



1 **Bacterioplankton turnover tracks hydrological connectivity and water history across the** 2 **Danube Delta**

3 Sylwia Lew^a, Paweł Burandt^b, Łukasz Pauksztó^a, Krystian Obolewski^c, Anna
 4 Goździewicz^d, Mirosław Grzybowski^d, Igor Cretescu^e, Katarzyna Glińska-Lewczuk^b

5
 6 ^aUniversity of Warmia and Mazury in Olsztyn, Department of Botany and Mycology, 1 Łódzki Sq., 10-719
 7 Olsztyn, Poland

8 ^bUniversity of Warmia and Mazury in Olsztyn, Department of Water Management and Climatology, 2 Łódzki
 9 Sq., 10-719 Olsztyn, Poland,

10 ^cKazimierz Wielki University in Bydgoszcz, Department of Hydrobiology, 10 Powstańców Wielkopolskich Str.,
 11 85-090 Bydgoszcz, Poland

12 ^dUniversity of Warmia and Mazury in Olsztyn, Department of Tourism, Recreation and Ecology, 5 Oczapowski
 13 Str., 10-719 Olsztyn, Poland

14 ^eGheorghe Asachi Technical University of Iași, Department of Environmental Engineering and Management, 73
 15 Prof. Dimitrie Mangeron Blvd., 700050 Iași, Romania

16
 17 Corresponding author: sylwia.lew@uwm.edu.pl

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 19 **Abstract.** Hydrological connectivity regulates water residence, resource availability and
 20 dispersal across freshwater networks, but its links with microbial community organisation
 21 remain difficult to resolve in spatially complex deltaic systems. We combined stable water
 22 isotopes, hydrochemistry and 16S rRNA gene amplicon sequencing to examine
 23 bacterioplankton turnover across riverine, channel and lake habitats in the Danube Delta.
 24 Twenty sites were sampled in September 2023. Surface-water $\delta^{18}\text{O}$ – $\delta^2\text{H}$ values defined a
 25 coherent evaporation line (slope = 5.22, $R^2 = 0.981$), ranging from isotopically depleted river
 26 waters to enriched lake waters and indicating increasing evaporative influence and effective
 27 hydrological retention. Alpha-diversity metrics did not differ significantly among habitats,
 28 whereas Bray–Curtis dissimilarities revealed pronounced community turnover along the river–
 29 lake continuum. The primary axis of bacterioplankton variation was strongly associated with
 30 $\delta^{18}\text{O}$ enrichment, linking microbial community organisation to independently resolved water
 31 history. Verrucomicrobiota increased towards isotopically enriched, DOC-rich lake waters,
 32 whereas Proteobacteria and Actinobacteriota were associated with more connected riverine
 33 conditions. A redundancy analysis constrained by DOC, conductivity, total nitrogen and total
 34 phosphorus explained 50.3% of Hellinger-transformed phylum-level variation. Together, these
 35 results show that hydrological connectivity and water history are closely coupled to microbial
 36 community reorganisation and associated carbon and nutrient conditions across a
 37 heterogeneous deltaic network. Stable water isotopes therefore provide a process-based
 38 hydrological context for interpreting bacterioplankton turnover in freshwater landscapes.

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 41 **Keywords:** hydrological connectivity; water residence; bacterioplankton turnover; 16S rRNA
 42 metabarcoding; beta-diversity; stable water isotopes; freshwater biogeochemistry; dissolved
 43 organic carbon; river–lake continuum; Danube Delta



44 **Introduction**

45 Deltaic ecosystems are characterised by strong spatial variation in hydrological connectivity,
46 water residence time and biogeochemical processing, and are particularly sensitive to climate
47 and anthropogenic pressures (Ellison, 2015; Ericson et al., 2006; Giosan et al., 2014; Renaud
48 et al., 2013; Tessler et al., 2015; van de Giesen et al., 2016; Deltares, 2023). Hydrological
49 connectivity regulates the exchange of water, solutes and organisms among river channels,
50 transitional waterways and shallow lakes, while variation in effective residence time alters
51 opportunities for evaporation, organic-matter processing and nutrient transformation.
52 Consequently, spatially heterogeneous deltaic networks provide natural settings in which
53 physical transport, biogeochemical conditions and microbial community organisation can be
54 examined along the same hydrological continuum. Conventional physicochemical
55 measurements describe contemporary water conditions, but alone provide limited information
56 on the hydrological history experienced by microbial communities.

57 Stable water isotopes ($\delta^{18}\text{O}$ – $\delta^2\text{H}$) integrate information on water origin, mixing and evaporative
58 history and can therefore resolve hydrological differences that are not readily captured by
59 instantaneous hydrochemical measurements (Gibson et al., 2005; Vystavna et al., 2021).
60 Microbial communities respond simultaneously to hydrological transport and local
61 environmental selection because dispersal, residence time, organic-carbon availability and
62 nutrient conditions influence community assembly across aquatic networks. Whereas alpha-
63 diversity describes local richness and evenness, beta-diversity reflects spatial community
64 turnover generated by the balance between dispersal and environmental filtering (Newton et al.,
65 2011; Langenheder and Lindström, 2019; Mori et al., 2018; Zhu et al., 2024). Specific microbial
66 taxa further reflect trophic status and organic matter availability (Newton et al., 2011;
67 Kirchman, 2018; Warter et al., 2024). Integrating water-isotope tracers with microbial
68 community data therefore provides an opportunity to distinguish the hydrological history of



69 water masses from the contemporary resource environment and to examine how these two
70 dimensions jointly structure freshwater microbiomes.

71 The Danube Delta Biosphere Reserve forms an extensive river–channel–lake network between
72 the lower Danube River and the Black Sea (UNESCO, 2024; Ramsar Secretariat, 2015; Radu
73 and Bostan, 2013; ICPDR, 2019; Vadim and Bostan, 1998). Its mosaic of active branches,
74 transitional channels, shallow lakes and reed-dominated wetlands generates strong gradients in
75 water exchange, effective residence time and biogeochemical processing. Riverine waters
76 receive continuous upstream inputs and experience comparatively rapid transport, whereas
77 hydrologically less connected lakes provide greater opportunities for evaporation, organic-
78 matter accumulation and internal nutrient transformation. The system therefore offers a natural
79 spatial gradient for examining how water history and contemporary biogeochemical conditions
80 are related to microbial community organisation. Although stable isotopes and microbial
81 approaches have been combined in groundwater and experimental studies (Boschker and
82 Middelburg, 2002; Neufeld et al., 2007; Pimentel et al., 2014; Daims et al., 2006; Hung et al.,
83 2024; Smercina et al., 2019; Stegen et al., 2024), studies coupling natural $\delta^{18}\text{O}$ – $\delta^2\text{H}$ gradients
84 with bacterioplankton turnover across large river-dominated deltaic networks remain scarce.

85 We investigated how bacterioplankton community organisation varies along an isotope-defined
86 gradient of water history across the Danube Delta and how this turnover relates to contemporary
87 carbon and nutrient conditions. Specifically, we aimed to: (i) use $\delta^{18}\text{O}$ – $\delta^2\text{H}$ composition to
88 resolve water-history gradients across riverine, channel and lake habitats; (ii) determine how
89 bacterioplankton community structure, alpha diversity and beta diversity vary along this
90 gradient; (iii) identify microbial groups associated with contrasting hydrological connectivity,
91 effective retention and DOC enrichment; and (iv) evaluate the complementary relationships of
92 hydrological history and contemporary hydrochemical conditions with microbial community
93 organisation across a heterogeneous freshwater network.



2. MATERIALS AND METHODS

2.1. Study area and sampling design

The study was conducted in the Danube Delta (Romania), one of Europe’s largest and most complex wetland systems. This region features high hydrological and habitat heterogeneity, comprising active river branches, interconnected channels, shallow lakes, expansive reed beds, and periodically inundated areas. This mosaic landscape drives spatial variation in biogeochemical processes, providing a suitable natural system to examine relationships among hydrological connectivity, water quality and bacterioplankton structure. In September 2023, water was sampled across 20 sites representing the delta’s primary aquatic habitats: 13 lakes, 2 channels, and 5 riverine sites spanning from Isaccea along the Sulina branch to the Black Sea (Figure 1). Each site constituted an independent spatial observation with no temporal replication. Identical water samples were partitioned for concurrent physicochemical, stable-isotope, and microbiome analyses.

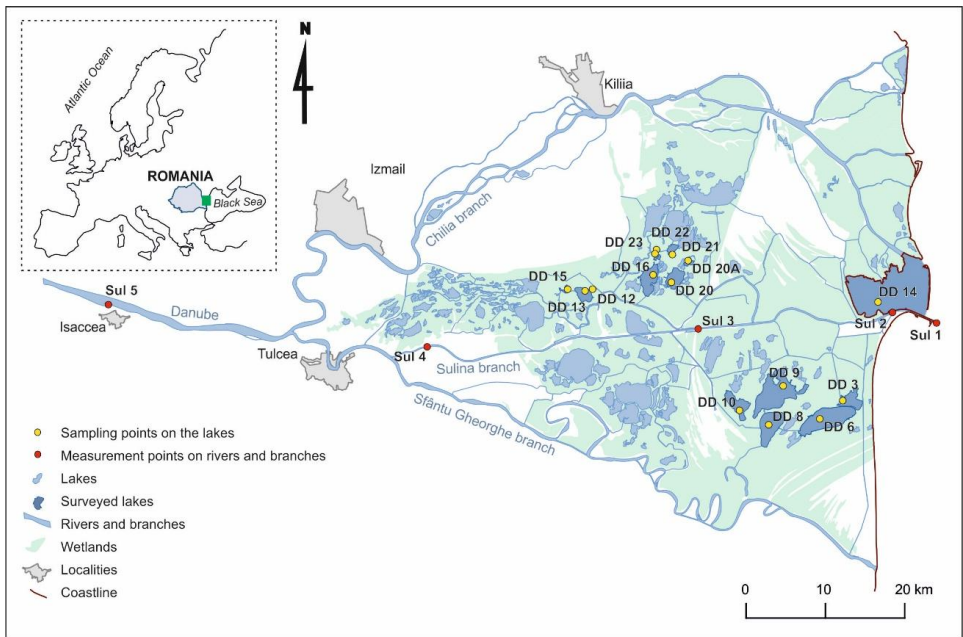


Figure 1. Location of the study area within the Danube Delta (Romania) and the spatial distribution of the 20 sampling sites monitored in September 2023. Yellow circles indicate sampling points located within the surveyed shallow lakes ($N=13$). Red circles represent measurement points positioned along active river channels and



112 branches, specifically spanning the main Danube stretch near Isaccea (Sul 5) down the Sulina branch (Sul 4 to Sul
 113 1) toward the Black Sea coast. The inset map illustrates the geographic positioning of the Danube Delta region
 114 within Europe and Romania.

