



Mechanisms shaping dissolved organic matter and microbial community in lake ecosystems

Katarina Kajan^{a,b}, Helena Osterholz^{c,d}, James Stegen^e, Marija Gligora Udovič^f, Sandi Orlić^{a,b,*}

^a Division of Materials Chemistry, Ruder Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

^b Center of Excellence for Science and Technology-Integration of Mediterranean Region (STIM), Split, Croatia

^c Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, Oldenburg, Germany

^d Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany

^e Pacific Northwest National Laboratory, 902 Battelle Boulevard, P. O. Box 999, Richland, WA 99352, USA

^f Department of Biology, Faculty of Science, University of Zagreb, 10000 Zagreb, Croatia

ARTICLE INFO

Keywords:

Dissolved organic matter

Bacterial community

Microeukaryotic community

Lakes

Community assembly

Ecological processes

ABSTRACT

Lakes are active components of the global carbon cycle and host a range of processes that degrade and modify dissolved organic matter (DOM). Through the degradation of DOM molecules and the synthesis of new compounds, microbes in aquatic environments strongly and continuously influence chemodiversity, which can feedback to influence microbial diversity. Developing a better understanding of the biodiversity patterns that emerge along spatial and environmental gradients is one of the key objectives of community ecology. A changing climate may affect ecological feedback, including those that affect microbial communities. To maintain the function of a lake ecosystem and predict carbon cycling in the environment, it is increasingly important to understand the coupling between microbial and DOM diversity. To unravel the biotic and abiotic mechanisms that control the structure and patterns of DOM and microbial communities in lakes, we combined high-throughput sequencing and ultra-high resolution mass spectrometry together with a null modeling approach. The advantage of null models is their ability to evaluate the relative influences of stochastic and deterministic assembly processes in both DOM and microbial community assemblages. The present study includes spatio-temporal signatures of DOM and the microbial community in six temperate lakes contrasting continental and Mediterranean climates during the productive season. Different environmental conditions and nutrient sources characterized the studied lakes. Our results have shown high covariance between molecular-level DOM diversity and the diversity of individual microbial communities especially with diversity of microeukaryotes and free-living bacteria indicating their dynamic feedback. We found that the differences between lakes and climatic regions were mainly reflected in the diversity of DOM at the molecular formula-level and the microeukaryota community. Furthermore, using null models the DOM assembly was governed by deterministic variable selection operating consistently and strongly within and among lakes. In contrast, microbial community assembly processes were highly variable across lakes with different trophic status and climatic regions. Difference in the processes governing DOM and microbial composition does not indicate weak coupling between these components, rather it suggests that distinct factors may be influencing microbial communities and DOM assemblages separately. Further understanding of the DOM-microbe coupling (or lack thereof) is key to formulating predictive models of future lake ecology and function.

1. Introduction

Lakes are hotspots of biodiversity covering only a small area of terrestrial landscape but are nonetheless active sites for the transport, production, transformation and storage of significant amounts of carbon

with a large impact on regional and global biogeochemical cycles (Drake et al., 2018; Li et al., 2021; Mendonça et al., 2017; Messenger et al., 2016; Tranvik et al., 2009). Within lakes, dissolved organic matter (DOM) is a primary driver of biogeochemical reactions. The complex and dynamic DOM pool includes thousands of molecules of diverse

* Corresponding author.

E-mail address: sorlic@irb.hr (S. Orlić).

<https://doi.org/10.1016/j.watres.2023.120653>

Received 21 December 2022; Received in revised form 17 July 2023; Accepted 18 September 2023

Available online 19 September 2023

0043-1354/© 2023 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

origins. Allochthonous DOM, derived from terrestrial sources, with a significant proportion originating from vascular plants, and autochthonous DOM, produced in situ by photosynthesis of phytoplankton, incomplete grazing of phytoplankton, and viral lysis or death of bacterial cells, are critical to the dynamics of the food web (Bertilsson and Jones, 2003; Thornton, 2014). The molecules in the DOM pool range from labile aliphatics to less bioavailable terrigenous phenolics and

different microbes exhibit different abilities to utilize the different molecules (Kritzberg et al., 2006). By degrading DOM constituents and synthesizing new compounds, the microbes of aquatic ecosystems extensively and continuously influence the chemical diversity of DOM, which in turn, again impacts the diversity of microbial communities (Orland et al., 2020). Microbe-mediated DOM transformation is partly influenced by physical and chemical conditions in the water column,

