain ecosystems. As major primary producers, cyanobacteria and microalgae play an essential role in biogeochemical transformations by sequestering CO_2, serving as a biomass reservoir which supports the heterotrophic microorganisms of the microbial loop, and by facilitating nutrient uptake and transfer (Lobus and Kulikovskiy 2023). Their composition and dynamics depend on various abiotic factors such as temperature, pH, light, nutrient and oxygen availability (Law 2011; Mihaljević et al. 2013). Hydrological variability in temperate floodplains is crucial for microbial diversity, as it influences nutrient distribution and niche availability (Mihaljević et al. 2015). Flooding has been reported to have a seasonal effect on phytoplankton dynamics: while it can stimulate ecosystem processes in early spring, it disrupts phytoplankton proliferation in early summer due to nutrient dilution (Mihaljević et al. 2009). Primary sources of organic matter within the dynamic river-floodplain system are phytoplankton biomass, standing riparian biomass, detrital biomass, soil organic carbon, litter and humus (Sutfin et al. 2016). Flood pulses affect the availability and quality of organic matter that regulate bacterial heterotrophic metabolism (Vidal et al. 2015), while low-water periods limit connectivity to inflows and reduce organic matter turnover which can constrain microbial activity and lower abundance (Boot et al. 2013). This interplay contributes to the development of a seasonal microbial community and the coalescence of floodplain microbial communities, leading to potentially highly complex and dynamic microbial assemblages. Microscopy-based research has shown that floods in the Danube shape microbial food webs by increasing the abundance and biomass of bacterioplankton during floods, while bacterial cell size is primarily influenced by biotic interactions, such as grazing, rather than hydrological fluctuations (Luef et al. 2007; Palijan 2012). However, high-throughput amplicon sequencing provides complementary insights by detecting morphologically indistinguishable taxa, capturing the rare biosphere, and offering higher taxonomic resolution and functional interpretation. This approach allows us to address microbial responses to seasonal flooding that remain inaccessible to microscopy-based studies.

Here, we assess how hydrological conditions shape the compositional stability and connectivity of FL and PA prokaryotes and microbial eukaryotes (ME) in the Danube floodplain. We used monitoring and high-throughput sequencing across a river-fed network of interconnected lakes and channels to understand the roles of flood intensity and low-water phases in structuring microbial communities. We hypothesize that flooding enhances microbial exchange and diversity, while low-water conditions limit dispersal and reduce compositional connectivity across floodplain ecosystems. By identifying key environmental drivers and determining core microbial assemblages as responses to changing hydrology, this study furthers the understanding of microbial communities in temperate floodplains and their adaptation and resilience in response to hydrological changes.

Materials and methods

Study sites and sampling procedure

The Kopački Rit (Fig. 1) floodplain on the Middle Danube (Croatia) is characterized by seasonal fluctuations in water flow mainly influenced by the Danube and to a lesser extent by the Drava (Bonacci et al. 2002). The floodplain covers an inundation area of more than 18 km^2 and includes various permanent and seasonal water bodies such as channels and lakes. Flooding usually occurs in spring and early summer (February–May) when the Danube water level rises above 3 m (Mihaljević et al. 1999). In this study, the hydrological conditions are defined by the water level (WL) of the Danube: low-water periods (< 3 m), minor flooding (3.0–3.5 m), moderate flooding (3.5–4.0 m), major flooding (4.0–5.0 m), and extremely high flooding (> 5 m). Minor floods inundate ~ 18% of the floodplain, while extremely high floods (> 5 m) inundate almost the entire area (Schwarz 2005).

Five interconnected water bodies (Fig. 1a) were sampled monthly from February to September 2021 to study changes in floodplain microbial communities in relation to the local hydrological regime. The first site, Danube River (1, at 1388 r. km) supplies the floodplain with overflow water channeled through the Hulovo Channel (2), ultimately reaching the permanent Lake Kopačko (3). The Hulovo Channel is also connected to the Čonakut Channel (4), which carries the overflow water further into the floodplain and flows into the eutrophic/hypertrophic Lake Sakadaš (5) (Mihaljević et al. 2015). Daily Danube water level data (Fig. 1b) were obtained from the gauge station at 1401.4 r. km. No extremely high flooding events were captured during the research period.

Water temperature (WT), pH, dissolved oxygen concentration (DO), and conductivity (Cond) were measured at each station using a multimeter (HQ4300, Hach, US). The water depth (WD) was determined with a handheld depth sounder (Uwitec GmbH, Austria) and the water transparency with a Secchi disk (SD). One liter of subsurface water was collected to analyze nutrient and chlorophyll concentrations, and total suspended solids (TSS). An additional 2 L of water were collected in sterile polycarbonate (PC) bottles from channels and the Danube 20 cm below the surface, while composite samples, comprising the entire water column, were taken from the two lakes (Uwitec GmbH, Austria). Sterile filtration equipment was used to sequentially filter the collected water until saturation through 10 and 0.2 μm PC filters (Whatman Nuclepore

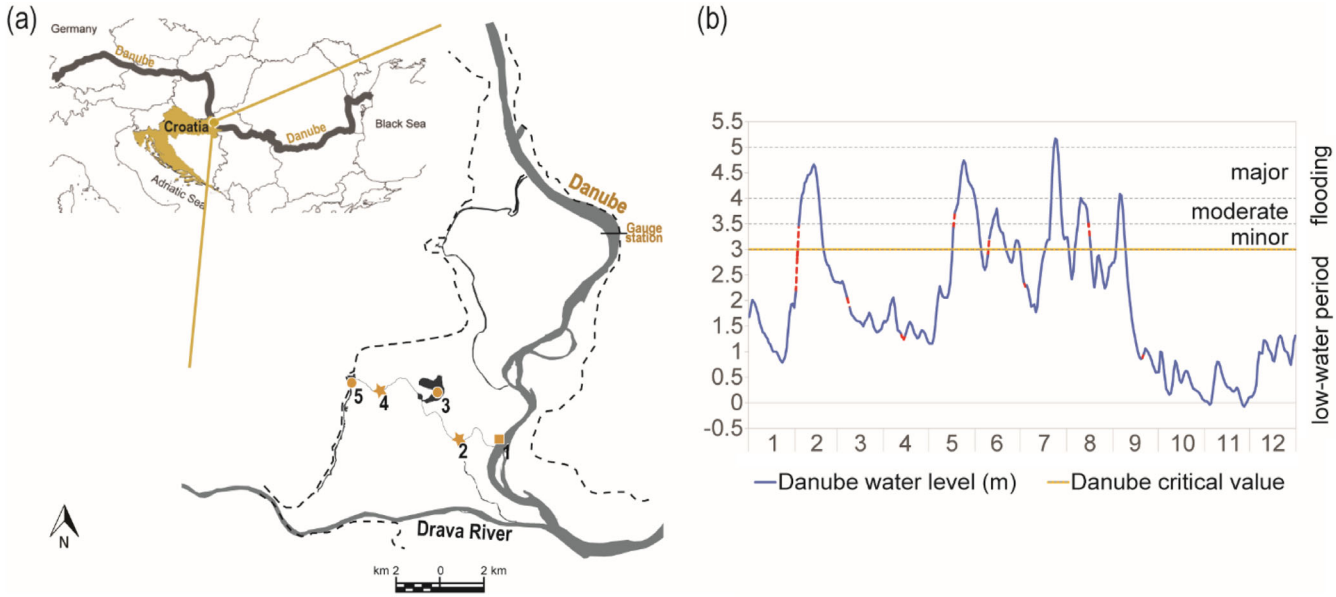

Fig. 1. Study site—Kopački Rit Nature Park (Croatia): (**a**) Locations of sampling points (1—Danube, 2—Hulovo Channel, 3—Lake Kopačko, 4—Čonakut Channel, 5—Lake Sakadaš). The numbering illustrates the spatial direction of flooding; shapes correspond to ecosystem types: river (rectangle), channels (star), and lakes (circle). (**b**) Daily changes in the Danube water level in 2021 at the gauge station. The dashed red line indicates monthly sampling. The line labeled "Danube critical value" delineates the low-water period from the flooding phase.