115 2.2. Physicochemical analyses

116 During sample collection, basic physicochemical parameters of the water were measured
 117 directly *in situ*. Water temperature ($^{\circ}\text{C}$), pH, dissolved oxygen (DO , mg L^{-1}), turbidity (NTU),
 118 electrical conductivity (EC, $\mu\text{S cm}^{-1}$), chlorophyll-a (Chl-a, $\mu\text{g L}^{-1}$), total dissolved solids
 119 (TDS, g L^{-1}) and salinity were measured using a calibrated YSI 6600V2 multiparameter probe
 120 (Yellow Springs, OH, USA). Water transparency was measured using a standard Secchi disk.
 121 Water samples for laboratory analyses were collected in 2.5 L polyethylene bottles, transported
 122 under cold and dark conditions, and processed within 12 hours of collection. Total nitrogen
 123 (TN, mg L^{-1}) was determined using a multi N/C 3100 TOC/TN_b analyser (Analytik Jena GmbH,
 124 Jena, Germany). Dissolved organic carbon (DOC, mg L^{-1}) was analysed on the same TOC
 125 system. Chemical oxygen demand (COD_{Cr} , mg L^{-1}) was determined via the dichromate closed-
 126 reflux method. Total phosphorus (TP, mg L^{-1}) was quantified colorimetrically using the
 127 ascorbic acid method following potassium persulfate digestion, in accordance with APHA
 128 (2017) guidelines. Orthophosphate ($\text{PO}_4\text{-P}$, mg L^{-1}) was determined colorimetrically using the
 129 ascorbic acid method on water samples filtered through $0.45\ \mu\text{m}$ membrane filters.

130 2.3. Stable water isotope analysis

131 The isotopic composition of water was determined from $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values. Analyses were
 132 performed using a Picarro L2140-i analyser (Picarro Inc., Santa Clara, CA, USA), operating on
 133 the principle of cavity ring-down spectroscopy (CRDS), a laser-spectroscopic approach widely
 134 applied to liquid-water isotope analysis when instrumental drift and memory effects are
 135 controlled (van Geldern and Barth, 2012). The analyser was coupled to an ALS-G autosampler
 136 and an A0211 High Precision Vaporizer. After introduction into the vaporizer, water samples
 137 were completely evaporated and transported into the optical cavity in a stream of dry carrier
 138 gas. Stable pressure conditions within the analytical system were maintained using an external



vacuum pump. Measurements were conducted in Standard/High Precision mode for high-precision analysis of liquid-water samples.

The results were calibrated against the international VSMOW-SLAP scale using certified reference materials USGS46, USGS47 and USGS48, following recommended terminology and reporting practice for stable-isotope-ratio measurements (Coplen, 2011; Brand et al., 2014). USGS47 and USGS48 were used as low and high normalisation standards, whereas USGS46 was used as a quality-control material. Each analytical sequence also included deionised water to monitor instrumental drift and memory effects. Corrections for memory effects and instrumental drift were applied before normalisation of the results to the VSMOW-SLAP scale. Results are reported in delta notation (‰) relative to VSMOW on the VSMOW–SLAP scale, consistent with standard isotope-hydrology and stable-isotope reporting conventions (Craig, 1961; Gat, 1996; Coplen, 2011):

$$\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \text{ [‰]}$$

where R_{sample} is the isotope ratio of the sample and R_{standard} is the isotope ratio of the reference standard. Deuterium excess (d-excess = $\delta^2\text{H} - 8 \times \delta^{18}\text{O}$) was calculated to capture non-equilibrium evaporation signals and complement the interpretation of the isotope-defined hydrological gradient.

2.4. Microbial community analysis

DNA extraction

For microbiological analyses, approximately 500 mL of water was collected from each sampling site. After transport to the laboratory, samples were filtered through sterile polycarbonate membranes with a pore size of 0.2 µm (Nucleopore, Whatman, UK), using low vacuum pressure not exceeding 200 hPa above atmospheric pressure. After filtration, the membranes were stored at –80°C until DNA extraction.

Genomic DNA was extracted from the biomass retained on the filters using the DNeasy PowerWater® Kit (Qiagen, Germany), following the manufacturer’s protocol and procedures



commonly applied in aquatic microbiome studies (Reynoso et al., 2019). DNA concentration was determined spectrophotometrically using a NanoDrop instrument (Thermo Scientific, USA). Amplicon library preparation and sequencing were performed by an external sequencing facility, Genomed SA.

Amplicon library preparation and 16S rRNA gene sequencing

Amplicon libraries were prepared for the V3–V4 region of the bacterial 16S rRNA gene using the universal primer pair 341F/785R with Illumina adapter sequences (Klindworth et al., 2013). PCR amplification was performed using Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs, USA), in accordance with the Illumina protocol for 16S Metagenomic Sequencing Library Preparation. Following amplification, PCR products were indexed using the Nextera XT Index Kit (Illumina, USA), purified, and the size distribution of the libraries was assessed using an Agilent Bioanalyzer 2100 with a High Sensitivity DNA chip. Sequencing was performed on an Illumina MiSeq platform in paired-end mode, 2×300 bp, using the MiSeq Reagent Kit v3. The target minimum sequencing depth was 50,000 paired-end reads per sample.

2.5. Bioinformatic processing

Raw sequence reads were demultiplexed and subjected to initial quality control using FastQC. Subsequent data processing was conducted in QIIME 2, version 2021.11 (Bolyen et al., 2019). Adapter sequences were removed using the q2-cutadapt plugin, and low-quality regions were trimmed based on read-quality profiles. Denoising, paired-end read merging, chimera removal and inference of amplicon sequence variants (ASVs) were performed using the DADA2 algorithm (Callahan et al., 2016). The use of ASVs enabled the reconstruction of exact biological sequence variants and reduced the influence of sequencing errors on the final dataset structure. Taxonomic classification of ASVs was performed using a Naïve Bayes classifier trained on the SILVA SSU rRNA database, release 138, at a 99% similarity threshold (Quast et al., 2013). Sequences assigned to chloroplasts, mitochondria and other non-prokaryotic lineages were removed before downstream analyses; prokaryotic ASVs assigned to Bacteria or Archaea



191 were retained where detected. To minimise the influence of differences in sequencing depth,
 192 samples were rarefied to 60,778 reads per sample prior to diversity analyses, provided that
 193 rarefaction curves indicated sufficient sequencing coverage. Phylogenetic relationships among
 194 ASVs were reconstructed using MAFFT, and a phylogenetic tree was generated with FastTree.
 195 Alpha-diversity metrics included Chao1 richness, the Shannon diversity index and the Simpson
 196 diversity index.

197 2.6. Statistical analyses

198 The sampling site was treated as the independent analytical unit, comprising riverine sites ($n =$
 199 5), channels ($n = 2$), and lakes ($n = 13$). Where more than one sequencing output was available
 200 for a given river site, technical replicates were combined before site-level analyses. Phylum-
 201 level read counts were summed and converted to relative abundances, whereas alpha-diversity
 202 values were averaged to obtain one value per site. Thus, technical sequencing replicates were
 203 not treated as independent environmental observations.

204 The relationship between $\delta^{18}\text{O}$ – $\delta^2\text{H}$ in surface waters was estimated using ordinary least-
 205 squares regression. The global meteoric water line (GMWL: $\delta^2\text{H} = 8 \times \delta^{18}\text{O} + 10$) was included
 206 only as a reference (Craig, 1961). Because the study did not include a time-integrated
 207 precipitation isotope series, the fitted relationship was interpreted as a surface-water
 208 evaporation line rather than a local meteoric water line, following isotope-hydrology guidance
 209 (Gat, 1996; Gibson et al., 2005; Crawford et al., 2014; Vystavna et al., 2021).

210 Differences in d -excess and alpha-diversity indices among habitat types were assessed using
 211 permutation-based Kruskal–Wallis tests. Bray–Curtis dissimilarities were calculated from site-
 212 level phylum relative-abundance profiles and visualised using principal coordinates analysis
 213 (PCoA). Habitat-associated differences in community composition were tested using
 214 PERMANOVA (Anderson, 2001), and homogeneity of multivariate dispersion was assessed to
 215 distinguish differences in community location from differences in within-habitat variability
 216 (Anderson, 2006).



217 Redundancy analysis (RDA) was used to relate microbial community composition to
 218 environmental variables. Prior to RDA, phylum relative abundances were Hellinger
 219 transformed, following Legendre and Gallagher (2001), and environmental predictors were
 220 centred and scaled. Because DOC and $\delta^{18}\text{O}$ were strongly correlated, $\delta^{18}\text{O}$ was excluded from
 221 the multivariate model to avoid collinearity. The final constrained RDA model thus included
 222 conductivity, DOC, TN, and TP. Collinearity among the remaining variables was verified using
 223 variance-inflation factors (VIF), and overall model significance was tested by permutation.
 224 Exploratory associations between bacterial phyla and isotope variables ($\delta^{18}\text{O}$, $\delta^2\text{H}$, and d -
 225 excess) were assessed using Pearson correlations based on site-level phylum relative
 226 abundances. Two-sided permutation p -values were adjusted using the Benjamini–Hochberg
 227 false-discovery-rate procedure (Benjamini and Hochberg, 1995). Because the phylum-level
 228 data are compositional and the study is observational, these correlations were interpreted as
 229 directional associations rather than independent taxon-specific effects. A detailed description
 230 of resampling procedures, ordination settings, treatment of technical replicates, and exploratory
 231 taxon–isotope correlations is provided in the Supplementary Methods S1.

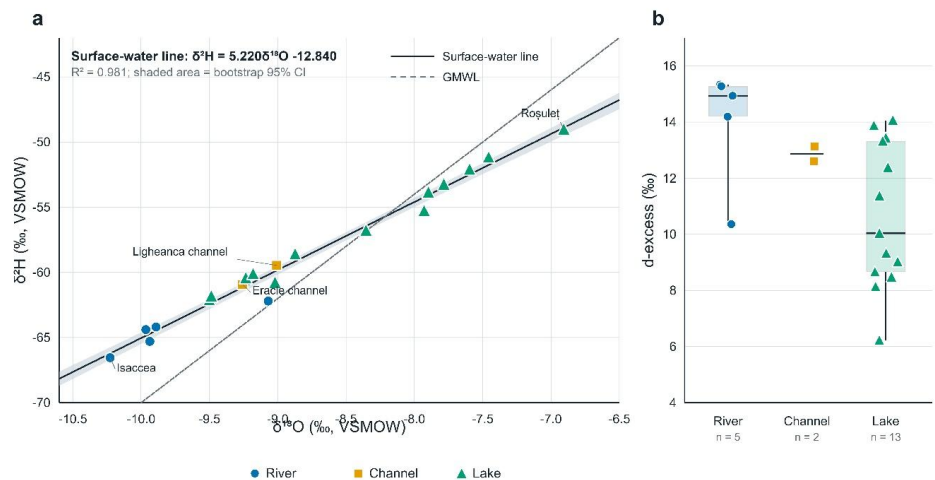
232 **3. RESULTS**

233 *3.1. Hydrological and isotopic gradient across the Danube Delta*

234 Stable-isotope composition resolved a clear river-to-lake gradient: riverine waters were
 235 isotopically depleted, channels showed intermediate values, and lakes were enriched along a
 236 surface-water evaporation line distinct from the Global Meteoric Water Line. Across habitats,
 237 mean $\delta^{18}\text{O}$ and $\delta^2\text{H}$ increased from rivers to lakes (rivers: -9.82‰ and -64.52‰ ; channels:
 238 -9.14‰ and -60.20‰ ; lakes: -8.40‰ and -56.57‰), reflecting progressive evaporative
 239 enrichment and retention. Mean d -excess declined from 14.02‰ in rivers to 12.87‰ in
 240 channels and 10.64‰ in lakes, and the overall habitat effect on d -excess was significant in the
 241 permutation rank test ($p = 0.0139$). Because the channel category is represented by only two
 242 sites, the corresponding estimates should be regarded as descriptive rather than representative.