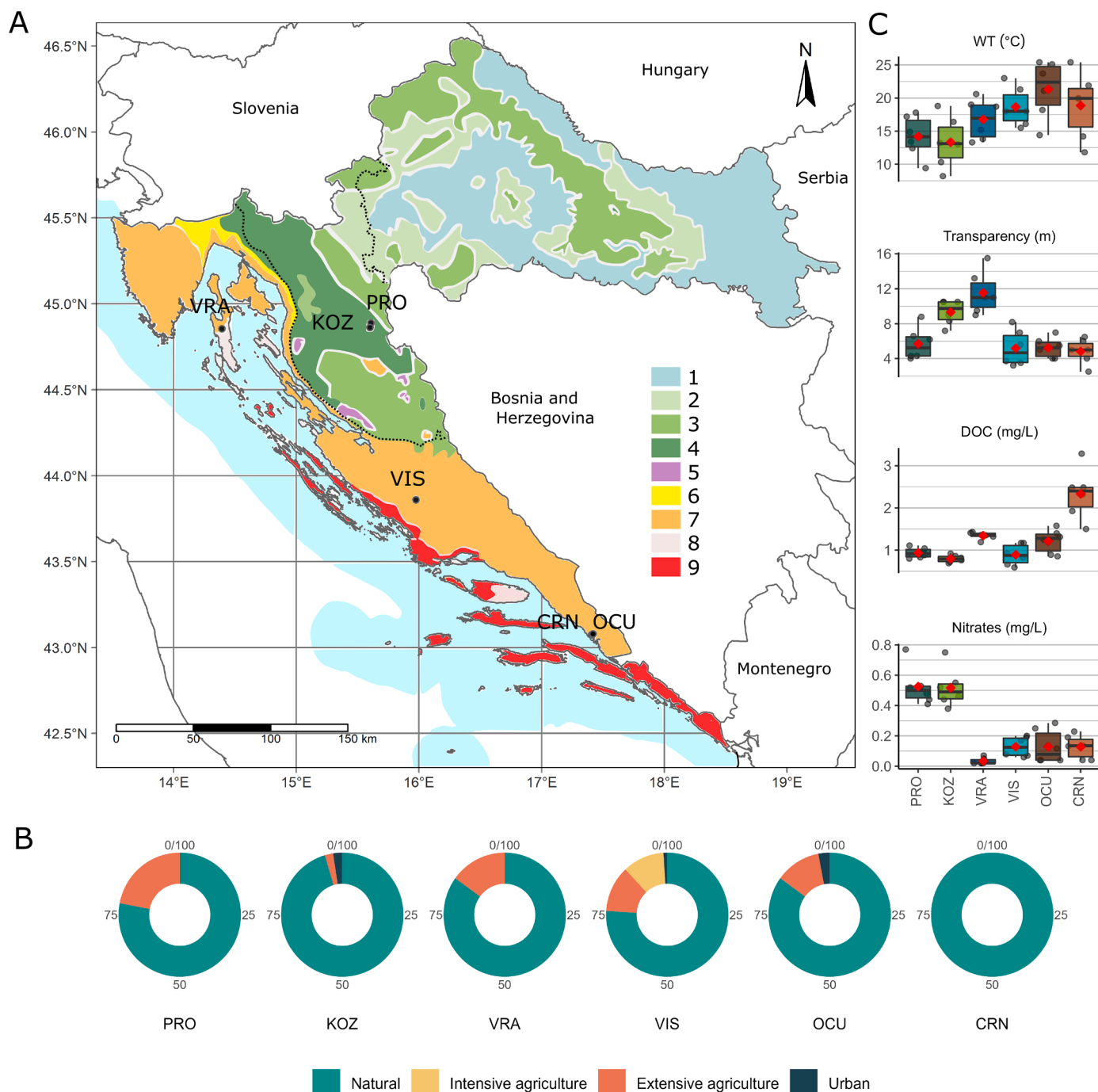


Fig. 1. Study area and environmental conditions. (A) Locations of the sampled lakes in the continental and Mediterranean climatic regions of Croatia. Colored areas present spatial distribution of major forest types in Croatia: (1) lowland deciduous forests, including flood-plains and swamps; (2) colline and submontane deciduous forests; (3) montane deciduous mesophilic forests; (4) altemontane mixed (coniferous/deciduous) forests; (5) subalpine deciduous and coniferous forests; (6) montane deciduous thermophilic forests; (7) Mediterranean deciduous forests; (8) Mediterranean mixed (evergreen / deciduous) forests; (9) Mediterranean evergreen forests (Antonić et al., 2000). Marked dashed lines separate continental and Mediterranean climatic regions. (B) Percentage of land cover elements in the lake area according to CLC illustrated nomenclature guidelines (Kosztra et al., 2017). (C) Average water temperature (°C), transparency (m), dissolved organic carbon (mg/L) and nitrate concentration during the sampling season. Boxplots represent the 1st and 3rd quartiles, the line represents the median, and the points are the established data. The red rectangle denotes the average value per lake.

such as temperature, inorganic nutrient content, and ultraviolet radiation intensity (Kujawinski, 2011).

The mechanisms that shape microbial composition, function, succession, and biogeography span niche (deterministic) and neutral (stochastic) processes (Zhou and Ning, 2017). Stochastic processes include probabilistic dispersal and random demographic and evolutionary dynamics (Chase and Myers, 2011). Deterministic processes are, in contrast, associated with ecological selection imposed by abiotic and biotic factors. The extent to which these processes control both DOM and microbial community composition have only recently begun to be quantified (Aguilar and Sommaruga, 2020; Danczak et al., 2020; Dini-Andreote et al., 2015; Llamas et al., 2017; Stegen et al., 2012; Tripathi et al., 2018; Yuan et al., 2019). There is a significant knowledge gap as only several studies have shown that DOM composition affects the diversity, structure, and functioning of prokaryotic communities in multiple environments (Chen et al., 2021; Li et al., 2018; Osterholz et al., 2018, 2016; Pérez and Sommaruga, 2006; Tanentzap et al., 2019). Previous studies have examined the relative importance of deterministic and stochastic assembly processes on microbial communities in a variety of aquatic environments (Jia et al., 2022; Lin et al., 2022; Logares et al., 2020; Santillan and Wuertz, 2022; Vass et al., 2020; Yan et al., 2022).