Track-Etch Membrane, 47 mm diameter) using a vacuum pump (Rocker 811, Rocker Scientific Co., Ltd., Taiwan). The filters were stored at −80°C until further analysis.

Water analysis and analytical procedures

Ammonium (NH_4-N; HRN ISO 7150-1:1998), nitrites (NO_2-N; HRN EN 26777:1998), nitrates (NO_3-N; HRN ISO 7890-3:1998), total nitrogen (TN; HRN ISO 5663:2001 + [NO_2-N + NO_3-N]), and total phosphorus (TP; HRN EN ISO 6878:2008) concentrations were determined. Total suspended solids and its content of inorganic (ash weight, AW) and organic (ash-free dry weight, AFDW) matter were determined following the APHA (1992) procedure.

Chlorophyll concentrations were determined by filtering 0.5–1 L water samples through Whatman GF/F glass microfiber filters, homogenizing the filters with 90% acetone, and extracting the pigments by incubating the filters at 4°C for 24 h. The pigment concentrations were then measured spectrophotometrically (S-200, BOECO Germany) following SCOR-UNESCO (1966) and Strickland and Parsons (1972) protocols.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from filters using the DNeasy Power Water Kit (Qiagen, Germany), adhering to the manufacturer's protocols. The hypervariable V4 region of the 16S rRNA gene was amplified by PCR using the primer pair 515F (Parada et al. 2015) and 806R (Apprill et al. 2015), which specifically target bacterial and archaeal taxa. The V4 region of the 18S rRNA gene was amplified for the microbial eukaryotic dataset using the primers TAReuk454FWD1 and TAReukREV3 (Stoeck et al. 2010). PCR conditions, DNA quantification, and gel electrophoresis were conducted following the protocols in Selak et al. (2022). The resulting amplicons were barcoded, pooled, library prepped and sequenced in a paired-end format (2 × 300 bp) on the MiSeq platform (V3 chemistry, 600 cycles, Illumina, San Diego, CA, USA) at the Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna (JMF ID-2201-10). Raw sequence data were extracted, trimmed and quality filtered as detailed in Pjevac et al. (2021). Amplicon sequence variants (ASVs) were inferred following the standard protocol implemented in the DADA2 R package, version 1.20.0 (Callahan et al. 2016). Taxonomy was assigned via the SILVA database (Ref NR 99 release 138.1) and Protist Ribosomal Reference (PR^2) database (v.4.12.0) using the SINA version 1.6.1 classifier (Pruesse et al. 2012). The nucleotide sequencing data are available in the NCBI repository under BioProject accession number PRJNA1238263.

Statistical analysis

Data filtration, statistical analysis, and visualization were performed in R (v.4.3.2) using phyloseq 1.46.0 (McMurdie and Holmes 2013), vegan 2.6-4 (Oksanen et al. 2022), microbiome 1.24.0 (Lahti and Shetty 2017), ampvis2 2.8.7 (Andersen et al. 2018), and ggplot2 2.8.7 packages (Wickham 2016).

A total of 160 floodplain samples were sequenced, comprising 80 from the prokaryote dataset and 80 from the ME dataset. Each dataset contained 40 samples belonging to the

FL and 40 to the PA fraction. Within each of the four resulting subsets, 5 samples corresponded to moderate flooding, 13 to minor flooding, and 22 to low-water conditions. Samples with < 1000 reads were removed, resulting in 158 samples used for the downstream analysis. Singleton and doubleton ASVs were removed prior to statistical analysis, except for alpha diversity analyses. Amplicon sequence variants unclassified at the Phylum (prokaryotes) or Supergroup (ME) level were excluded from further analyses. Prokaryote datasets were further filtered to exclude mitochondria and chloroplast ASVs, and ME datasets to exclude Metazoa. The filtered datasets contained 2468 (FL ME), 2577 (PA ME), 3565 (FL prokaryotes), and 3644 (PA prokaryotes) ASVs.

"Core" microbiome was defined based on 2 metadata factors: ecosystem (channel, lake, river) and hydrological condition (low-water, minor flooding, moderate flooding). A "group" refers to one combination of these two factors (e.g., channel-low water, lake-moderate flooding). To account for variable library sizes, an ASV was considered present if its relative abundance was ≥ 0.1% or its read count was ≥ 5. Within each group, core taxa were those with prevalence ≥ 0.6 and occurring in at least two samples. A prevalence confidence filter was applied to avoid overestimating cores in small groups: for groups with $n \geq 5$ samples, the lower 95% Wilson confidence bound on prevalence had to be ≥ 0.6; for $n < 5$, this filter was skipped. Equal-n bootstrapping was applied because group sample sizes differed. For each analysis, the number of samples per group was set to the minimum available (≥ 2 samples), with 200 iterations without replacement (seed = 1). All rules were applied per replicate. Core size was defined as the mean number of core ASVs per group across bootstrap replicates, with 95% confidence intervals (CI). Core coverage measured the dominance of core taxa within each group. For each replicate, coverage was the fraction of reads assigned to a group's core taxa per sample, summarized by the sample median, then reported the bootstrap mean of these medians with 95% CI. For each hydrological condition, ecosystem-specific cores using Jaccard similarity were compared and averaged across bootstraps. Comparisons with < 2 samples in any ecosystem were excluded (reported as NA). Shared core taxa were identified for each group by their mean relative abundance (averaged across ecosystems) and visualized at their lowest assigned taxonomic level.

The datasets containing singletons and doubletons were rarefied to the smallest library size (~ 1500 reads) and used to estimate alpha diversity using ASV richness and the Shannon–Wiener diversity index (Shannon 1948). An ANOVA test with a post-hoc Tukey test was applied for normally distributed datasets, while Kruskal–Wallis and Wilcoxon tests were used for non-normally distributed datasets to test for the significance of differences in alpha diversity of FL and PA prokaryote and ME communities between sites. As sites were repeatedly sampled, observations are not independent; results were therefore interpreted with caution, emphasizing consistent hydrology-driven patterns.

Amplicon sequence variant read counts were converted to relative abundances and aggregated at the Genus level to identify the most abundant taxa, grouped by site. Community structure was summarized at the Division level for ME and the Phylum level for prokaryotes.