243 Across all 20 surface-water samples, $\delta^2\text{H}$ was strongly related to $\delta^{18}\text{O}$ ($\delta^2\text{H} = 5.220 \times \delta^{18}\text{O} -$
244 12.840 ; $R^2 = 0.981$). The bootstrap 95% confidence interval for the slope was 4.954 – 5.445 . The
245 slope was substantially lower than the GMWL slope of 8 , consistent with evaporative
246 fractionation along the sampled river-channel-lake gradient (**Figure 2**). The fitted line is
247 therefore interpreted as the Danube Delta surface-water evaporation line, not an LMWL.



248 **Figure 2.** Stable-isotope composition of Danube Delta surface waters in September 2023. (a) Relationship between
249 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for riverine ($n = 5$), channel ($n = 2$) and lake ($n = 13$) sites. The solid line is the fitted surface-water
250 evaporation line ($\delta^2\text{H} = 5.220 \times \delta^{18}\text{O} - 12.840$; $R^2 = 0.981$) and shading is the bootstrap 95% confidence band; the
251 dashed line represents the Global Meteoric Water Line (GMWL). (b) Site-level d -excess by habitat. Boxes show
252 the interquartile range and median for groups with $n \geq 3$; all independent site observations are displayed as
253 individual points.

254

256 3.2. Physicochemical background of the hydrological gradient

257 The physicochemical properties of the investigated waters varied among riverine, channel and
258 lake habitats and mirrored the isotope-defined hydrological gradient (**Table 1**). Rivers showed
259 relatively stable conditions, with pH of 8.20 ± 0.07 , electrical conductivity of $394.60 \pm 5.32 \mu\text{S}$
260 cm^{-1} and DOC concentration of $3.42 \pm 0.19 \text{ mg L}^{-1}$. Channels were characterised by lower
261 conductivity ($371.00 \pm 5.66 \mu\text{S cm}^{-1}$), higher Secchi depth ($2.27 \pm 1.80 \text{ m}$) and intermediate
262 DOC values ($4.74 \pm 1.61 \text{ mg L}^{-1}$). Lake habitats showed higher mean pH (8.46 ± 0.35),



dissolved oxygen concentration ($10.83 \pm 2.27 \text{ mg L}^{-1}$), DOC ($6.92 \pm 2.43 \text{ mg L}^{-1}$) and COD_{Cr} ($29.44 \pm 13.43 \text{ mg L}^{-1}$) compared with riverine sites.

Table 1. Physicochemical characteristics of surface waters across different macrohabitat types in the Danube Delta during the September 2023 sampling campaign.

Parameter	Unit	River (n = 5)	Channel (n = 2)	Lake (n = 13)	Total (n = 20)	<i>p</i>
Secchi depth	m	$0.54 \pm 0.10^*$	2.27 ± 1.80	0.79 ± 0.38	0.87 ± 0.71	0.169
		8.20 ± 0.07	8.07 ± 0.02	8.46 ± 0.35	8.36 ± 0.32	
pH	–	23.69 ± 0.25	21.62 ± 0.43	22.37 ± 0.72	22.63 ± 0.89	0.003
		8.28 ± 0.36	9.59 ± 0.26	10.83 ± 2.27	10.07 ± 2.13	
DO	mg L^{-1}	12.76 ± 4.20	3.60 ± 2.97	5.92 ± 4.91	7.40 ± 5.48	0.023
		394.60 ± 5.32	371.00 ± 5.66	398.23 ± 54.15	394.60 ± 43.90	
EC	$\mu\text{S cm}^{-1}$	0.26 ± 0.00	0.24 ± 0.00	0.26 ± 0.04	0.26 ± 0.03	0.115
		0.19 ± 0.00	0.18 ± 0.00	0.19 ± 0.03	0.19 ± 0.02	
TDS	g L^{-1}	66.88 ± 41.21	64.10 ± 38.61	76.76 ± 23.47	73.03 ± 28.49	0.202
		3.42 ± 0.19	4.74 ± 1.61	6.92 ± 2.43	5.83 ± 2.52	
Salinity	ppt	0.98 ± 0.14	0.40 ± 0.45	0.72 ± 0.33	0.76 ± 0.33	0.772
		0.50 ± 0.11	0.76 ± 0.06	0.76 ± 0.13	0.70 ± 0.16	
Chla	$\mu\text{g L}^{-1}$	0.16 ± 0.04	0.25 ± 0.02	0.25 ± 0.04	0.23 ± 0.05	0.006
		6.02 ± 1.08	16.65 ± 10.82	29.44 ± 13.43	22.30 ± 15.11	
DOC	mg L^{-1}	0.50 ± 0.11	0.76 ± 0.06	0.76 ± 0.13	0.70 ± 0.16	0.009
		0.16 ± 0.04	0.25 ± 0.02	0.25 ± 0.04	0.23 ± 0.05	
TN	mg L^{-1}	0.50 ± 0.11	0.76 ± 0.06	0.76 ± 0.13	0.70 ± 0.16	0.009
		0.16 ± 0.04	0.25 ± 0.02	0.25 ± 0.04	0.23 ± 0.05	
TP	mg L^{-1}	0.50 ± 0.11	0.76 ± 0.06	0.76 ± 0.13	0.70 ± 0.16	0.009
		0.16 ± 0.04	0.25 ± 0.02	0.25 ± 0.04	0.23 ± 0.05	
$\text{PO}_4\text{-P}$	mg L^{-1}	0.50 ± 0.11	0.76 ± 0.06	0.76 ± 0.13	0.70 ± 0.16	0.009
		0.16 ± 0.04	0.25 ± 0.02	0.25 ± 0.04	0.23 ± 0.05	
COD_{Cr}	mg L^{-1}	0.50 ± 0.11	0.76 ± 0.06	0.76 ± 0.13	0.70 ± 0.16	0.003
		0.16 ± 0.04	0.25 ± 0.02	0.25 ± 0.04	0.23 ± 0.05	

Values are presented as mean $\pm$ standard deviation (SD). The *p*-values are derived from permutation-based exploratory Kruskal–Wallis tests evaluating differences across River, Channel, and Lake habitats (bold font indicates statistical significance at $p < 0.05$). DO: dissolved oxygen; EC: electrical conductivity; TDS: total dissolved solids; Chla: chlorophyll-a; DOC: dissolved organic carbon; TN: total nitrogen; TP: total phosphorus; $\text{PO}_4\text{-P}$: orthophosphate phosphorus; COD_{Cr} : chemical oxygen demand.

Nutrient concentrations also increased from rivers to lakes. Total phosphorus and orthophosphate phosphorus were lower in riverine waters (0.50 ± 0.11 and $0.16 \pm 0.04 \text{ mg L}^{-1}$, respectively) than in channels and lakes, where both parameters reached $0.76 \pm 0.06\text{--}0.13 \text{ mg L}^{-1}$ for TP and $0.25 \pm 0.02\text{--}0.04 \text{ mg L}^{-1}$ for $\text{PO}_4\text{-P}$. Significant differences among habitat types

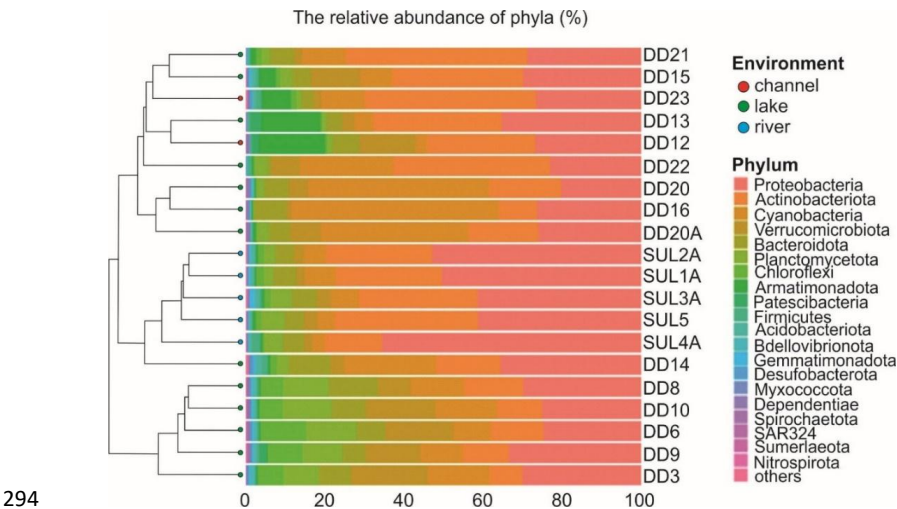


278 were observed for temperature, turbidity, DOC, TP, PO₄-P and COD_{Cr}, whereas EC, TDS,
279 salinity and chlorophyll-a showed no significant variation.

280 *3.3. Microbial community composition along the hydrological gradient*

281 The bacterial community was dominated by Proteobacteria, Actinobacteriota and
282 Cyanobacteria, which together represented the main component of the microbiome across the
283 investigated sites. Mean relative abundances were 39.4% for Proteobacteria, 23.8% for
284 Actinobacteriota and 12.6% for Cyanobacteria. Bacteroidota, Verrucomicrobiota and
285 Planctomycetota were consistently present as additional important components of the bacterial
286 community (**Figure 3**).

287 Marked differences in microbial composition were observed among habitat types. Riverine
288 waters were mainly associated with higher contributions of Proteobacteria and
289 Actinobacteriota, whereas lake habitats showed increased relative abundance of Cyanobacteria,
290 Bacteroidota and Verrucomicrobiota. Channel sites displayed an intermediate microbial
291 composition, consistent with their transitional hydrological character. Spatial variability of
292 microbial composition was lower within the riverine system, while lake habitats showed greater
293 heterogeneity in community structure.