In order to understand deeper, the assembly processing impacting DOM and microbial composition, we investigated the relationship between the molecular composition of DOM and the communities of free-living bacteria, particle-attached bacteria and microeukaryotes accompanied by lake morphology and abiotic factors in freshwater lakes during the productive season. The studied lakes were characterized by diverse climate region, environmental conditions, nutrient sources and lake characteristics. Working across diverse lake systems is ideal for generating transferable knowledge on the assembly processes and relationships between DOM and microbial communities. Our main aims were to determine (1) the influence of environmental selection on DOM and microbial composition, (2) the association of DOM and microbial communities across season, and (3) the quantitative assessment of ecological processes driving the molecular-level composition of DOM and microbial communities.

2. Materials and methods

2.1. Study area and sampling

Our research covered six natural freshwater temperate lakes in Croatia, two in the Croatian continental region and four in the Mediterranean region characterized by a carbonate geological background (Fig. 1A, B, Table S1). The size of the catchment areas of the lakes ranged from 4.9 to 1223 km² and water retention time was from ca. 27 days to 32 years with a trophic gradient from oligotrophic to mesotrophic. Water samples of the entire epilimnion were collected monthly between April and September 2017 using a tube sampler (Ruttner type) with the sampling location set in open water above the deepest lake point. In the continental region, two dimictic lakes were sampled located in the mountain region. The mountain region has predominantly continental climatic conditions, characterized by mild, sunny summers and relatively long, snowy winters. Plitvice Lakes are a lake complex separated by travertine barriers, with lake Prošće (PRO) being the uppermost and second largest lenticular lake, connected by channels, cascades, waterfalls and smaller lakes with the largest and deepest lake Kozjak (KOZ). The surrounding terrestrial area of the continental lakes is covered by beech and beech-fir forests with smaller areas of pine and black alder (Antonić et al., 2000). Four monomictic lakes were sampled in the Mediterranean region with a temperate climate characterized by mild, relatively wet winters and hot, dry summers. The deepest oligotrophic lake in the northern Mediterranean is Vransko (VRA), a cryptodepression rain-fed lake in the central part of the island of Cres with the longest retention time. The southern Mediterranean vegetation zone with evergreen forests is dominated by mixed holm oak and black ash

forests. Lake Visovac (VIS) is a barrage lake supplied with water from the upper flow and the largest catchment area. The Baćina lakes are a complex of interconnected coastal cryptodepression lakes, with the largest lake Oćuša (OCU) and the deepest lake Crniševno (CRN), storing precipitation from the surrounding area. During this study, a partial inflow of seawater from the nearby sea through porous karst rock into CRN was detected.

2.2. Environmental characterization

Water temperature, conductivity, salinity, pH, total dissolved solids, dissolved oxygen and oxidation–reduction potential were measured *in situ* with a Hach HQ40D Portable Multi Meter for Water (Hach Lange GmbH, Berlin, Germany). Water transparency was determined with a 30 cm Secchi disk. Samples for chemical analyses were taken simultaneously with samples for DOM and microbiological analyses. They were stored in bottles and analyzed in the laboratory. Oxygen and total suspended particles were analyzed following the APHA (Tarigan, 2013). Total phosphorus and dissolved silicates were determined using standard methods (Strickland and Parsons, 1970). The absorbance readings were detected using a UV–VIS spectrometer (Perkin Elmer Lambda 25) at different wavelengths for each nutrient. Total nitrogen and total organic carbon were analyzed using a Shimadzu TOC-VCPH. Chlorophyll *a* (Chl *a*) was filtered with Whatman GF/F glass filters, extracted in 96% ethanol and measured using a UV–VIS spectrophotometer (Perkin Elmer Lambda 25) according to standard guidelines (Tarigan, 2013).

2.3. Dissolved organic matter characterization

Between 750 and 2000 mL of sample was filtered through 47 mm GF/F filters (Whatman, Germany) acidified to pH 2 and stored frozen in acid-washed polycarbonate bottles. Samples were slowly thawed before analysis. Here, replicate 20 mL samples were used for DOC and TDN quantification via high temperature catalytic oxidation on a Shimadzu TOC-VCPH instrument. Accuracy was tested against deep seawater reference material (D.A. Hansell, University of Miami, FL, USA) and was better than 5%. The remaining water was solid-phase extracted with 1 g PPL Bond Elut columns (Agilent, Santa Clara, California, USA), according to Dittmar et al. (2008). The DOM was eluted in 6 mL methanol (ULC grade) and stored frozen until analysis. The concentration of the extract DOC was determined from aliquots after the complete removal of the methanol and redissolution in ultrapure water. Extraction efficiencies were 35±8% on a carbon basis, including all samples, but 22±10% for CRN. The methanol extract was diluted with ultrapure water and methanol to yield a final concentration of 5 ppm in methanol and water 1:1 (v/v) and filtered through a 0.2 µm polycarbonate syringe filter. Mass spectrometric analysis was done on a solarix FT-ICR-MS (Bruker Daltonik GmbH, Bremen, Germany) equipped with a ParaCell and connected to a 15 T magnet (Bruker Biospin, Wissembourg, France). Samples were injected at 120 µL h⁻¹ into the electrospray source (Apollo II, Bruker Daltonik GmbH, Bremen, Germany) operated at 200 °C in negative mode and with the capillary voltage at 4 kV. Data acquisition was done in broadband mode using 8 molecular weight datasets over a scanning range of 92 to 2000 Da and 250 transients were co-added per spectrum. Process blanks (filtration, extraction) were done with ultrapure water.