Rarefied datasets were Hellinger-transformed prior to generating dissimilarity matrices (Bray and Curtis 1957) to assess beta diversity by principal coordinate analysis (PCoA). PERMANOVA (adonis2[]) was applied to test whether variation in z-score-normalized environmental parameters (prior tested for normality) explained significant differences ($p < 0.05$) in community composition. Significant environmental variables were projected onto PCoA plots as vectors, indicating their direction of maximum correlation with ordination scores.

Spearman rank correlation correlations were calculated between the Hellinger-transformed dataset of the 25 most abundant Genera and z-score-normalized environmental parameters. p values ≤ 0.001 were marked with an asterisk. Compositional stability was calculated from count tables, aggregated at the Phylum (prokaryotes) or Division (ME) level for consecutive sampling months (Yuan et al. 2021). A generalized additive model was applied for fitting. Differential abundance between flood and low-water conditions, and among three ecosystem types, was tested with the Wilcoxon rank-sum test, followed by false discovery rate correction (FDR, $p \leq 0.01$). A co-occurrence network analysis was constructed using the trans_network() function from the microeco package (v1.15.0) with Spearman correlations. After optimization, networks retained correlations with $p < 0.01$, and correlation coefficients ≥ 0.7. Microbial modules, clustered on highly connected taxa, were identified by fast greedy modularity optimization. Network properties (links per taxon, cross-phyla associations) were summarized at the Phylum (prokaryotes) or Division (MEs) level to assess changes in microbial associations between flood and low-water conditions.

Results

Hydrological dynamics and water chemistry of the floodplain

During the study, the Danube floodplain experienced short floods in February, May and June, followed by prolonged flooding from July to September before becoming isolated from the Danube (water level < 3 m) in the autumn. These hydrological shifts strongly shaped water column chemistry and led to spatial variations within the floodplain (Supporting Information Table S1; Fig. 2).

In early spring, the floodplain water column exhibited high DO levels (11.08 mg L^{-1} in Sakadaš to 19.18 mg L^{-1} in Kopačko), coinciding with the lowest water temperatures (Pearson's $r = -0.67$, $p < 0.05$; Fig. 2). Summer was characterized by high water temperatures (> 30°C in Čonakut) and reduced DO, with hypoxia occurring in Sakadaš and Hulovo. Ammonium levels rose in Sakadaš during summer (mean ≈ 0.6 mg L^{-1}, fivefold higher than other sites; Fig. 2). Nitrate peaked in

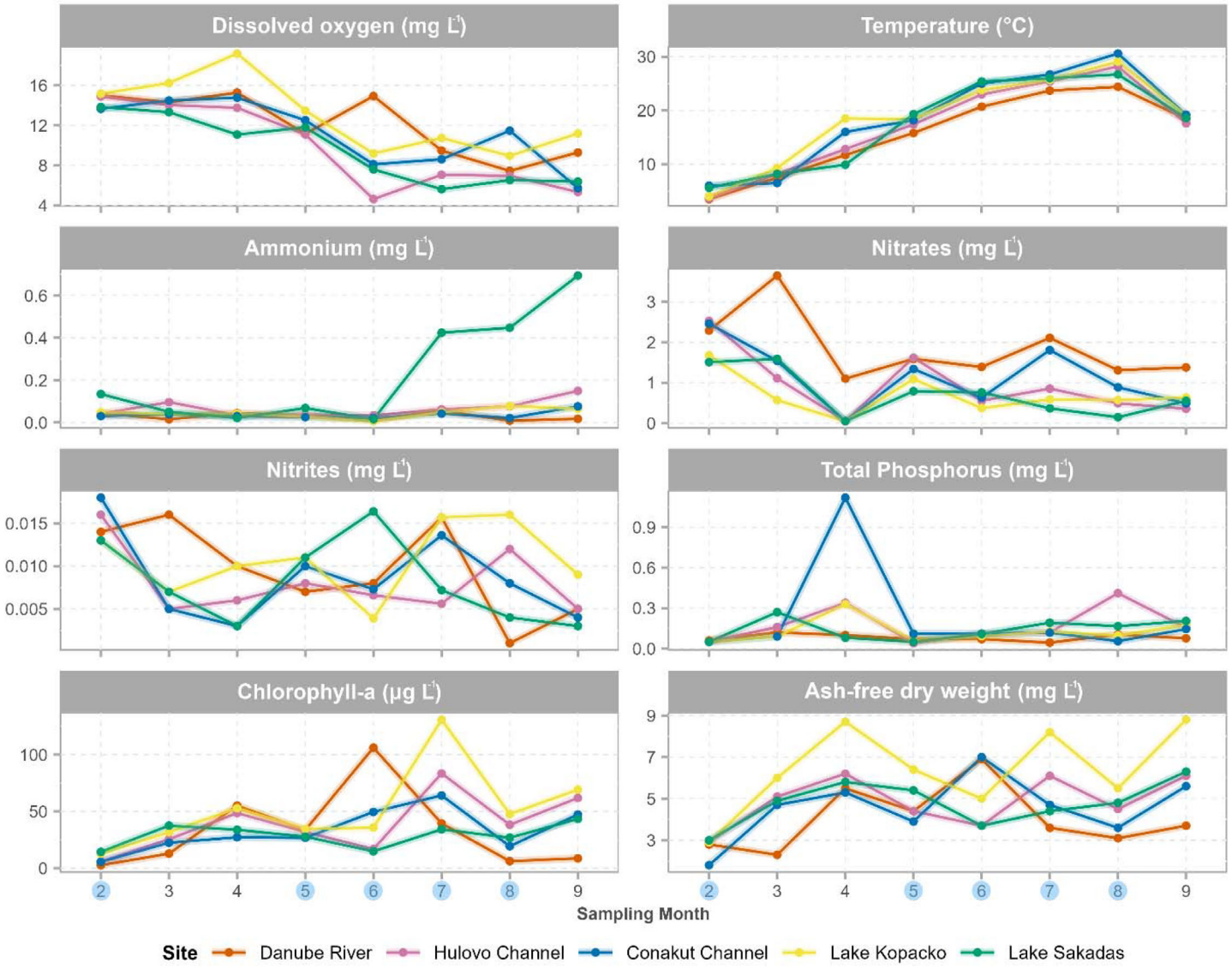

Fig. 2. Spatiotemporal dynamics of the most important physicochemical parameters in the studied water bodies. Different colors represent distinct water bodies. The blue circles on the x-axis denote the sampling months in which flooding was observed.

February and again locally in summer, while nitrite levels exhibited spatial uniformity during minor floods, and asynchronous site-specific fluctuation during summer floods. Total phosphorus values remained < 1 mg L^{-1} at all sites. Chlorophyll a (*Chl-a*) increased seasonally with temperature (Pearson's $r = 0.35$, $p < 0.05$), reaching maxima in June–July, especially in Lake Kopačko (130.8 μg L^{-1}), consistent with high biomass (AFDW values, 2.9–8.8 mg L^{-1}; Pearson's $r = 0.77$, $p < 0.05$).