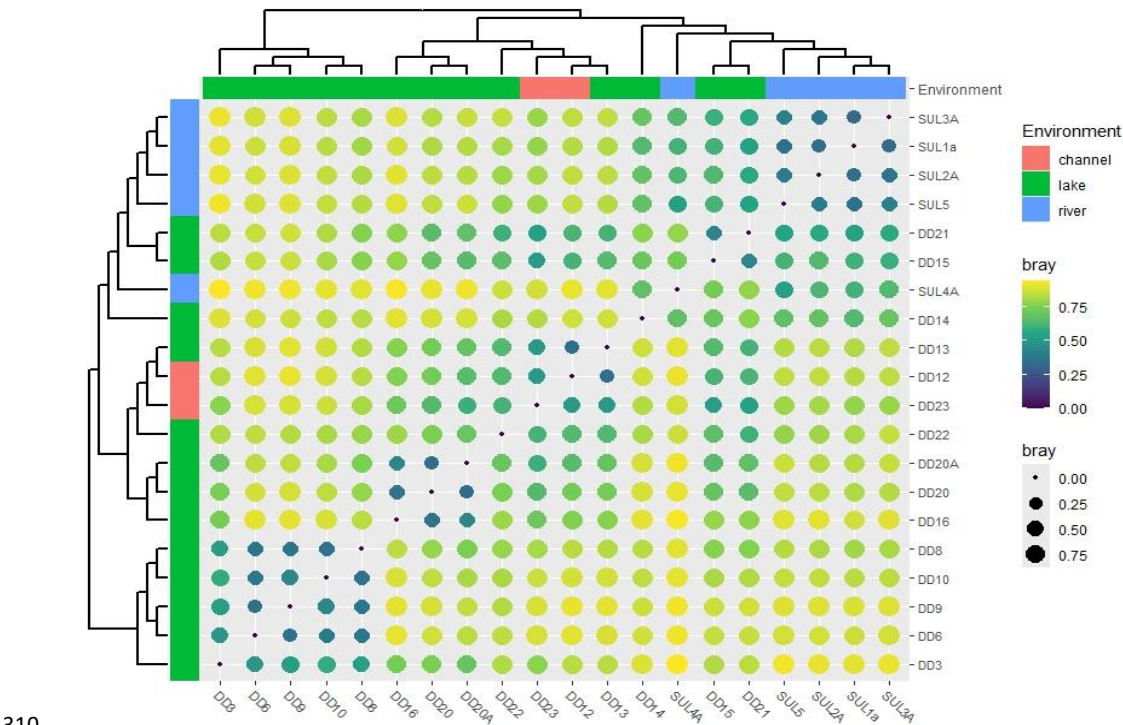


294
295 **Figure 3.** Taxonomic composition and hierarchical clustering of bacterioplankton communities across the Danube
296 Delta sampling sites ($N = 20$). The dendrogram (left) illustrates site-level community similarity based on Bray–



297 Curtis dissimilarities of phylum-level relative abundances. Colorful dots at the dendrogram tips denote the
298 macrohabitat type (red: channel, green: lake, blue: river). The stacked bar chart (right) displays the relative
299 abundance (%) of bacterial phyla for each site, with the key dominant groups (e.g., Proteobacteria,
300 Actinobacteriota, Cyanobacteria, and Verrucomicrobiota) differentiated by colour. Rare taxa are grouped under
301 the "others" category.

302 The heatmap of dominant bacterial taxa (**Figure 4**) confirmed a non-random distribution of
303 microbial communities across the investigated habitats. Hierarchical clustering separated
304 groups of samples with similar taxonomic composition and partly reflected the division into
305 riverine, channel and lake habitats. Riverine samples showed a more compact clustering pattern,
306 indicating relatively higher similarity of bacterial communities, whereas lake and channel
307 samples displayed greater taxonomic heterogeneity. Several bacterial groups showed habitat-
308 related patterns of occurrence, supporting the interpretation that microbial community
309 composition reflects the hydrological gradient across the deltaic system.



310
311 **Figure 4.** Double-hierarchical clustering matrix of bacterioplankton community beta-diversity across the Danube
312 Delta sampling sites ($N = 20$). Both axes and associated dendrograms represent individual sampling locations
313 organized by community similarity based on pairwise Bray–Curtis dissimilarities. Colored annotation bars along
314 the margins indicate macrohabitat classifications (red: channel, green: lake, blue: river). The scale bar defines the
315 Bray–Curtis dissimilarity values, where both the gradient colour (from dark purple to bright yellow) and circle



size correspond to the degree of compositional divergence between compared pairs (values closer to 0.00 indicate identical communities, while values approaching 0.75 denote maximum observed dissimilarity).

3.4. Alpha diversity patterns

Alpha-diversity indices varied within habitats but did not differ significantly among rivers, channels and lakes. Median Chao1 richness was 975.9 in rivers (IQR 946.4–1002.1), 890.6 in channels (individual values 846.3 and 934.9) and 894.3 in lakes (IQR 681.1–941.4). The global habitat effect was not significant (permutation Kruskal-Wallis $p = 0.382$) (**Figure 5**).

Median Shannon diversity was 5.60 in rivers (IQR 5.45–5.65), 4.89 in channels and 5.29 in lakes (IQR 4.85–5.62); the habitat effect was not significant ($p = 0.255$). Median Simpson diversity was 0.989 in rivers (IQR 0.988–0.990), 0.971 in channels and 0.986 in lakes (IQR 0.970–0.990), again without a significant habitat effect ($p = 0.314$). Thus, alpha-diversity indices did not show a robust habitat-level response in this single-campaign dataset, despite several low-diversity lake observations.

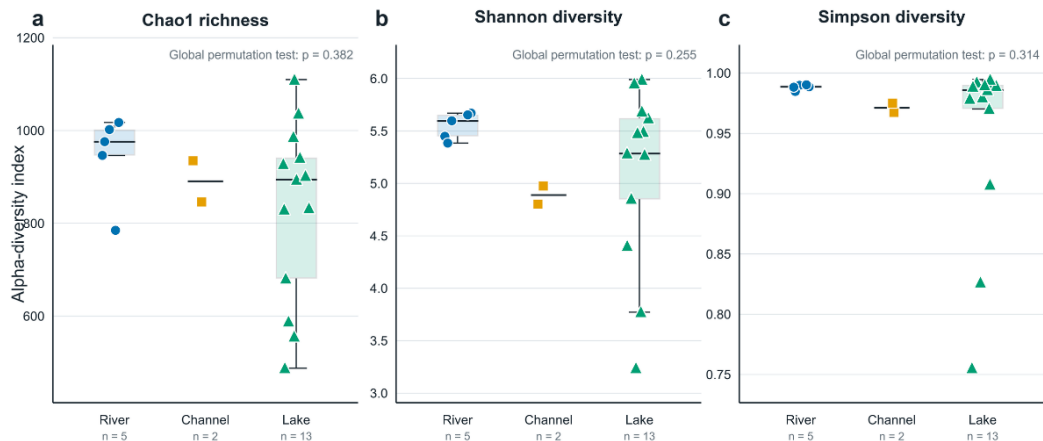
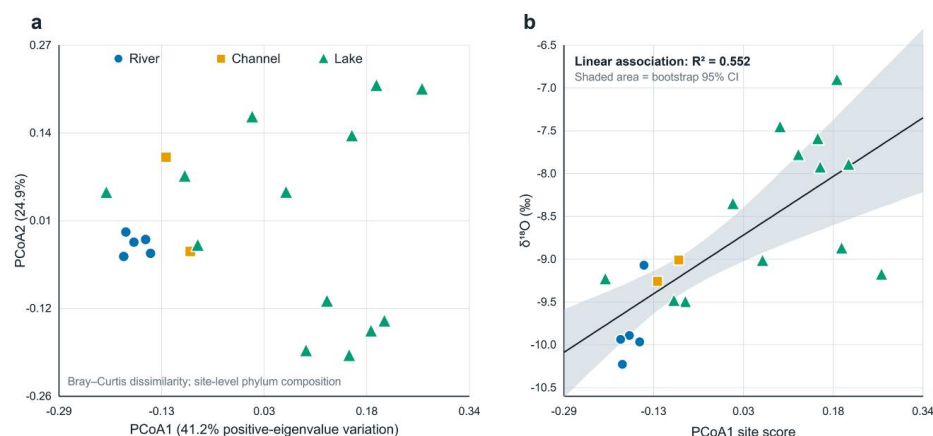


Figure 5. Alpha-diversity indices of bacterioplankton communities across the primary macrohabitat types in the Danube Delta (September 2023): (a) Chao1 richness, (b) Shannon diversity index, and (c) Simpson diversity index. Individual points represent averaged, site-level data for River ($n = 5$), Channel ($n = 2$), and Lake ($n = 13$) habitats. Boxplots illustrate the median and interquartile range (IQR) for habitats with $n \geq 3$. Global statistical differences among the three macrohabitats were evaluated using permutation-based Kruskal–Wallis tests, with the respective non-significant p -values indicated in the upper right corner of each panel.



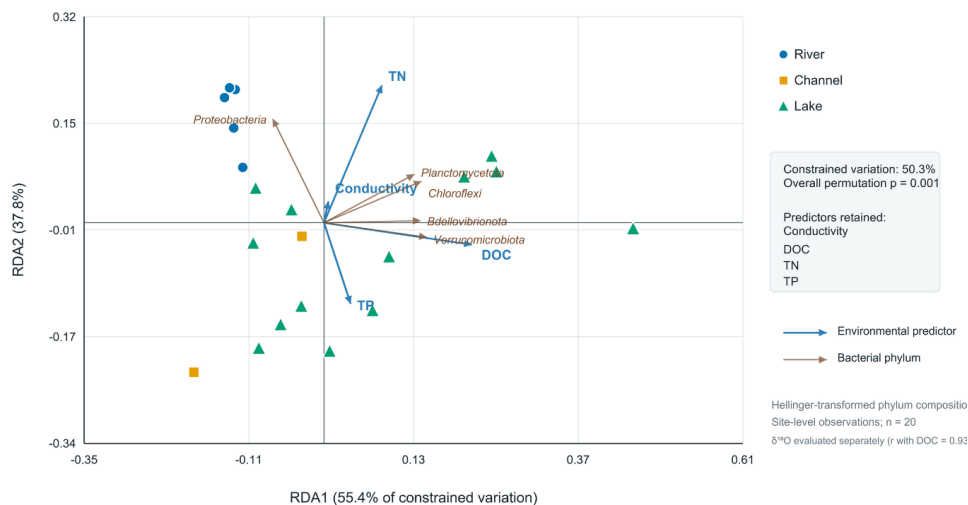
339 3.5. *Integrated environmental gradient and microbial community organisation*
 340 The first two PCoA axes represented 41.2% and 24.9% of the positive-eigenvalue variation in
 341 Bray-Curtis dissimilarity, respectively. River sites occupied a compact region at negative
 342 PCoA1 scores, whereas lake sites extended across a broader ordination space; the two channel
 343 sites were intermediate (**Figure 6**).
 344 Habitat explained 34.5% of Bray-Curtis variation (PERMANOVA pseudo-F = 4.47, p =
 345 0.0003). However, multivariate dispersion also differed among habitats (F = 17.54, p = 0.0001),
 346 mainly because lake communities were more heterogeneous than river communities. The
 347 PERMANOVA result therefore reflects a combination of habitat-associated location and
 348 dispersion effects and should not be interpreted as centroid separation alone.
 349 PCoA1 was positively associated with $\delta^{18}\text{O}$ (r = 0.743, R^2 = 0.552, permutation p = 0.0004).
 350 The fitted slope was 4.41‰ per PCoA1 unit, with a bootstrap 95% confidence interval of 2.36–
 351 6.81. This association links microbial community turnover with the isotope-defined
 352 hydrological gradient without implying that isotope enrichment directly caused community
 353 change.



354 **Figure 6.** Ordination and isotope-coupling analysis of bacterioplankton communities across the Danube Delta
 355 macrohabitats: **(a)** Principal Coordinates Analysis (PCoA) based on pairwise Bray–Curtis dissimilarities of site-
 356 level phylum relative abundances. The first axis (PCoA1) explains 41.2% of the positive-eigenvalue variation,
 357 capture-marking a clear compositional gradient from stable riverine locations to highly variable lake environments.
 358 **(b)** Linear regression between individual PCoA1 site scores and surface-water $\delta^{18}\text{O}$ values (‰, VSMOW). The
 359 solid black line indicates the fitted ordinary least-squares linear association (R^2 = 0.552), with the shaded area
 360



361 representing the bootstrap 95% confidence interval, indicating a strong association between isotope-defined
362 hydrological gradients and beta-diversity turnover.
363
364
365 *3.6. Environmental drivers of bacterial community structure*
366 The RDA constrained by conductivity, DOC, TN and TP explained 50.3% of total Hellinger-
367 transformed phylum-level variation (adjusted $R^2 = 0.371$; overall permutation $p = 0.001$). RDA1
368 and RDA2 represented 55.4% and 37.8% of constrained variation, respectively (**Figure 7**). VIF
369 values were 1.19 for conductivity, 1.38 for DOC, 1.39 for TN and 1.34 for TP, indicating low
370 collinearity in the retained model.