Obtained spectra were internally calibrated with a list of >50 masses spanning the mass range of the samples, achieving a mass error of <0.1 ppm. Masslists were exported from Data Analysis (Bruker Daltonik). The masslists were joined and molecular formulas were attributed to peaks above the method detection limit (Riedel and Dittmar, 2014) using ICBM ocean (Merder et al., 2020) with the following restrictions: ¹²C₁₋₁₃₀ ¹H₁₋₂₀₀ ¹⁶O₁₋₅₀ ¹⁴N₀₋₄ ³²S₀₋₂ ³¹P₀₋₂. Masses detected in less than 2 samples were removed prior to further analysis, peaks present in blanks above a signal-to-method detection limit of 10 were removed as well. Each sample was normalized to the sum of remaining FT-ICR-MS

signal intensities and a new detection limit was set as described in Osterholz et al. (2014). Molar ratios, double bond equivalencies (DBE) and indices such as the modified aromaticity index (AI_{mod}) (Koch and Dittmar, 2006) and the lability index (MLB_w) (D'Andrilli et al., 2015a) were calculated. Elemental compositions and indices are reported as averages weighted by peak intensity per sample.

The molecular formulas (MFs) were assigned to molecular compound classes based on van Krevelen diagrams (Kim et al., 2003) as lipids ($O/C = 0 - 0.3$, $H/C = 1.5 - 2.0$), proteins ($O/C = 0.3 - 0.55$, $H/C = 1.5 - 2.2$), amino sugars ($O/C = 0.55 - 0.67$, $H/C = 1.5 - 2.2$), carbohydrates ($O/C = 0.67 - 1.2$, $H/C = 1.5 - 2$), unsaturated hydrocarbons ($O/C = 0 - 0.1$, $H/C = 0.7 - 1.5$), lignin ($O/C = 0.1 - 0.67$, $H/C = 0.7 - 1.5$), tannin ($O/C = 0.67 - 1.2$, $H/C = 0.5 - 1.5$), and condensed aromatics ($O/C = 0 - 0.67$, $H/C = 0.2 - 0.7$). MFs that could not be assigned to a class were designated as unclassified.

The package *fmsRanalysis* (Bramer et al., 2020) was used to detect significant differences between DOM molecules of continental and Mediterranean lakes with the set default uniqueness_gtest=list(pres_fn="nsamps", pres_thresh=2, pvalue_thresh=0.05) visualized in van Krevelen diagrams. Venn diagrams were used to detect the difference between the continental and Mediterranean S-containing organic molecules (CHOS, CHONS and CHOPS).

To apply dendrogram-based β -diversity and ecological null modeling, three different metabolite dendrograms were generated based on the molecular characteristics and biochemical transformations: the molecular characteristics dendrogram (MCD), transformation-based dendrogram (TD), and transformation-weighted characteristics dendrogram (TWCD) using methods outlined in Danczak et al. (2020) with scripts and transformation database. The MCD was generated based on the elemental composition, calculated molar ratios and indices (e.g. DBE, AI_{mod} , Kendrick's mass defect) for each assigned MF using the package *fmsRanalysis* (Bramer et al., 2020) and scripts available on Github. The TD was generated based on the potential biochemical transformations of each assigned MF using a transformation database to map mass differences (gains and losses) considering any between-metabolite mass difference within 1 ppm of the expected mass of a transformation to be a match. The TWCD was partially composed of MCD and TD dendrogram using simple matrix multiplication, the molecular characteristics matrix was combined with the standardized transformation matrix. These metrics were combined to estimate a pairwise Euclidean distance matrix on which a UPGMA hierarchical cluster analysis was performed with the function *hclust* (package *vegan*).

Based on molecular trait dimensions of reactivity and activity, we parsed MFs into four contrasting partitions: labile-active, recalcitrant-active, labile-inactive, and recalcitrant-inactive (Hu et al., 2022). The molecular reactivity was quantified based on the H/C ratio, where the cutoff of H/C ratio = 1.5 is a threshold of molecular lability vs recalcitrance (D'Andrilli et al., 2015b). The molecular activity was quantified based on the number of molecular transformations accounted for each MF. The molecules involved in less than 1 and more than 10 transformations were considered inactive and active. The cutoff of molecules with transformations between 2 and 10 was based on the threshold of Hu et al. (2022). Overall, the MFs were grouped into four partitions as following: labile-active ($H/C \geq 1.5$, transformations > 10), recalcitrant-active ($H/C < 1.5$ transformations > 10), labile-inactive ($H/C \geq 1.5$, transformations ≤ 1) and recalcitrant-inactive ($H/C < 1.5$, transformations ≤ 1) (Table S2).