Microbial alpha diversity patterns

Free-living and PA fractions each generated ~ 1 million reads. Across all sites, prokaryotes showed higher richness than MEs (Fig. 3). Prokaryotic PA communities had higher alpha diversity than FL (ANOVA, $p < 0.001$). Richness was highest in the Danube, the main water supplier, and declined from channels to lakes, with significant differences between Hulovo channel and the lakes (Tukey HSD tests, $p < 0.05$). Diversity was lowest during spring low water but rose during flooding. In contrast, ME diversity showed no fraction-based difference (FL and PA, $p > 0.05$), but followed hydrology patterns, with higher values during floods than low water, except in June when flooding coincided with a decline (Fig. 3).

Microbial core taxa and community composition

Flooding expanded core size and coverage across most microbial groups, except PA prokaryotes, where the effect was weaker (Fig. 4a,b; Supporting Information Figs. S1a,b, S2a,b, S3a,b). During low-water, FL prokaryote core communities were

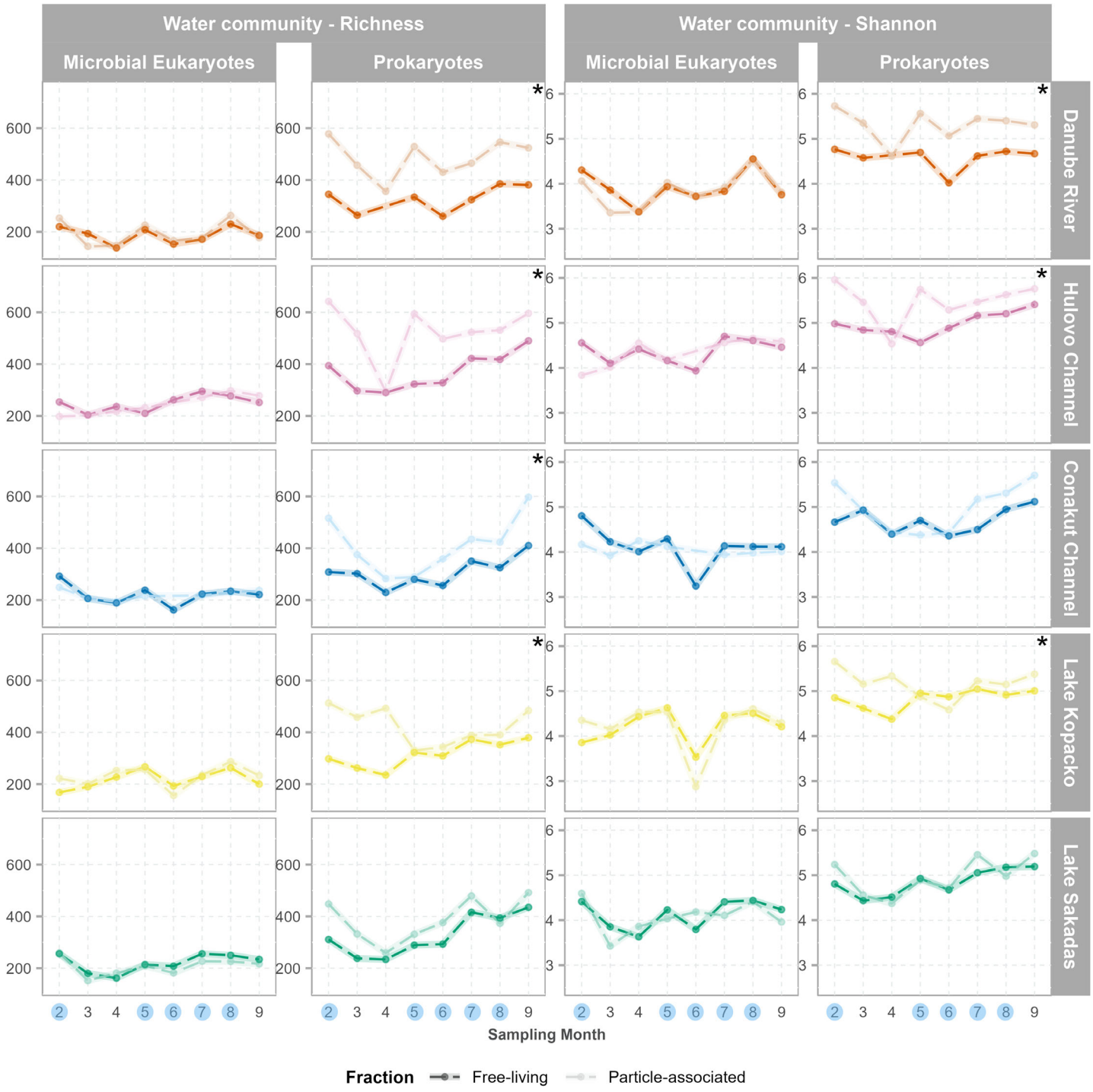

Fig. 3. Alpha diversity of richness and Shannon index within the prokaryotic and ME communities in the FL and PA communities during the study period. An asterisk (*) in the upper right corner indicates a statistically significant difference ($p < 0.05$) between the FL and PA communities at a specific site. The blue circles on the x-axis indicate the sampling months in which flooding was observed.

enriched in SAR11 Clade III (channels), *Candidatus Planktophila* (lakes), and Sporichthyaceae hgcI clade (river). Under flooding, *Limnohabitans* and Sporichthyaceae (hgcI) dominated river cores, *Luteolibacter* and Sporichthyaceae (hgcI) dominated lake cores, and *Polynucleobacter difficilis* together with *Luteolibacter* dominated channel cores (Fig. 4c). Particle-associated prokaryote cores contained few abundant ASVs during low-water and minor flooding contrasting with moderate flooding. Here, *Armatimonas* was