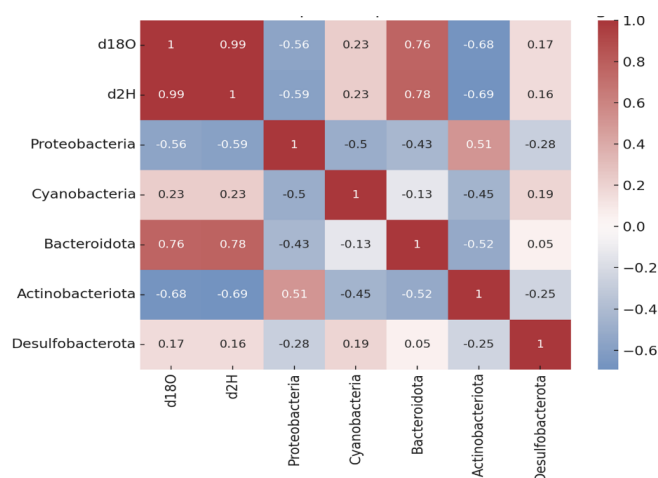


371
372 **Figure 7.** Redundancy analysis (RDA) biplot illustrating the relationships between constrained environmental
373 predictors and bacterioplankton community composition at the phylum level across the Danube Delta ($N = 20$).
374 Environmental predictors (blue vectors) include conductivity, dissolved organic carbon (DOC), total nitrogen
375 (TN), and total phosphorus (TP). Brown vectors represent the coordinates and directional response of key bacterial
376 phyla (e.g., Proteobacteria, Planctomycetota, Chloroflexi, Bdellovibrionota, and Verrucomicrobiota). The final
377 model explains 50.3% of the total Hellinger-transformed taxonomic variation, with an overall highly significant
378 statistical structure determined by permutation tests ($p = 0.001$). Individual stations are shape- and colour-coded
379 by macrohabitat type (blue circles: river, yellow squares: channel, green triangles: lake). Note: $\delta^{18}\text{O}$ was evaluated
380 separately due to strong collinearity with DOC ($r = 0.933$).
381
382 DOC and conductivity were aligned mainly with positive RDA1 scores, TN with positive
383 RDA2 scores and TP with negative RDA2 scores. Among the five longest displayed taxon
384 vectors, Planctomycetota, Chloroflexi, Verrucomicrobiota and Bdellovibrionota were oriented



385 toward positive RDA1, whereas Proteobacteria was oriented toward negative RDA1 and
 386 positive RDA2. These vectors represent multivariate associations rather than taxon-specific
 387 causal responses. DOC was retained because of its ecological role in carbon availability; $\delta^{18}\text{O}$
 388 was excluded from this same model because its correlation with DOC was high ($r = 0.933$) and
 389 was evaluated separately.

390 At the phylum level, isotope associations were directional but uneven among taxa (**Figure 8**).
 391 Verrucomicrobiota showed the strongest positive association with the isotope-enrichment axis
 392 ($\delta^{18}\text{O}$: $r = 0.760$, FDR-adjusted $p = 0.002$; $\delta^2\text{H}$: $r = 0.773$, FDR-adjusted $p = 0.002$) and a
 393 negative association with d-excess ($r = -0.699$, FDR-adjusted $p = 0.004$). Proteobacteria and
 394 Actinobacteriota were negatively associated with $\delta^{18}\text{O}$ and $\delta^2\text{H}$ after FDR correction, consistent
 395 with their greater contribution in more connected, isotopically depleted riverine waters.
 396 Bacteroidota and Cyanobacteria showed positive but weaker relationships with isotope
 397 enrichment that were not significant after FDR correction, indicating that their ecological
 398 interpretation should rely on the broader retention-DOC-trophic gradient rather than on isotope
 399 values alone.

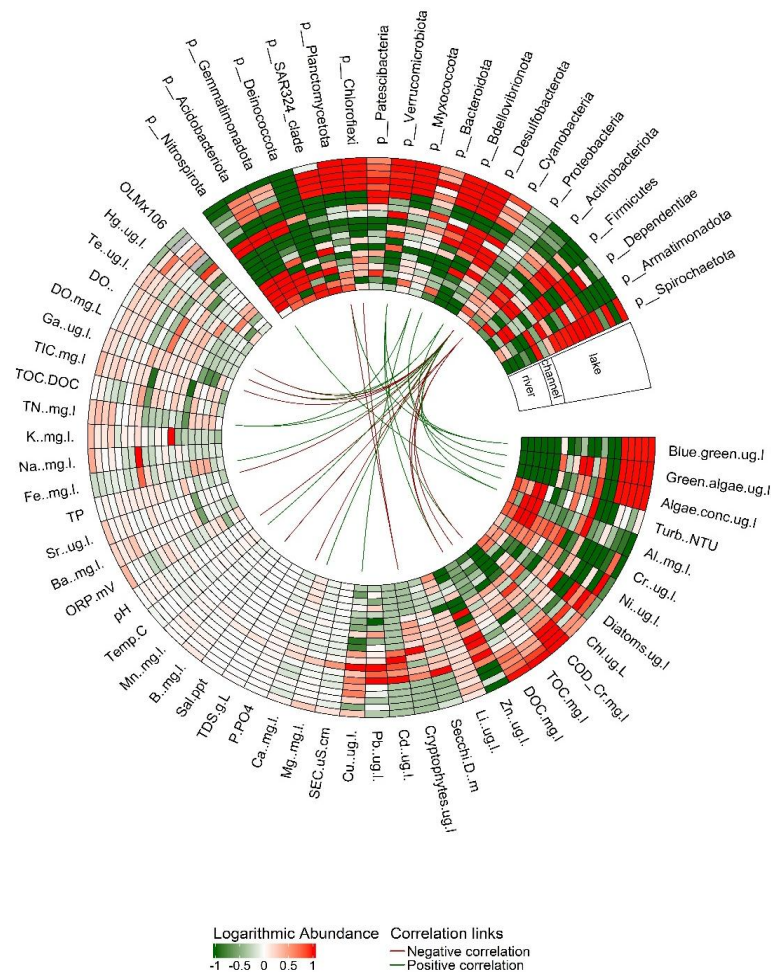


400

401 **Figure 8.** Correlation matrix between water isotope composition and dominant bacterial phyla in the Danube
 402 Delta. Colours indicate the direction and strength of Pearson correlation coefficients calculated from site-level
 403 phylum relative abundances and isotope variables. Reported associations should be interpreted as directional
 404 relationships rather than independent taxon-specific effects, owing to the compositional nature of the data and
 405 the observational design of the study.



406 The circular correlation map showed that dominant bacterial phyla varied across the measured
407 environmental gradient (**Figure 9**). Positive and negative links were observed between selected
408 microbial groups and physicochemical or isotopic variables, indicating non-random
409 associations between bacterioplankton composition and water properties. These patterns were
410 consistent with the RDA results and supported the observed separation of riverine, channel and
411 lake habitats.



412

413 **Figure 9.** Circular correlation map showing associations between dominant bacterioplankton phyla and
414 environmental variables in Danube Delta waters. The outer heatmap represents log-transformed relative abundance
415 patterns of bacterial phyla and environmental variables across sampling sites. Green and red colours indicate lower
416 and higher values, respectively. Internal links indicate significant positive or negative correlations between
417 microbial groups and environmental parameters.



3.7. Microbial associations with hydrological and trophic conditions

The observed patterns identify microbial associations with hydrological and trophic conditions rather than validated operational indicators (**Table 2**). Riverine waters, characterised by depleted isotope values and higher hydrological connectivity, were associated mainly with Proteobacteria and Actinobacteriota. Retention-dominated lake habitats were associated with isotope enrichment, higher DOC and greater contributions of Verrucomicrobiota, Cyanobacteria and Bacteroidota. Channel habitats showed a transitional signal, but the channel group contained only two sites and should not be used to define channel-specific indicator taxa. Thus, microbial community composition, particularly beta-diversity and selected dominant phyla, provides a stronger biological signal of hydrological connectivity, retention and trophic transformation than alpha diversity in this dataset.

Table 2. Candidate microbial associations with hydrological and trophic conditions in the Danube Delta. Associations are exploratory and require independent temporal and spatial validation before operational use as indicators.

Habitat type	$\delta^{18}\text{O}$ range	Dominant microbial groups	Ecological interpretation
Lakes: Roşuleţ, Puiu, Roşu	−6.9 to −7.9‰	Cyanobacteria, Proteobacteria, Bacteroidota	Evaporation, retention, retention-dominated conditions, organic-matter accumulation and trophic development
Channels: Mila 23, Eracle	ca. −9.0 ± 0.2‰	Firmicutes, Gemmatimonadota, Desulfobacterota	Transitional conditions and mixed hydrological influence, possible anaerobic microzones
River: Sulina	−9.9 to −10.2‰	Proteobacteria, Actinobacteriota, Nitrospirota	Riverine flow, higher connectivity, shorter residence time, oxygenated conditions

4. Discussion

4.1. Isotope-defined hydrological gradient in a deltaic setting

The stable-isotope composition of surface waters in the Danube Delta revealed a coherent hydrological gradient from connected riverine sites to retention-dominated lakes. River waters were consistently depleted in $\delta^{18}\text{O}$ and $\delta^2\text{H}$, lakes were enriched, and channel sites occupied an



intermediate position, so each macrohabitat can be placed along a continuum of connectivity and retention rather than treated as isolated units. The fitted relationship between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ with a slope well below the Global Meteoric Water Line (slope 5.220, $R^2 = 0.981$) and high goodness-of-fit, is consistent with increasing evaporative influence and greater effective retention along the river–channel–lake continuum.. Interpreting this relationship as a surface-water evaporation line, rather than as a local meteoric water line, is therefore a necessary and defensible choice for a single-campaign surface-water dataset.

This pattern is consistent with frameworks in which lakes act as evaporative end-members within river networks and lake $\delta^{18}\text{O}$ integrates both catchment-scale climate and local hydrological setting (Gibson et al., 2005; Vystavna et al., 2021). In the Danube Delta, however, the value of these isotopic metrics extends beyond descriptive hydrology. The steep enrichment gradient observed over a relatively short distance shows that connectivity, residence time and evaporation vary strongly even within a protected wetland, and that $\delta^{18}\text{O}$, $\delta^2\text{H}$ and d-excess provide a process-based hydrological context for interpreting nutrient retention, DOC accumulation and microbiome turnover.

Integrating stable water isotopes with biological data has previously been recognised as a useful way to link hydrological processes with ecological responses in aquatic systems (Warter et al., 2024). present study extends this idea by using natural isotope variation in surface waters as an organising axis for microbial community structure. Rather than treating isotopes as ancillary physicochemical variables, we use them as integrated tracers of water history that help explain why bacterioplankton communities diverge among riverine, channel, and lake habitats.