2.4. Microbial community characterization

The integrated epilimnion samples were filtered with a peristaltic pump on polycarbonate membrane filters (type GTTP; Whatman, UK) with 3 μ m pore size to retain particle-attached bacteria until the filters clogged. Free-living bacteria were collected by filtering 3 μ m filtrate onto filters with 0.2 μ m pore size, while samples for microeukaryota were sampled by filtering water directly on filters with 0.2 μ m pore size.

Filters were immediately stored on dry ice and transferred to -80°C until further processing.

Total genomic DNA was extracted with the DNeasy PowerWater kit (Qiagen GmbH Hilden, Germany) according to the manufacturer's guidelines. The hypervariable V4 region of 16S rRNA gene was amplified using primer pair 515F / 806R (Caporaso et al., 2011). The hypervariable V9 region of the eukaryotic SSU rRNA gene was amplified using the primer pair 1,391F / Euk Br following the protocol of Stoeck et al. (2010). To minimize PCR-bias, three individual reactions per sample were prepared. Samples were further processed and sequenced by SeqIT GmbH & Co. KG (Kaiserslautern, Germany). Purified 16S V4 amplicons were paired-end sequenced on an Illumina MiSeq platform and 18S V9 amplicons on Illumina NextSeq platform.

Paired-end reads were quality trimmed using the *bbduk* function and merged using *bbmerge* function of the BBMap package (v38.71; <https://sourceforge.net/projects/bbmap/>). Merged reads were quality-filtered again using QIIME v1.8.0 (Caporaso et al., 2010). Only reads with exact barcodes, primers and unambiguous nucleotides were retained. Chimera filtering was done by using UCHIME (Edgar et al., 2011). Non-chimeric reads were clustered with SWARM v3.0.0 (Mahé et al., 2015) with default settings clustering sequences with one difference in their nucleotide into Operational Taxonomic Units (OTUs). The 16S reads were blasted against the SILVA database (SILVA v138.1) (Quast et al., 2013) and the microeukaryotic reads to PR2 reference database (Guillou et al., 2013) by using *blastn* (BLAST, v2.9.0). Nontarget OTUs (metazoans, embryophytes in the 18S dataset; chloroplasts, mitochondria in the 16S dataset), as well as singletons and doubletons, were excluded. In total, 2316,862 high-quality sequences of free-living bacteria were clustered in 7914 OTUs, while sequencing of particle-attached bacteria resulted in a total of 2650,747 high-quality sequences clustered into 11,963 OTUs. The sequencing of the microeukaryota resulted in 14,216 OTUs after clustering 12,360,929 high-quality sequences. Based on the taxonomic assignment of microeukaryota, each OTU was assigned to a functional group (autotrophs, mixotrophs, parasites, osmotrophs, and phagotrophs) according to (Adl et al., 2019). Prior to analyses of microbial communities, we applied standardization among samples using the *rrarefy* function (*vegan* package) (Oksanen et al., 2020) by randomly subsampling the abundance OTU table to the minimum read level in order to minimize biases associated with sequencing and allow comparisons between samples. The phylogenetic tree of microbial communities was generated using Clustal Omega (Sievers et al., 2011) for alignment and FastTree (Price et al., 2010) to estimate phylogeny.

2.5. Diversity analyses

Differences in environmental factors among lakes and time points were displayed with a principal component analysis (PCA). Of the total dataset, the main environmental factors were used for the principal component analysis after excluding co-correlating parameters evaluated by Pearson's correlation. Environmental heterogeneity was estimated by computing the average dissimilarity between lakes based on standardized environmental factors (Huber et al., 2020a; Ranjard et al., 2013). We computed a Euclidean distance matrix for each lake and calculated the mean dissimilarity between months.

The alpha diversity was estimated as the richness, Shannon-Wiener index, and Faith's phylogenetic distance for molecular DOM composition and microbial community using packages *vegan* and *picante* (Kembel et al., 2010). The abundance-occupancy analysis was conducted to detect a core microbiome difference in microbial communities between continental and Mediterranean lakes (Shade and Stopnisek, 2019).

Before beta diversity analysis, Hellinger transformation was applied to rarefied microbial community datasets. Average beta diversity based on the Bray-Curtis dissimilarity distance of molecular DOM composition and microbial communities was calculated to obtain information on the inter- and intra-lake variability. Principal Coordinate Analysis (PCoA)

based on Bray-Curtis dissimilarity was carried out to identify DOM composition and microbial communities diversity patterns using package *ade4* (Dray and Dufour, 2007). Permutational multivariate analysis of variance (PERMANOVA) with Bonferroni p-value adjustment was used to test whether the partitioning of microbial communities was affected significantly by the location, climate, season and trophic status. The function *envfit* (vegan package) was applied to the results of PCoA to evaluate the correlations with environmental factors and the significance of this regression by permutation.

The effect of seasonal changes on molecular DOM and microbial community structure was assessed by calculating the community temporal variability in each lake (Huber et al., 2020b; Ranjard et al., 2013). We computed a Bray-Curtis dissimilarity distance for each lake and calculated the mean dissimilarity between months.