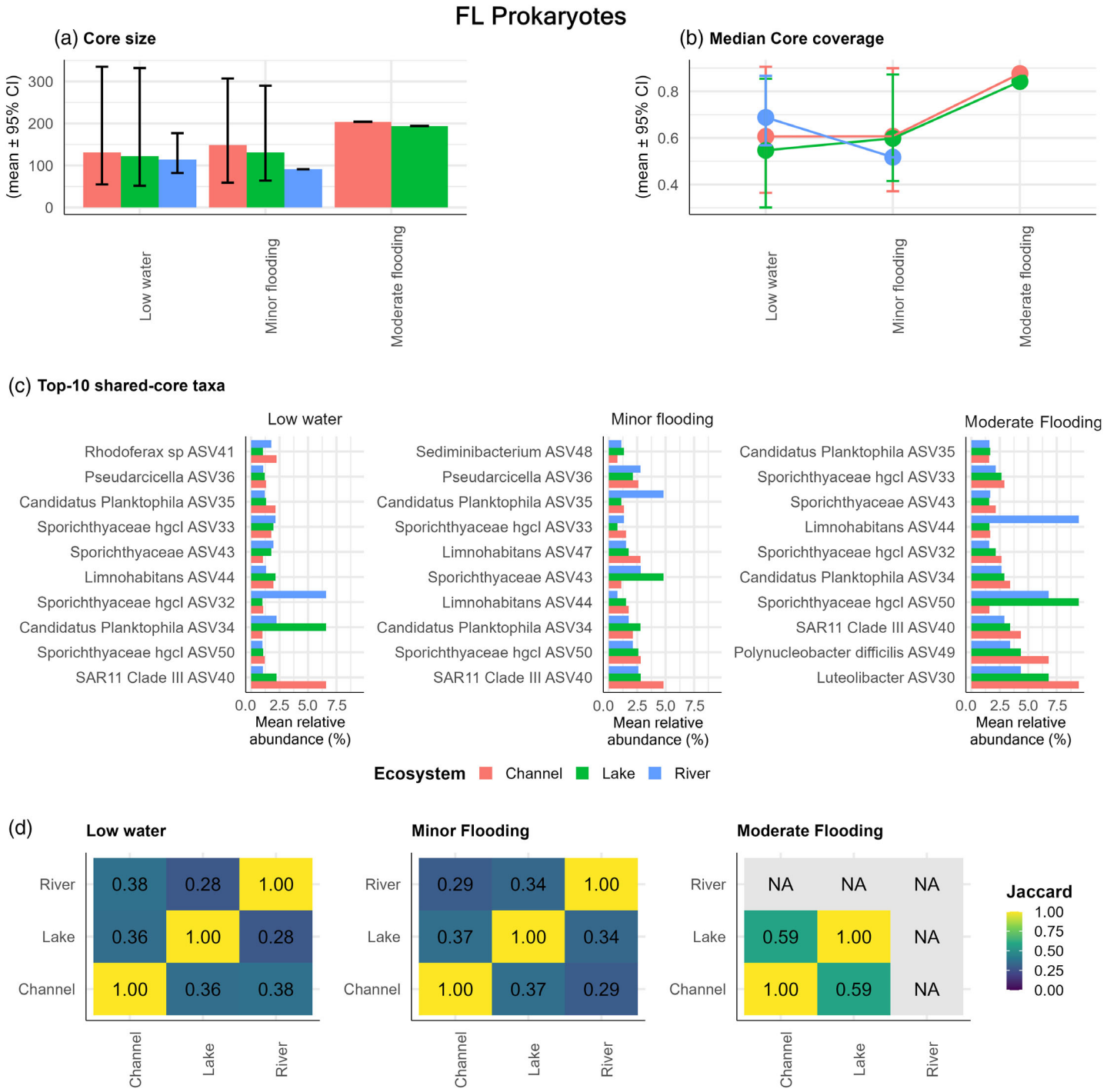

Fig. 4. Core taxa of FL prokaryote communities. (**a**) Core size (mean number of core ASVs across bootstrap replicates with 95% confidence interval (CI)). (**b**) Median core coverage (bootstrap mean of sample-level medians with 95% CI), shown by hydrological condition and ecosystem. (**c**) Ten most abundant core ASVs with their lowest assigned taxonomy, across hydrological conditions (colors = ecosystems). (**d**) Jaccard similarity of core communities between ecosystems under each hydrological condition.

specific to river cores, while *Luteolibacter* dominated both channel and lake cores (Supporting Information Fig. S1c). In both fractions, core similarity was highest between habitats during moderate flooding (Fig. 4d; Supporting Information Fig. S1d).

In both fractions of MEs, channel and river communities were dominated by *Cryptomonas* and Rhyzophidiales under low-water and minor flooding, while *Stephanodiscus*, *Peniculida*, and Chrysophyceae became dominant during moderate flooding

(Supporting Information Figs. S2c, S3c). Particle-associated MEs in lakes shifted from *Cyclotella* (minor flooding) to *Stephanodiscus* and *Tintinnidium* (moderate flooding) (Supporting Information Fig. S3c). Core taxa were generally more abundant during moderate flooding than under other conditions (Supporting Information Figs. S2c, S3c), while their similarity was lowest during minor flooding (Supporting Information Figs. S2d, S3d).

Hydrology strongly structured prokaryotic communities. Proteobacteria (FL: 16.21–52.54%; PA: 14.07–47.40%) peaked in the Danube after flooding (Aug), while Bacteroidota (up to 44.26%) dominated the Čonakut Channel in winter (Feb) but declined during low-water (Supporting Information Fig. S4a). Cyanobacteria increased during warm flooding periods, especially in the PA communities of channels and lakes. Chloroflexi increased during moderate flooding in Lake Sakadaš, alongside site-specific enrichments in Firmicutes and Desulfobacterota. The most abundant genera showed temporal, spatial, and fraction-specific differences (Supporting Information Fig. S5). In prokaryotes, FL actinobacterial hgcl clade (1–12%) was stable across sites and time, but other dominant groups shifted with season and hydrology. During cold, low-water months (Feb–Mar), *Flavobacterium* (up to 22%) and *Pseudarcicella* (up to 10.1%) dominated FL communities across habitats. From late spring onward, *Candidatus Planktophila* and *Luteolibacter* increased in relative abundance, while summer flooding and favored Alphaproteobacterial clade III, especially in the river. In PA fractions, *Luteolibacter* remained abundant across all lotic ecosystems until May, while from April onward, *Cyanobium* PCC-6307 dominated all floodplain habitats and both microbial fractions. Spatial contrasts were clear: *Cyanobium* PCC-6307 (and LD29 in PA) dominated lentic systems, while river communities were enriched in Gammaproteobacteria (OM60/NOR5, *Halioglobus* clades, OLB12, and *Agitococcus lubricus*; Supporting Information Figs. S6, S7).

The ME communities were highly diverse, with 30 divisions identified (Supporting Information Fig. S4b), with Ochrophyta and Cryptophyta dominant year-round. During moderate flooding dinoflagellates dominated lakes (PA: up to 22.16%) and channels (FL: Čonakut—15.14%, Hulovo—10.43%). During floodings Perkinsea dominated in the Danube and a month later lentic sites, while episodic blooms of Centroheliozoa and Apicomplexa were site-specific but absent from the Danube. Low-water periods were marked by Katablepharidophyta in lentic habitats. Fungi and Pseudofungi showed river-linked peaks, with Pseudofungi also displaying seasonal enrichment in Lake Kopačko (Supporting Information Fig. S4b). In ME, diatoms and cryptophytes dominated but shifted with hydrology (Supporting Information Fig. S8). In the river, *Stephanodiscus* was most abundant until June, after which *Cyclotella* prevailed. *Cryptomonas* was present across all sites, peaking in June. Summer floods were dominated by *Rhodomonas* (Aug–Sep, up to 20%). River FL communities also hosted distinct *Thalassiosira* and *Micronuclearia*, absent from lakes (Supporting Information Fig. S9).

Taxa in lower relative abundance also reflected hydrology-related changes (Supporting Information Fig. S10). In FL prokaryotes, Nitrospinota and P9X2b3D02 favored low water, while in PA Armatimonadota, *Sandarakinorhabdus*, and *Rhizobium* were more common during floods. Among FL eukaryotes, *Bicoecea*, Pseudodendromonadales, *Siluania*, *Chytriomyces*, and *Siluania monomastiga* became more prevalent during flooding, whereas PA eukaryotes did not show inundation-related shifts.