460

4.2. Invariance of alpha-diversity along the gradient and the prominence of beta turnover

Our results showed that alpha-diversity metrics have limited diagnostic value along the isotope-defined gradient. Richness and evenness differed only weakly among habitats, and the few lake sites with lower diversity did not produce a consistent habitat-level signal. Shannon, Simpson



465 and Pielou's evenness covered broad ranges but did not show significant relationships with
466 $\delta^{18}\text{O}$, $\delta^2\text{H}$ or d-excess, indicating that local bacterial richness and evenness did not directly track
467 the hydrological history of the sampled waters.

468 This lack of a strong alpha-diversity response is ecologically plausible. In connected aquatic
469 networks, dispersal from regional species pools can maintain local richness even when habitats
470 differ in water residence time, DOC availability or isotope composition. Alpha diversity may
471 therefore remain relatively stable while community composition changes substantially. Similar
472 patterns have been reported from river systems and lake–river continua where alpha-diversity
473 often fails to distinguish habitats or stress gradients, whereas beta-diversity reveals marked
474 turnover along environmental or hydrological axes (Mori et al., 2018; Savio et al., 2015;
475 Sadeghi et al., 2024).

476 For ecological assessment, the implication is clear: alpha-diversity alone is unlikely to provide
477 a robust indicator of hydrological connectivity in this deltaic setting, and its apparent stability
478 should not be interpreted as ecological uniformity. The contrast between weak alpha-diversity
479 responses and strong compositional turnover suggests that microbiome-based indicators
480 should emphasise taxonomic replacement, ordination patterns and dominant community
481 components rather than richness metrics alone. This interpretation is consistent with the view
482 that beta-diversity better captures environmental selection, spatial reorganisation and
483 ecosystem functioning than local taxonomic richness in heterogeneous systems (Mori et al.,
484 2018; Tang et al., 2020; Xiao et al., 2024).

485

486 *4.3. Microbial beta diversity and community reorganization along the hydrological gradient*

487 In contrast to the invariance of alpha diversity, Bray–Curtis dissimilarities and ordination
488 analyses revealed a pronounced reorganization of bacterioplankton communities along the
489 hydrological gradient. Riverine communities formed a compact, homogenous cluster at
490 negative PCoA1 scores, whereas lake communities occupied a broader ordination space



491 associated with enriched $\delta^{18}\text{O}$ values, leaving channel stations in an intermediate position.
492 Macrohabitat type explained a substantial fraction of beta-diversity variation, and the primary
493 axis of community turnover was strongly and linearly associated with $\delta^{18}\text{O}$ enrichment. Thus,
494 taxonomic turnover closely tracked the stable-isotope signature of surface-water history, even
495 when local richness and evenness were similar across habitats.

496 The simultaneous detection of distinct habitat clustering and higher beta-dispersion within
497 lacustrine communities is particularly informative. It shows that retention-dominated lakes
498 differ from riverine channels not only in mean taxonomic composition, but also in the overall
499 heterogeneity of their microbiomes (Crevecoeur et al., 2023). In lotic environments,
500 downstream transport and physical homogenization exert a dominant control; conversely,
501 prolonged water residence times in lentic basins allow local environmental filtering, organic-
502 matter processing, and within-lake niche selection to become more prominent (Newton et al.,
503 2011; Langenheder and Lindström, 2019). Thus, higher hydrological connectivity is consistent
504 with stronger dispersal and mass effects, whereas greater effective retention may increase the
505 opportunity for local environmental filtering and organic-matter processing.

506 Taken together, these results show that beta-diversity and ordination-based patterns provide
507 sensitive measures of microbial community reorganisation along gradients of hydrological
508 connectivity and effective retention. The tight coupling between microbial beta-diversity and
509 hydrological context aligns with previous frameworks showing that physical hydrology and
510 local hydrochemistry jointly drive bacterioplankton biogeography across freshwater networks
511 (Niño-García et al., 2016). Similar patterns have been documented in complex river–lake
512 continua, where microbial community structure reflects the balance between hydrological
513 dispersal and local environmental selection (Stadler et al., 2022; Sadeghi et al., 2024). The main
514 advance here is that the underlying hydrological gradient was independently and continuously
515 constrained by $\delta^{18}\text{O}$ and $\delta^2\text{H}$ signatures, reducing reliance on arbitrary, discrete habitat
516 categories.



Integrating natural $\delta^{18}\text{O}$ and $\delta^2\text{H}$ variation with microbial community turnover represents a central contribution of this study. Stable isotopes are routinely coupled with microbial analyses in groundwater, sediments or experimental stable-isotope probing (Boschker and Middelburg, 2002; Neufeld et al., 2007; Warter et al., 2024), but here ambient isotope composition of surface waters is used to define a landscape-scale hydrological gradient in a large river delta. This shifts the interpretation of bacterioplankton communities: rather than viewing them solely as local responders to current chemistry, they can be used as biological records of water history, connectivity and catchment-scale retention.

4.4. Environmental controls and taxon-specific associations

The phylum-level bacterioplankton composition was typical of freshwater networks but varied systematically across macrohabitats. The dataset was dominated by Proteobacteria (mean relative abundance 39.4%), Actinobacteriota (23.8%), and Cyanobacteria (12.6%), with Bacteroidota, Verrucomicrobiota, and Planctomycetota persisting as secondary components, aligning with established freshwater models (Newton et al., 2011; Savio et al., 2015). These groups displayed distinct spatial sorting: riverine sites were predominantly associated with Proteobacteria and Actinobacteriota, whereas enriched, lentic habitats exhibited higher contributions of Cyanobacteria, Bacteroidota, and Verrucomicrobiota.

Redundancy analysis constrained by conductivity, DOC, TN, and TP accounted for 50.3% of the Hellinger-transformed taxonomic variation, identifying DOC and conductivity as the primary drivers of community reorganization. Because DOC and $\delta^{18}\text{O}$ were highly correlated ($r = 0.933$), analyzing DOC within the RDA model while evaluating $\delta^{18}\text{O}$ via complementary isotope-ordination analyses successfully resolved collinearity while maintaining the mechanistic distinction between carbon availability and water history.

Among the responsive taxa, Verrucomicrobiota showed the strongest isotope-linked response, increasing along the enriched $\delta^{18}\text{O}$ – $\delta^2\text{H}$ axis and decreasing with d -excess after FDR correction. This association is consistent with previous reports linking some Verrucomicrobiota lineages



543 to the degradation of complex polysaccharides and high-molecular-weight substrates (Cardman
544 et al., 2014). Conversely, Bacteroidota, while characteristic of lake habitats, exhibited weaker
545 direct correlations with isotope variables that did not remain significant after multiple-testing
546 correction; given their established role in processing labile and complex organic compounds
547 (Newton et al., 2011; Kirchman, 2018), they are better viewed as indicators of organic-matter
548 and trophic processing under high-retention conditions than as direct isotope indicators.

549 Cyanobacteria expanded in lake communities, under the combined influence of prolonged
550 residence times, hydrological stability and nutrient accumulation in shallow lentic basins (Paerl
551 and Otten, 2013; Reinl et al., 2021) and should likewise be considered part of the broader
552 biological response to retention and trophic status rather than markers of water origin.

553 In contrast, the riverine microbiome was defined by flowing, isotopically depleted waters that
554 favor high connectivity. Negative correlations of Actinobacteriota and Proteobacteria with $\delta^{18}\text{O}$
555 and $\delta^2\text{H}$ reflect fresh riverine inputs, active mixing, and transport. The success of
556 Actinobacteriota in these lotic zones likely stems from their small cell size and efficient
557 utilization of low-concentration substrates under reduced local retention (Newton et al., 2011;
558 Salcher et al., 2011). Because Proteobacteria encompass highly diverse metabolic lineages,
559 their dominance in riverine environments reflects physical hydrological transport and
560 continuous water exchange rather than a single metabolic process; reduced flow and
561 fragmentation can otherwise restrict dispersal and alter composition by modifying redox
562 conditions and DOM quality (Fazi et al., 2013; Stadler and del Giorgio, 2022).

563 These taxon-specific associations reveal contrasting microbial signatures along the
564 hydrological–biogeochemical gradient. Proteobacteria and Actinobacteriota mark connected,
565 isotopically depleted river waters, whereas Verrucomicrobiota, Cyanobacteria, and
566 Bacteroidota characterize enriched, retention-dominated lakes. Channel communities display
567 intermediate signatures, but their limited sample size precludes defining channel-specific
568 indicators. Focusing on phylum-level patterns is an appropriate, conservative choice for this



dataset, reducing the risk of overinterpreting finer taxonomic responses that are more sensitive to sample size and compositional effects.

4.5. *Towards a process-based framework for microbial community reorganisation across deltaic networks*

The combined analysis of stable water isotopes, hydrochemical variables and bacterioplankton turnover supports a process-based conceptual model of microbial community reorganisation across deltaic networks. (**Figure 10**). Hydrological connectivity influences effective water retention and exposure to evaporation, which are reflected in $\delta^{18}\text{O}$, $\delta^2\text{H}$ and d-excess. DOC and nutrients characterise the contemporary resource environment associated with that water history, whereas bacterioplankton turnover captures biological reorganisation along the same gradient. Together, these components describe a coupled hydrological–biogeochemical continuum rather than independent environmental signals.

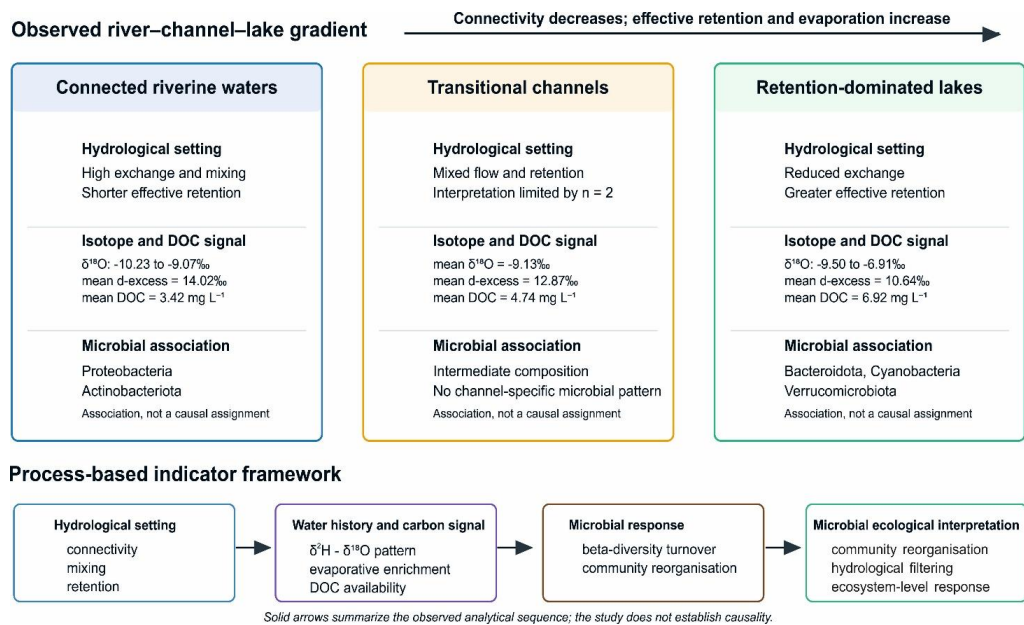


Figure 10. Conceptual diagram illustrating the observed environmental gradient and the process-based indicator framework in the Danube Delta. Top panel: Characteristics of the spatial continuum from connected riverine waters ($n = 5$), through transitional channels ($n = 2$), to retention-dominated lakes ($n = 13$). The arrow denotes the directional gradient of decreasing hydrological connectivity and increasing effective water retention and evaporation, coupled with shifting stable isotope signatures ($\delta^{18}\text{O}$, d-excess), dissolved organic carbon (DOC) concentrations, and dominant bacterioplankton phyla. Bottom panel: The sequential, process-based framework



588 linking the physical hydrological setting to chemical tracer signals, multi-taxon microbial responses (community
589 turnover), and their linking physical hydrological conditions, chemical tracer signals and microbial community
590 reorganisation. Solid arrows represent the analytical sequence observed in this study and do not imply direct,
591 experimental causality.