To test whether the molecular DOM composition correlates to microbial communities and environmental parameters, the coinertia analysis (CIA) was conducted on the ordinations of datasets using package *ade4* (Dray and Dufour, 2007). We used the results of PCoAs covering the axes accounting for a minimum of 70% variance. The significance of the RV coefficient was assessed with the Monte-Carlo test with 999 permutations. The RV coefficient is a measure of global similarity between the datasets (between 0 and 1), the closer to 1 the greater the global similarity between the datasets.

Prior to the null model approach, the presence of a phylogenetic signal in microbial communities was tested using Mantel correlograms, as described in Stegen et al. (2013). The relationship between the phylogenetic distance of pairwise OTUs and their optimal DOC and WT differences were tested using *mantel.correlog* (package *vegan*) with 999 permutations based on Pearson's correlation.

The differences between the lakes were tested by one-way ANOVA, followed by Tukey's multiple comparison tests. Correlations between the parameters were calculated based on Pearson's correlation with Bonferroni p-value adjustment. All analyses and visualizations were performed in R v 4.0.4 (R Core Team, 2020) if not stated otherwise.

2.6. Ecological null modeling of DOM and microbial communities

We performed the β -diversity ecological null modeling to evaluate processes driving molecular DOM composition implemented by (Danczak et al., 2020) and microbial communities implemented by (Stegen et al., 2015, 2013). The approach was applied to the presence/absence dataset of the molecular DOM composition to each of the three metabolite dendrograms (MCD, TD, and TWCD) using methods outlined in (Danczak et al., 2020) and scripts available on Github. Conversely, β -nearest taxon index (β NTI) values of microbial communities (FL, PA, and ME) were conducted on the rarified abundance OTU table and amplicon phylogenetic tree using methods outlined in (Stegen et al., 2013). First, the β -mean nearest taxon index (β MNTD) was calculated to test significant dendrogram-based turnover between the observed molecular and phylogenetic turnover between the observed microbial assemblages. Further, the β NTI was derived from the difference between the observed β MNTD and the null distribution. Deterministic processes dominate when β NTI is greater than 2 or less than -2 , which refers to variable or homogeneous selection. The values within the range of $2 > \beta$ NTI > -2 indicate the dominance of stochastic processes (homogenizing dispersal or dispersal limitation) or random drift. To distinguish stochastic processes, we calculated the Raup-Crick (RC) beta diversity based on the identity of molecular DOM and the abundance of microbial communities, respectively. The null distribution of dissimilarity values was then compared with the observed Bray-Curtis value to determine how far from the null expectation the observed value deviated. Based on these deviations, the final RC metric was standardized to range between -1 and 1 . Assemblies were structured by dispersal limitation if RC $> +0.95$, homogenizing dispersal if RC < -0.95 , or random processes acting alone (ecological drift) if RC falls between -0.95 and $+0.95$. Consequently, no single assembly process is able to dominate in such

'undominated' conditions. ANOSIM was used to identify significant differences in distributions of NTI and RC values across lakes and climatic regions. Correlations between metabolite and microbial β NTI values were performed by relating β NTI values within a lake to evaluate correspondence of assembly processes. Additionally, the null model approach was applied to determine processes governing the identified DOM fractions: labile-active (LA), recalcitrant-active (RA), labile-inactive (LI), recalcitrant-inactive (RI), and transformation 2–10 (TR).

3. Results

3.1. Environmental characterization of lakes on a spatial and temporal scale

During the productive season, each lake showed a distinct profile of physical and chemical properties (PERMANOVA, lake: $R^2 = 0.69$, climate: $R^2 = 0.24$, $p = 0.001$, Fig. S1). Lakes in the Croatian continental region had lower water temperature ranging from 8 to 19 °C, whereas Mediterranean lakes had higher water temperature ranging from 13 to 26 °C (ANOVA, $p = 0.001$; Fig. 1C). The dissolved organic carbon concentration (DOC) remained low and stable in sampled lakes during the vegetation period ($\leq 2 \text{ mg L}^{-1}$), except in Mediterranean lake CRN, where the concentration prevailed at an average of $2.3 \pm 0.6 \text{ mg C L}^{-1}$ (ANOVA, $p < 0.001$). Nitrate concentrations were significantly higher in Croatian continental lakes (ANOVA, $p < 0.001$), whereas sulfate concentrations were higher in Mediterranean lakes except for VRA (ANOVA, $p < 0.001$). The presence of saline waters in the summer months also caused CRN lake to differ chemically from other lakes (ANOVA, $p < 0.001$; Fig. S2). The saturation level of DO was high in all lakes and varied from 5.9 mg L^{-1} to 12.9 mg L^{-1} . The highest concentration of chlorophyll *a* (Chl *a*) was measured in mesotrophic lakes (PRO ($4.4 \pm 2.2 \mu\text{g L}^{-1}$) and VIS ($4.0 \pm 1.9 \mu\text{g L}^{-1}$); ANOVA, $p < 0.01$), respectively, while it remained low in other lakes ($0.9 \pm 0.5 \mu\text{g L}^{-1}$). Overall, transparency, nitrates and DO of lakes positively correlated to latitude (Pearson's $r = 0.61$; $r = 0.50$; $r = 0.56$, $p < 0.001$), while negatively to sulfates, salinity, WT, and DOC (Pearson's $r = -0.91$; $r = -0.61$; $r = -0.55$; $r = -0.52$, $p < 0.001$).