Community beta diversity, stability, and microbial networks based on hydrological conditions

Hydrological changes, rather than site identity, were the main driver of both prokaryotic and eukaryotic FL and PA compositional changes: communities clustered by hydrological phase, separating low-water brief floods (Feb–Jun) from prolonged summer flooding (Jul–Sep) (Fig. 5). During moderate flooding, the Danube formed a distinct cluster, more evidently in prokaryotic than in eukaryotic communities. Environmental parameters strongly structured these dynamics. PERMANOVA ($p < 0.05$) and Spearman correlations identified water temperature (R^2: 15.8–92.4%), water level (R^2: 56–58.7%), nitrate (R^2: 9.4–10%) and DO (R^2: 14.7–17.1%) as the strongest predictors of community shifts (Fig. 5; Supporting Information Fig. S11–S14). For example, DO was positively associated with *Luteolibacter* and *Stephanodiscus* but negatively with alphaproteobacterial clade III, *Cyclotella* and *Rhodomonas* (Supporting Information Figs. S11–S14). *Synura* and *Stephanodiscus* decreased with increasing temperature, while *Cyclotella* and *Rhodomonas* showed the opposite trend.

Hydrology also strongly influenced community stability across ecosystems. Free-living prokaryotic communities generally became more stable over time, except in the Danube where flooding reduced stability. The reduced variability, reflected as the tight confidence intervals, indicated greater reliability of these trends (Supporting Information Fig. S15). Particle-associated prokaryotes displayed consistently high stability, with only minor temporal fluctuations.

FL ME communities followed a U-shaped trend, with stability decreasing in spring (May), and recovering during moderate floods (Aug) (Supporting Information Fig. S15). Particle-associated eukaryotes exhibited overall increasing stability, with the Danube maintaining the highest resilience throughout the study period.

Microbial networks showed distinct shifts in patterns of co-occurrence and inter- and intra-taxon associations between low- and high-water periods (Fig. 6). Under low-water conditions, Cyanobacteria emerged as a keystone taxon in prokaryotic networks, forming more diverse connections, while Actinobacteriota lost associations. Furthermore, Firmicutes, Chloroflexi, and Nanoarcheaota increased in the number of associations, contributing to a more diverse and interconnected microbial network. Flooding shifted networks toward more balanced associations, especially among Proteobacteria, Bacteroidota, and Verrucomicrobiota (Fig. 6a).

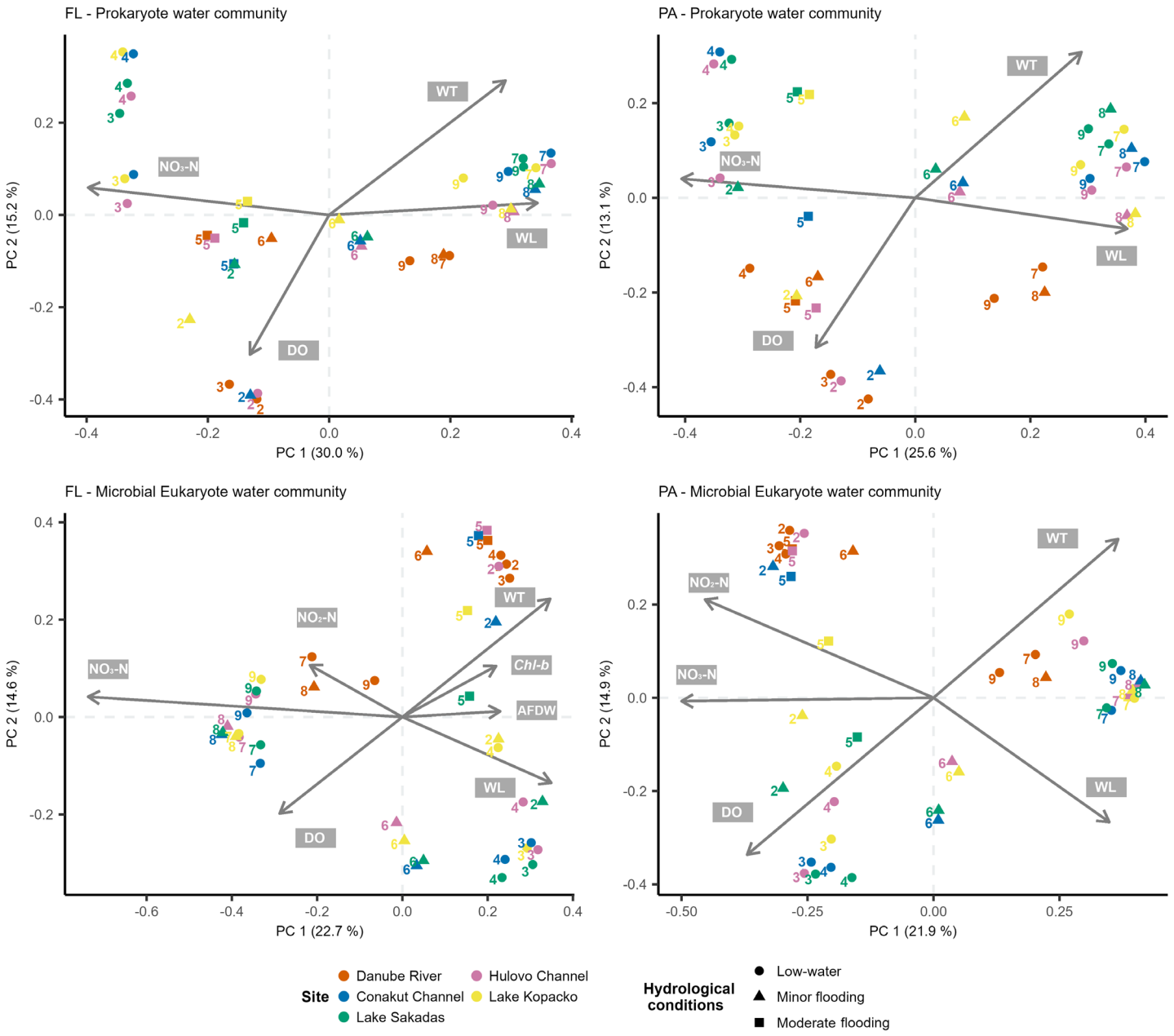

Fig. 5. Principal coordinate analysis (PCoA) of FL and PA prokaryote and eukaryote aquatic communities based on Bray-Curtis distance matrix. The arrows represent environmental parameters significantly associated with the respective microbial community (WT—water temperature, WL—Danube water level, DO—dissolved oxygen concentration, NO_3-N—nitrates, NO_2-N—nitrites, AFDW—ash-free dry weight, *Chl-b*—chlorophyll-b).

In eukaryotic communities, low-water conditions led to compartmentalized networks dominated by Ochrophyta and Fungi, whereas flooding promoted broader connectivity, with Cryptophyta having more than twice as many associations with other MEs and Dinoflagellates forming robust cross-taxa links (Fig. 6b). During low-water periods specialized taxa, such as Choanoflagellida, Katablepharidophyta, Perkinsea, and Centroheliozoa formed new co-occurrences with keystone taxa, while PA non-keystone taxa such as Perkinsea, Apicomplexa, and Pseudofungi favored a greater diversity of associations (Fig. 6b).