592 This integrated approach offers several advantages for environmental monitoring. First,
593 isotopes provide a physically grounded metric of hydrological history that distinguishes
594 external inputs, mixing, evaporation and retention. Second, microbiome turnover aggregates
595 complex biological responses across many taxa, integrating cumulative hydrological and
596 biogeochemical conditions more effectively than snapshot nutrient measurements. Third,
597 responsive phyla associated with distinct combinations of connectivity and DOC can serve as
598 early-warning signals of emerging retention and trophic shifts before they manifest as overt
599 disturbances such as algal blooms, severe oxygen depletion or high chlorophyll-a.

600 Our multivariate analyses reinforce this conceptualisation by showing that microbiome
601 structure is associated with an integrated gradient combining water history, retention,
602 mineralisation and trophic state.. Hydrology may influence bacterioplankton directly via
603 transport and mixing (mass effects) and indirectly by shaping substrate availability, consistent
604 with previous river-network frameworks (Niño-García et al., 2016; Stadler and del Giorgio,
605 2022).

606 *Study limitations and future directions*

607 Several limitations define the scope of our inferences. Based on a single late-summer campaign
608 with 20 sites, the study captures spatial gradients but not seasonal or interannual dynamics that
609 may modulate isotope–DOC–microbiome couplings. Water isotopes reflect source and
610 evaporative history but do not directly quantify residence times without explicit water-balance
611 modelling. Taxon–environment correlations may partly reflect shared habitat covariation and
612 the compositional nature of relative-abundance data. Phylum-level analyses offer robustness
613 for the available sample size, but may mask functionally important responses at finer taxonomic
614 or genomic resolution. Moreover, broad taxonomic groups may encompass lineages with
615 markedly different growth dynamics and ecological strategies, as demonstrated for freshwater



Actinobacteria and Verrucomicrobia (Piwosz et al., 2018), reinforcing the need for caution when inferring ecological function from phylum-level relative-abundance patterns. Future work should implement multi-season longitudinal sampling and increase replication in transitional channels. Integrating multi-omic approaches (e.g. metagenomics, metatranscriptomics) with additional tracers such as $\delta^{13}\text{C}$ of dissolved inorganic and organic carbon or nutrient-specific isotopes would help separate external catchment inputs from in situ processing. Linking microbial indicator structures to direct ecosystem-function metrics – such as respiration rates, primary production and greenhouse-gas fluxes – will further clarify the functional consequences of community shifts. Extending this framework across seasons and hydrological conditions will help determine how persistent these isotope–DOC–microbiome relationships are and clarify their relevance for understanding the biogeochemical functioning of deltaic freshwater networks.

5. Conclusions

Stable water isotopes ($\delta^{18}\text{O}$, $\delta^2\text{H}$) and bacterioplankton community composition provided complementary evidence for a clear river-to-lake gradient in hydrological connectivity and water retention across the Danube Delta. The surface-water evaporation line (slope = 5.220) and systematically declining *d*-excess values indicated increasing evaporative enrichment in lake habitats, while microbial beta diversity and dominant phylum-level shifts captured the corresponding biological reorganization along this hydrological continuum. In contrast, alpha-diversity metrics remained invariant and insensitive to the gradient, demonstrating limited standalone diagnostic value.

Together, these results identify the Danube Delta as a spatially structured hydrological–biogeochemical continuum in which differences in water history and effective retention coincide with pronounced microbial community reorganisation. Integrating stable-water-isotope tracers with bacterioplankton community analysis provides a process-based approach



641 for linking hydrological history with biological organisation across heterogeneous freshwater
642 networks.

643 **6. Data availability**

644 Raw 16S rRNA gene amplicon reads have been deposited in the European Nucleotide Archive
645 under accession number PRJEB112832.

646 **Author contributions.** SL: Conceptualization, Methodology, Formal analysis, Data curation,
647 Visualization, Supervision, Writing – original draft, Writing – review and editing. PB:
648 Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization.
649 ŁP: Methodology, Software, Data curation, Visualization. KO: Conceptualization, Writing –
650 original draft, Writing – review and editing. AG: Investigation, Methodology, Writing –
651 original draft. MG: Investigation, Methodology, Writing – original draft. IC: Conceptualization,
652 Resources, Project administration. KG-L: Conceptualization, Investigation, Data curation,
653 Supervision, Funding acquisition, Project administration, Writing – original draft, Writing –
654 review and editing.

655

656 **Conflict of Interest:**

657 None declared.

658 **Ethical Statement:**

659 The research was done according to ethical standards.

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668 References

- 669 Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance.
 670 *Austral Ecology* 26, 32-46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>
- 671 Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions.
 672 *Biometrics* 62, 245-253. <https://doi.org/10.1111/j.1541-0420.2005.00440.x>
- 673 APHA, AWWA, WEF, 2017. *Standard Methods for the Examination of Water and Wastewater*,
 674 23rd ed. American Public Health Association, Washington, DC.
- 675 Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: A practical and
 676 powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B* 57,
 677 289-300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- 678 Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A.,
 679 Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K.,
 680 Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase,
 681 J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M.,
 682 Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons,
 683 S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H.,
 684 Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang,
 685 K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciulek, T., Kreps, J.,
 686 Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz,
 687 C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C.,
 688 Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T.,
 689 Peoples, S.L., Petras, D., Preuss, M.L., Priesse, E., Rasmussen, L.B., Rivers, A., Robeson,
 690 M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R.,
 691 Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-
 692 Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M.,
 693 Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D.,
 694 Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible,
 695 interactive, scalable and extensible microbiome data science using QIIME 2. *Nature*
 696 *Biotechnology* 37, 852-857. <https://doi.org/10.1038/s41587-019-0209-9>
- 697 Boschker, H.T.S., Middelburg, J.J., 2002. Stable isotopes and biomarkers in microbial ecology.
 698 *FEMS Microbiology Ecology* 40, 85-95. [https://doi.org/10.1016/S0168-6496\(02\)00194-0](https://doi.org/10.1016/S0168-6496(02)00194-0)
- 699 Brand, W.A., Coplen, T.B., Vogl, J., Rosner, M., Prohaska, T., 2014. Assessment of
 700 international reference materials for isotope-ratio analysis (IUPAC Technical Report). *Pure and*
 701 *Applied Chemistry* 86, 425-467. <https://doi.org/10.1515/pac-2013-1023>
- 702 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016.
 703 DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13,
 704 581-583. <https://doi.org/10.1038/nmeth.3869>
- 705 Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A.D., Teske, A., 2014.
 706 Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an Arctic
 707 fjord. *Applied and Environmental Microbiology* 80, 3749-3756.
 708 <https://doi.org/10.1128/AEM.00899-14>



- 709 Coplen, T.B., 2011. Guidelines and recommended terms for expression of stable-isotope-ratio
 710 and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry* 25, 2538-
 711 2560. <https://doi.org/10.1002/rcm.5129>
- 712 Craig, H., 1961. Isotopic variations in meteoric waters. *Science* 133, 1702-1703.
 713 <https://doi.org/10.1126/science.133.3465.1702>
- 714 Crawford, J., Hughes, C.E., Lykoudis, S., 2014. Alternative least squares methods for
 715 determining the meteoric water line, demonstrated using GNIP data. *Journal of Hydrology* 519,
 716 2331-2340. <https://doi.org/10.1016/j.jhydrol.2014.10.033>
- 717 Crevecoeur, S., Vincent, W.F., Lovejoy, C., Mohit, V., Culley, A.I., 2023. Spatio-temporal
 718 connectivity of the aquatic microbiome in the Laurentian Great Lakes. *Frontiers in*
 719 *Microbiology* 14, 1073753. <https://doi.org/10.3389/fmicb.2023.1073753>
- 720 Daims, H., Pelz, O., Hanert, B., Wagner, M., 2006. Linking microbial community structure to
 721 in situ activity using stable isotope probing and fluorescence in situ hybridization.
 722 *Environmental Microbiology* 8, 1218-1230. <https://doi.org/10.1111/j.1462-2920.2006.01007.x>
- 723 Deltares, 2023. Understanding the delta response to climate change. Impact Report:
 724 Delivering Impact 2023. Deltares, Delft.
 725 [https://specials.deltares.nl/impact_report_2023/understanding_the_delta_response_to_climate](https://specials.deltares.nl/impact_report_2023/understanding_the_delta_response_to_climate_change)
 726 [change](https://specials.deltares.nl/impact_report_2023/understanding_the_delta_response_to_climate_change) (date: 12/05/2026)
- 727 Ellison, J.C., 2015. Vulnerability assessment of mangroves to climate change and sea-level rise
 728 impacts. *Wetlands Ecology and Management* 23, 115-137. [https://doi.org/10.1007/s11273-014-](https://doi.org/10.1007/s11273-014-9397-8)
 729 [9397-8](https://doi.org/10.1007/s11273-014-9397-8)
- 730 Ericson, J.P., Vörösmarty, C.J., Dingman, S.L., Ward, L.G., Meybeck, M., 2006. Effective sea-
 731 level rise and deltas: Causes of change and human dimension implications. *Global and*
 732 *Planetary Change* 50, 63-82. <https://doi.org/10.1016/j.gloplacha.2005.07.004>
- 733 Fazi, S., Vazquez, E., Casamayor, E.O., Amalfitano, S., Butturini, A., 2013. Stream
 734 hydrological fragmentation drives bacterioplankton community composition. *PLOS ONE* 8,
 735 e64109. <https://doi.org/10.1371/journal.pone.0064109>
- 736 Gat, J.R., 1996. Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review of Earth*
 737 *and Planetary Sciences* 24, 225-262. <https://doi.org/10.1146/annurev.earth.24.1.225>
- 738 Gibson, J.J., Edwards, T.W.D., Birks, S.J., 2005. Progress in isotope tracer hydrology.
 739 *Hydrological Processes* 19, 303-327. <https://doi.org/10.1002/hyp.5766>
- 740 Giosan, L., Syvitski, J.P.M., Constantinescu, S., Day, J., 2014. Climate change: Protect the
 741 world's deltas. *Nature* 516, 31-33. <https://doi.org/10.1038/516031a>
- 742 ICPDR, 2019. Significant Water Management Issues in the Danube River Basin District -
 743 Nutrient Pollution Factsheet. International Commission for the Protection of the Danube River,
 744 Vienna. https://www.danubesurvey.org/jds3/files/nodes/documents/factsheet4-jds3_0.pdf
- 745 Kirchman, D.L., 2018. *Processes in Microbial Ecology*, second ed. Oxford University Press,
 746 Oxford.
- 747 Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Gloeckner, F.O.,
 748 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-