3.2. Seasonal DOM chemical characterization

We assigned 8015 molecular formulas (MFs) with masses ranging from 101 to 750 Da in our dataset of 36 lake DOM samples. Lakes in the Croatian continental and Mediterranean climatic region showed a high similarity of identified formulas (6008 common, $\sim 75\%$ count; Fig. 2A). The number of assigned MFs per sample ranged from 3909 to 5303 and increased with the decrease of lake depth, volume, surface area, retention time and transparency (Pearson's $r = -0.91$, $r = -0.77$, $r = -0.52$, $r = -0.87$, $r = -0.76$, $p < 0.001$; Fig. S3). Elemental formulas with CHO and CHON were the most numerous formula classes in all lakes, with a significantly higher count of CHO in Croatian continental and CHON in Mediterranean lakes (ANOVA, $p < 0.001$; Fig. S4). There was a significant increase in S:C and N:C molar ratios in Mediterranean lakes (ANOVA, $p < 0.001$), but P:C ratios were variable (Fig. S5). A higher proportion of S-containing organic molecules were found in Mediterranean lakes, with S:C molar ratios showing a strong correlation with latitude, WT, and sulfates (Pearson's $r = 0.80$, $r = 0.73$, $r = 0.86$, $p < 0.001$). In total, Mediterranean lakes had 886 (52% of count) unique S-containing organic molecules with the molecular formation of CHOS, CHONS and CHOPS, and 690 (40.5% of count) in common with Croatian continental lakes, consistent with the higher sulfate concentrations.

A majority of MFs were lignin-like molecules in all lakes ($58 \pm 3.1\%$ counts), while the other compounds, protein- and carbohydrate-like, contributed in lower abundance to the total molecular composition ($11.4 \pm 3.3\%$ and $8.9 \pm 2.6\%$ counts, Fig. S6). The Mediterranean lake VRA had a distinct composition compared to Croatian continental and