Discussion

Hydrological changes drive environmental conditions within the floodplain

Floodplain habitats were primarily shaped by their hydrological connection to the Danube and seasonal temperature

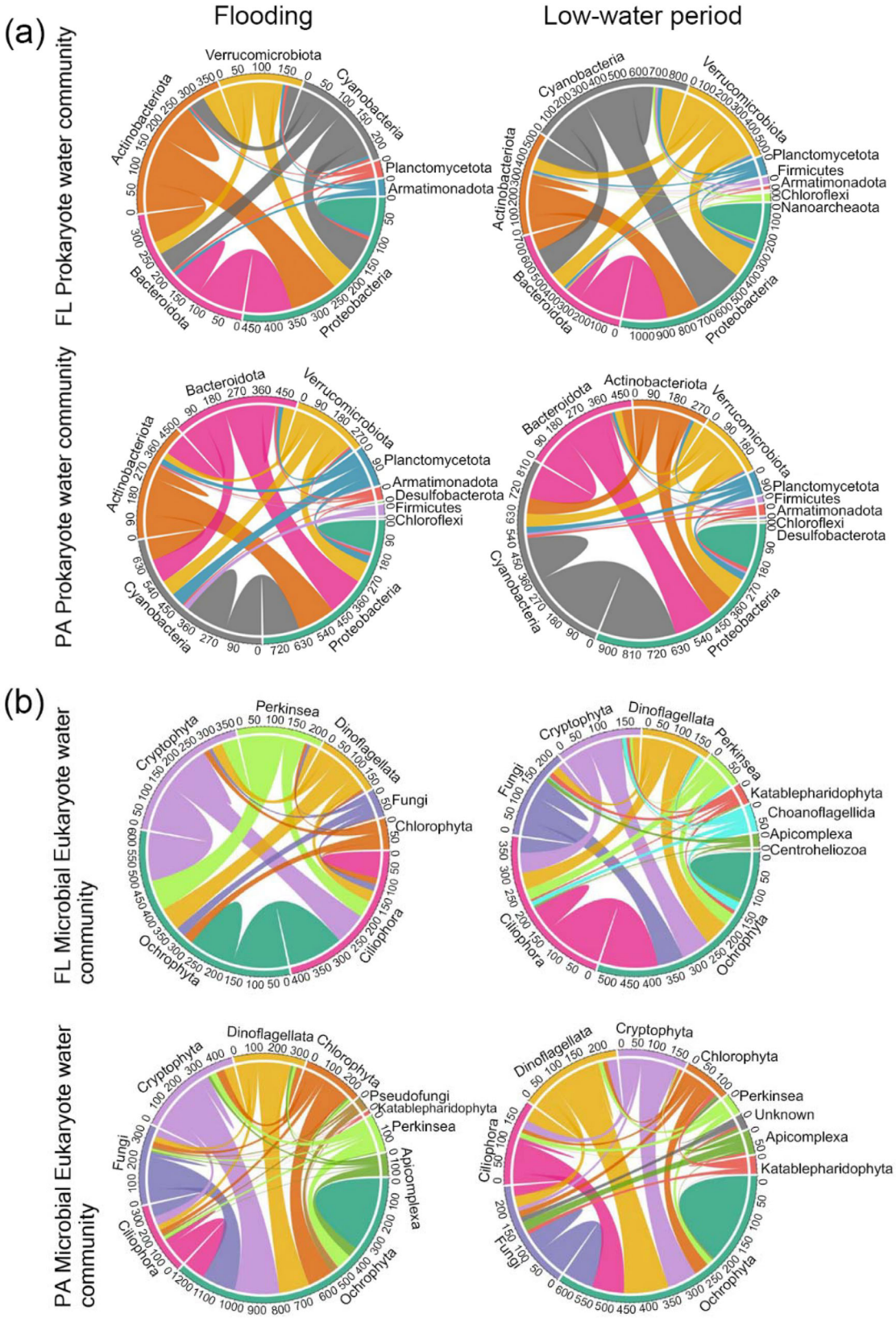

Fig. 6. The microbial community networks, visualized by chord diagrams, show distinct shifts in the composition of PA and FL (**a**) prokaryote (**b**) ME communities during flooding and low-water periods.

fluctuations. Minor spring floods, a moderate summer flood, and intervening low-water phases caused strong shifts in water chemistry (Fig. 2). Although these conditions did not capture the highest reported fluctuations (Mihaljević et al. 2024), they showcase a pattern in which habitats closer to the Danube respond more strongly to hydrological changes, particularly the Hulovo Channel, while lakes showed more buffered responses (Fig. 2). Temperature gradients regulated DO and primary production, with *Chl*-a peaking in shallow lentic systems such as Lake Kopačko. Nutrient dynamics reflected floodplain connectivity: the river transported substantial amounts of sediments and nutrients into the floodplain (Lehotský et al. 2010; Kovacs and Zavadsky 2021). Nitrate, a typical dominant N species of rivers (Malagó et al. 2017; Tschikof et al. 2022) dominated the Danube but declined across the floodplain. Nitrite was homogenized during minor floods but heterogeneous during moderate floods, and ammonium accumulated under hypoxic conditions in Lake Sakadaš, likely as a product of intensive organic matter decomposition (Beutel 2006). These patterns point to enhanced mixing and connectivity during minor floods and ecosystem disruption during moderate floods. The latter occurs not only due to excessive water inflow, but also due to the redistribution of sediments, nutrients, and organic matter (Ostojić et al. 2013).

Floodplain dynamics shape microbial diversity and core community complexity

Across habitats, PA prokaryotic and ME communities had higher alpha diversity than FL communities, reflecting broader niche space and nutrient availability on particles (Urvoy et al. 2022, and the references within). Proximity to the Danube enhanced microbial diversity, with a cross-floodplain decline from river to channels and lakes, reflecting stronger terrestrial inputs, sediment transport, and periphyton influx near the river (Tang et al. 2020, and the Ref within). During low-water periods, stagnant phases in channels and lakes reduced diversity in both MEs and prokaryotes. Isolation from inflow and slowed organic matter turnover can further reduce microbial activity and abundance (Boot et al. 2013). Flooding reversed these trends: both alpha diversity indices increased (Fig. 3), as terrestrial inputs and nutrient pulses promoted microbial growth, especially in lentic habitats of the floodplain. Flooding homogenized microbial communities by expanding their core size, and similarity across habitats (FL prokaryotes only), while low-water promoted habitat-specific core communities (Fig. 4; Supporting Information Figs. S1–S3). During moderate floods, all communities showed higher core coverage, reflecting dominance by fewer taxa. In FL prokaryotes, moderate floods also produced the highest cross-habitat similarity, pointing to strong dispersal and homogenization, whereas minor floods didn't follow that pattern, suggesting weaker dispersal and greater divergence likely promoted by local resuspension processes. Particle-associated prokaryotes showed greater resistance to these effects, maintaining lower similarity than FL fractions. Together, these results indicate that flood intensity determines whether microbial communities converge or differentiate, putting hydrology as the central driver of succession and core community dynamics in the floodplain.