- 749 generation sequencing-based diversity studies. *Nucleic Acids Research* 41, e1.
750 <https://doi.org/10.1093/nar/gks808>
- 751 Langenheder, S., Lindström, E.S., 2019. Factors influencing aquatic and terrestrial bacterial
752 community assembly. *Environmental Microbiology Reports* 11, 306-315.
753 <https://doi.org/10.1111/1758-2229.12731>
- 754 Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of
755 species data. *Oecologia* 129, 271-280. <https://doi.org/10.1007/s004420100716>
- 756 Mori, A.S., Isbell, F., Seidl, R., 2018. beta-Diversity, community assembly, and ecosystem
757 functioning. *Trends in Ecology & Evolution* 33, 549-564.
758 <https://doi.org/10.1016/j.tree.2018.04.012>
- 759 Neufeld, J.D., Vohra, J., Dumont, M.G., Lueders, T., Manefield, M., Friedrich, M.W., Murrell,
760 J.C., 2007. DNA stable-isotope probing. *Nature Protocols* 2, 860-866.
761 <https://doi.org/10.1038/nprot.2007.109>
- 762 Newton, R.J., Jones, S.E., Eiler, A., McMahon, K.D., Bertilsson, S., 2011. A guide to the natural
763 history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews* 75, 14-49.
764 <https://doi.org/10.1128/MMBR.00028-10>
- 765 Niño-García, J.P., Ruiz-González, C., Del Giorgio, P.A., 2016. Interactions between hydrology
766 and water chemistry shape bacterioplankton biogeography across boreal freshwater networks.
767 *The ISME Journal* 10, 1755-1766. <https://doi.org/10.1038/ismej.2015.226>
- 768 Paerl, H.W., Otten, T.G., 2013. Harmful cyanobacterial blooms: Causes, consequences, and
769 controls. *Microbial Ecology* 65, 995-1010. <https://doi.org/10.1007/s00248-012-0159-y>
- 770 Pimentel, M., Botrel, M., Pegoraro, E., Perez, C.A., Cerri, C.E.P., Couto, H.T.Z., Carmo, J.B.,
771 Cerri, C.C., 2014. Using stable isotopes to link microbial community function and structure in
772 soils. *Rapid Communications in Mass Spectrometry* 28, 2533-2542.
773 <https://doi.org/10.1002/rcm.7040>
- 774 Piwosz, K., Shabarova, T., Tomasch, J., Šimek, K., Kopejtká, K., Kahl, S., Pieper, D.H.,
775 Koblížek, M., 2018. Determining lineage-specific bacterial growth curves with a novel
776 approach based on amplicon reads normalization using internal standard (ARNIS). *The ISME*
777 *Journal* 12, 2640-2654. <https://doi.org/10.1038/s41396-018-0213-y>
- 778 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Gloeckner,
779 F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and
780 web-based tools. *Nucleic Acids Research* 41, D590-D596. <https://doi.org/10.1093/nar/gks1219>
- 781 Radu, V.M., Bostan, V., 2013. Nutrients and trophic status of the Danube Delta waters. *Revue*
782 *Roumaine de Chimie* 58, 241-250.
783 <http://bch.ro/pdfRC/RADU%20V%20M.pdf%203%2013.pdf>
- 784 Ramsar Secretariat, 2015. Danube Delta celebrates 50 years of European Diploma for Protected
785 Areas. *Ramsar News*, 27 September 2015. <https://www.ramsar.org/news/danube-delta-celebrates-50-years-european-diploma-protected-areas>
- 787 Reinl, K.L., Brookes, J.D., Carey, C.C., Harris, T.D., Ibelings, B.W., Morales-Williams, A.M.,
788 de Senerpont Domis, L.N., Atkins, K.S., Isles, P.D.F., Mesman, J.P., North, R.L., Rudstam,



- 789 L.G., Stelzer, J.A.A., Weng, W., Yokota, K., Adrian, R., 2021. Cyanobacterial blooms in
790 oligotrophic lakes: Shifting the high-nutrient paradigm. *Freshwater Biology* 66, 1846-1859.
791 <https://doi.org/10.1111/fwb.13791>
- 792 Renaud, F.G., Syvitski, J.P.M., Sebesvari, Z., Werners, S.E., Kremer, H., Kuenzer, C., Ramesh,
793 R., Jeuken, A., Friedrich, J., 2013. Tipping from the Holocene to the Anthropocene: How
794 threatened are major world deltas? *Current Opinion in Environmental Sustainability* 5, 644-
795 654. <https://doi.org/10.1016/j.cosust.2013.11.007>
- 796 Reynoso, G., Smith, M.R., Holmes, C.P., Keelan, C.R., McGrath, S.E., Alvarez, G.H.,
797 Coceano, M.A., Eldridge, K.A., Fried, H.I., Gilbert, N.E., Harris, M.T., Kohler, L.R., Modolo,
798 C.M., Murray, E.A., Polisetti, S.M., Sales, D.J., Walsh, E.S., Steffen, M.M., 2019. Bacterial
799 community structure and response to nitrogen amendments in Lake Shenandoah (VA, USA).
800 *Water Science and Technology* 80, 675-684. <https://doi.org/10.2166/wst.2019.311>
- 801 Sadeghi, J., Venney, C.J., Wright, S., Watkins, J., Manning, D., Bai, E., Frank, C., Heath, D.D.,
802 2024. Aquatic bacterial community connectivity: The effect of hydrological flow on
803 community diversity and composition. *Environments* 11, 90.
804 <https://doi.org/10.3390/environments11050090>
- 805 Salcher, M.M., Pernthaler, J., Posch, T., 2011. Seasonal bloom dynamics and ecophysiology of
806 the freshwater sister clade of SAR11 bacteria ‘that rule the waves’ (LD12). *The ISME Journal*
807 5, 1242-1252. <https://doi.org/10.1038/ismej.2011.8>
- 808 Savio, D., Sinclair, L., Ijaz, U.Z., Parajka, J., Reischer, G.H., Stadler, P., Blaschke, A.P.,
809 Blöschl, G., Mach, R.L., Kirschner, A.K.T., Farnleitner, A.H., Eiler, A., 2015. Bacterial
810 diversity along a 2600 km river continuum. *Environmental Microbiology* 17, 4994-5007.
811 <https://doi.org/10.1111/1462-2920.12886>
- 812 Smercina, D.N., Evans, S.E., Friesen, M.L., Tiemann, L.K., 2019. To fix or not to fix: Controls
813 on free-living nitrogen fixation in the rhizosphere. *Applied and Environmental Microbiology*
814 85, e02546-18. <https://doi.org/10.1128/AEM.02546-18>
- 815 Stadler, M., Del Giorgio, P.A., 2022. Terrestrial connectivity, upstream aquatic history and
816 seasonality shape bacterial community assembly within a large boreal aquatic network. *The*
817 *ISME Journal* 16, 937-947. <https://doi.org/10.1038/s41396-021-01146-y>
- 818 Stegen, J.C., Johnson, T., Fredrickson, J.K., Balsley, S., Swenson, T., Jaffe, R., Miller, M.,
819 Crump, A.R., Kennedy, D.W., Nelson, W.C., Fansler, S.J., Chu, R.K., Tfaily, M.M., 2024.
820 Microbial community storm dynamics signal sources of “old” stream water. *PLOS ONE* 19,
821 e0306896. <https://doi.org/10.1371/journal.pone.0306896>
- 822 Tang, X., Xie, G., Shao, K., Hu, Y., Cai, J., Bai, C., Gong, Y., Gao, G., 2020. Contrast diversity
823 patterns and processes of microbial community assembly in a river-lake continuum across a
824 catchment scale in northwestern China. *Environmental Microbiome* 15, 10.
825 <https://doi.org/10.1186/s40793-020-00356-9>
- 826 Tessler, Z.D., Vörösmarty, C.J., Grossberg, M., Gladkova, I., Aizenman, H., Syvitski, J.P.M.,
827 Foufoula-Georgiou, E., 2015. Profiling risk and sustainability in coastal deltas of the world.
828 *Science* 349, 638-643. <https://doi.org/10.1126/science.aab3574>



- 829 UNESCO, 2024. Danube Delta Biosphere Reserve. Man and the Biosphere (MAB) Programme.
830 United Nations Educational, Scientific and Cultural Organization, Paris.
831 <https://www.unesco.org/en/mab/danube-delta>
- 832 Vadim, V., Bostan, V., 1998. Nutrients and trophic status of the Danube Delta lakes.
833 *GeoEcoMarina* 3, 97-106. [https://www.geoecomar.ro/website/publicatii/Nr.3-](https://www.geoecomar.ro/website/publicatii/Nr.3-1998/9.VadimBostan.pdf)
834 [1998/9.VadimBostan.pdf](https://www.geoecomar.ro/website/publicatii/Nr.3-1998/9.VadimBostan.pdf)
- 835 van de Giesen, N., Stive, M.J.F., Tilmans, W.M.K., Dijkstra, J.T., Hoitink, A.J.F., 2016.
836 Climate change impact on freshwater resources in a deltaic environment. In: EGU General
837 Assembly 2016, Vienna, Austria, Abstract EGU2016-14898.
838 https://presentations.copernicus.org/EGU2016/EGU2016-14898_presentation.pdf
- 839 van Geldern, R., Barth, J.A.C., 2012. Optimization of instrument setup and post-run corrections
840 for oxygen and hydrogen stable isotope measurements of water by isotope ratio infrared
841 spectroscopy (IRIS). *Limnology and Oceanography: Methods* 10, 1024-1036.
842 <https://doi.org/10.4319/lom.2012.10.1024>
- 843 Vystavna, Y., Harjung, A., Monteiro, L.R., Matiatos, I., Wassenaar, L.I., 2021. Stable isotopes
844 in global lakes integrate catchment and climatic controls on evaporation. *Nature*
845 *Communications* 12, 7224. <https://doi.org/10.1038/s41467-021-27569-x>
- 846 Warter, M.M., Tetzlaff, D., Ring, A.M., Christopher, J., Kissener, H.L., Funke, E., Sparmann,
847 S., Mbedi, S., Soulsby, C., Monaghan, M.T., 2024. Environmental DNA, hydrochemistry and
848 stable water isotopes as integrative tracers of urban ecohydrology. *Water Research* 250,
849 121065. <https://doi.org/10.1016/j.watres.2023.121065>
- 850 Xiao, P., Wu, Y., Zuo, J., Grossart, H.-P., Sun, R., Li, G., Jiang, H., Cheng, Y., Wang, Z., Geng,
851 R., Zhang, H., Ma, Z., Yan, A., Li, R., 2024. Differential microbiome features in lake-river
852 systems of Taihu basin in response to water flow disturbance. *Frontiers in Microbiology* 15,
853 1479158. <https://doi.org/10.3389/fmicb.2024.1479158>
- 854 Zhu, L., Luan, L., Chen, Y., Wang, X., Zhou, S., Zou, W., Han, X., et al., 2024. Community
855 assembly of organisms regulates soil microbial functional potential through dual mechanisms.
856 *Global Change Biology* 30, e17160. <https://doi.org/10.1111/gcb.17160>