Compositional changes during variable hydrological conditions

Microbial community composition was strongly shaped by seasonal and hydrological fluctuations, which altered temperature, flood intensity, habitat connectivity, and nutrient availability. During winter low-water, FL communities were dominated by *Flavobacterium* (Supporting Information Fig. S5), a genus linked to complex organic matter degradation (Fernandez-Gomez et al. 2013), likely benefiting from reduced microbial turnover under stagnant conditions. In lakes, the FL prokaryote core communities were enriched with *Candidatus Planktophila* during low-water conditions, whereas under flooding *Luteolibacter* dominated the cores of both lakes and channels, as well as in the PA prokaryote cores. The prevalence of these planktonic taxa, linked to the degradation of plant-derived organic matter, is characteristic of wetland ecosystems (Zhang et al. 2014; Rakitin et al. 2024). The hydrological connections between the Danube and its floodplain also increase the amount of dissolved organic content in the river (Besemer et al. 2005), which promotes blooms of bacteria included in the organic degradation process, as shown in our study. Summer floods and higher temperatures favored Alphaproteobacterial Clade III in the Danube and triggered widespread blooms of the cyanobacterium *Cyanobium* PCC-6307, likely due to nutrient-rich, sunlit conditions (Kramer et al. 2024). The members of this genus are typically FL single cells (Huber et al. 2017), and due to their small size, classic morphological analyses have previously not identified this genus in the plankton communities of the studied floodplain area.

Flood intensity also reshaped rarely abundant nitrogen-cycling taxa: nitrite-oxidizing Nitrospinota and P9X2b3D02 clade thrived during low water, coinciding with reduced nitrite, whereas floods disrupted nitrification and enriched for PA taxa such as Armatimonadota, *Sandarakinorhabdus*, and *Rhizobium*, which likely benefited from flood-driven organic inputs (Lee et al. 2014). This suggests a shift in nitrification dynamics, where slower hydrological turnover may allow for more efficient nitrite oxidation. During floods however, rapid water movement and increased organic inputs could disrupt this process and increase particulate matter content, potentially favoring denitrification or ammonium assimilation over nitrite oxidation. Carlton et al. (2023) also linked Armatimonadota to polysaccharide degradation, suggesting that the flood-driven input of terrestrial organic material stimulates its proliferation. However, the observed results of differentially abundant taxa only consisted of rare taxa, making the conclusions not sufficiently reliable, with the need for further experimental confirmation.

Floodplain channels, with their intermittent connectivity to the Danube, showed strong microbial responses to

hydrological fluctuations. In the Čonakut Channel, Bacteroidota and Proteobacteria declined during low water (Supporting Information Fig. S4), reflecting responses to nutrient depletion and shifts in organic matter quality (Zeglin 2015). Moderate floods triggered blooms of Apicomplexa and Centroheliozoa in channels and Lake Kopačko, while prolonged summer flooding (Jul–Sep) increased the abundance of Chloroflexi, Firmicutes, and Desulfobacterota in Lake Sakadaš. As waters receded in September, Dinoflagellates peaked, consistent with increasing isolation and stratification (Reynolds et al. 2002). These patterns highlight how alternating nutrient pulses during floods and retention during low water drive microbial succession and redox dynamics (Battin et al. 2016). Flood pulses increased similarity between floodplain and riverine communities, emphasizing the homogenizing role of connectivity (Fig. 4; Supporting Information Figs. S1–S3, S15). They restructure microbial diversity by altering oxygen levels, nutrients, and hydrodynamics, with channels acting as transitional hotspots where summer flooding most strongly shaped community composition.

Although riverine ME were present in the floodplain, they were mainly dominated by two consistent Danube Bacillariophyta genera: *Stephanodiscus* and *Thalassiosira*, linked to high nutrient levels (Tolotti et al. 2010), and low water temperatures (Rynearson et al. 2020). Shifts to *Cyclotella* during warm, nutrient-rich conditions highlight temperature and hydrology-driven phytoplankton succession. Since moderate flooding homogenized conditions in floodplain habitats (Mihaljević et al. 2015), it was unsurprising that the dominant Danube bacillariophytes (Kiss et al. 2012), *Cyclotella* and *Discostella*, occurred in both the river and the rest of the floodplain (Weilhoefer et al. 2008). *Cryptomonas* was widespread, consistent with previous seasonal observations (Mihaljević et al. 2024), while floods supported *Rhodomonas*, adapted to turbid, low-light environments (Karnjanapak et al. 2021).

Rare MEs, such as *Bicoecea*, Pseudodendromonadales, *Siluania*, and chytrid fungi, increased during floods, reflecting the influence of hydrological connectivity and enhanced organic matter availability that can boost heterotrophic and mixotrophic protists (Amoros and Bornette 2002; Gleason et al. 2008). *Bicoecea* and Pseudodendromonadales, known bacterivorous flagellates (Boenigk and Arndt 2002), proliferated during floods, probably in response to increased bacterial production due to nutrient influx. In contrast, PA ME communities showed weaker flood responses, likely buffered by particle association. Perkinsea and Fungi exhibited dispersal-linked gradients, peaking in the Danube and declining in their abundance further from the river.

Species interaction and community stability changes under different conditions in the floodplain

The stability of FL and PA prokaryotic communities reflected resilience and adaptation to shifting hydrology. Free-living taxa were less stable during flooding, likely due to increased turbulence and dispersal effects, while PA taxa remained more resilient (Besemer et al. 2005; Yan et al. 2024), potentially due to their higher density and the protective role of particles against environmental fluctuations.

The FL ME stability declined during alternating minor floods and low water but recovered during moderate floods, suggesting that community reorganization depends on the nature and intensity of flood dynamics. In contrast, PA MEs exhibited an overall higher trend in compositional stability, although site-specific responses to flooding introduced variability. The Danube community maintained consistently high stability, probably due to the absence of drought-flood cycles compared to floodplain sites (Besemer et al. 2005).

Species associations also shifted with hydrology. Cyanobacteria were keystone taxa during low-water periods that introduced tightly interconnected networks in FL fraction with Chloroflexi, Firmicutes and Nanoarchaeota. Floods promoted balanced, cross-phyla associations and greater taxonomic evenness by facilitating microbial dispersal and enabling more uniform species associations. Particle-associated prokaryotes followed a similar pattern, with dominant Cyanobacteria-Proteobacteria associations under low water, replaced by more even networks during flooding. In eukaryotes, floods enhanced dispersal-driven connectivity among Ochrophyta, Dinoflagellates, and Cryptophyta (FL), while low water created compartmentalized networks where Fungi expanded their role in interspecies associations during ecosystem stability. We note that co-occurrence does not imply direct ecological interaction (Blanchet et al. 2020), but these networks provide a useful proxy for assessing how associations within microbial communities reorganize under contrasting hydrological conditions, complementing the diversity and compositional analyses.

Flood intensity and its duration play a central role in microbial community dynamics in the Danube floodplain. Floods acted as homogenizing agents by enhancing cross-system connectivity, expanding microbial core communities, and restructuring networks toward balanced, cross-taxa associations. In contrast, low-water conditions led to the selection of specialized taxa and associations partitioning, with Cyanobacteria and Fungi dominating, likely due to competitive selection and more stagnant environmental conditions. Particle-associated communities were less affected by short-term hydrological fluctuations than their free-living counterparts. Our results show that the magnitude of hydrological pulses influences microbial diversity, stability, and cross-system connectivity within floodplain environments.

Author Contributions

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Conflicts of Interest

None declared.

Data Availability Statement

The obtained raw sequencing data of microbial reads have been submitted to the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) with the accession number PRJNA1238263.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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