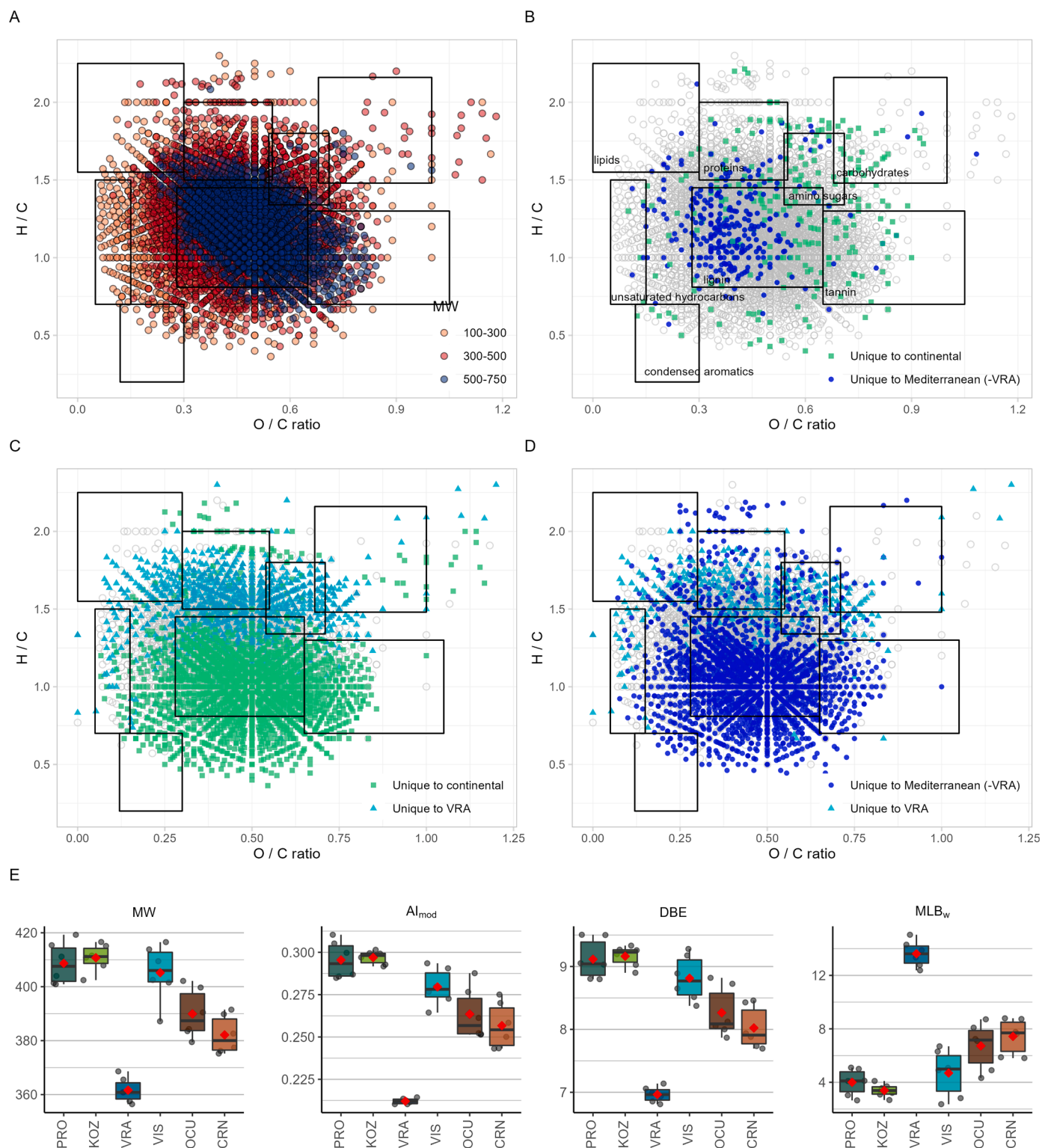


Fig. 2. Molecular characteristics of shared MFs in lakes Van Krevelen plots of compounds found in (A) all samples categorized by compound class, and unique compounds in comparison (B) PRO-KOZ and VIS-OCU-CRN, (C) PRO-KOZ and VRA, and (D) VIS-OCU-CRN and VRA. The peak-intensity weighted average of chemical characteristics per lake is marked with the red rectangle. The different colors represent three molecular weight ranges. Dissolved organic matter (DOM) molecular composition plotted in van Krevelen diagrams. The van Krevelen diagrams are plotted as a function of hydrogen to carbon (H/C) and oxygen to carbon (O/C) ratios. Gray symbols in the background (B-D) are the molecular formulas shared between all samples. (E) Seasonal average of molecular weight (MW), modified aromaticity index (AI_{mod}), double-bond equivalents (DBE) and molecular lability boundary (MLB_w). Boxplots represent the 1st and 3rd quartiles, the line represents the median, and the points are the established data.

remaining Mediterranean lakes (Fig. 2B-D). MFs unique to VRA included bioavailable compound classes (lipid-, protein-, amino sugar- and carbohydrate-like; vs continental: 1092 MFs, vs Mediterranean: 345 MFs), whereas Croatian continental and remained Mediterranean lakes significantly differed in more recalcitrant compounds (lignin- and tannin-like; continental: 1935 MFs, Mediterranean: 2015 MFs), respectively.

The relative contribution of molecular formulas to the lability index MLB_w decreased with increasing nitrate concentration (Pearson's $r = -0.77$, $p < 0.001$), while terrigenous character, assessed via AI_{mod} , DBE and average molecular weight (MW) decreased with nitrate concentration from Croatian continental to Mediterranean lakes (Pearson's $r = 0.78$, $r = 0.77$, $r = 0.74$, $p < 0.001$), except in VRA (Fig. 2E).

We note that the extraction efficiencies of the DOM were low to moderate in the present study (max. 46%), likely due to the contribution of fresh, phytoplankton derived polysaccharides, colloidal material, and small organics as discussed in Selak et al. 2020 and also shown by (Liu et al., 2020) for a eutrophic lake. As the extractable DOM fraction covers compounds of different quality, but with some preference for the more recalcitrant pool (Jerusalén-Lleó et al., 2023), we believe this analytical approach to cover not the complete variability of the dissolved carbon pool, yet a reproducibly accessible portion of the DOM pool.

3.3. Seasonal microbial community composition

Different patterns of microbial community richness were observed over the sampling period (Fig. S3). Particle-attached bacterial communities showed the greatest variation between lakes, with OCU significantly differing from other lakes (ANOVA, $p < 0.05$). The richness of free-living and particle-attached communities increased with the decrease in lake depth (Pearson's $r = -0.46$, $r = -0.55$, $p < 0.01$), whereas the diversity of microeukaryotes decreased with increase in lake surface (Pearson's $r = -0.35$, $p < 0.05$). The community of free-living bacteria at the phylum level was dominated by Actinobacteriota (55±24%), Proteobacteria (23±13%), Bacteroidota (12±13%), Verrucomicrobiota (5 ± 11%), and Cyanobacteria (3 ± 4%) (Fig. S7). All lakes were predominated by Actinobacteriota, except KOZ, in which Proteobacteria were equally abundant. There were significant differences in the relative abundance of Actinobacteriota, Proteobacteria, and Bacteroidota between Croatian continental and Mediterranean lakes (ANOVA, $p = 0.01$). The most abundant phyla of particle-attached bacteria were Proteobacteria (35±23%) followed by Bacteroidota (23%), Actinobacteriota (12±17%), Verrucomicrobiota (11±10%), Cyanobacteria (11 ± 12%), and Planctomycetota (5 ± 10%) (Fig. S8). A significant difference in the composition of Croatian continental lake KOZ between May and June compared with other lakes was recorded, OTUs assigned to Bacteroidota having the highest average relative abundance (59%; ANOVA, $p < 0.05$). The community of microeukaryota was dominated by Ochrophyta (33%), Dinoflagellata (19%), Ciliophora (18%), Cryptophyta (13%), and Fungi (7%) (Fig. S9). Ciliophora was significantly more abundant in Mediterranean lake OCU (ANOVA, $p < 0.001$), while Dinoflagellates dominated KOZ and VRA (ANOVA, $p < 0.001$). The relative abundance of Chlorophyta had a significant difference between the climatic regions (ANOVA, $p < 0.05$). Overall, the main microeukaryota functional groups determined in lakes were autotrophs (45%), mixotrophs (24%), phagotrophs (22%), osmotrophs (7%), and parasites (2%), where only relative abundance of parasites differed between climatic regions (ANOVA, $p < 0.001$; Fig. S9).

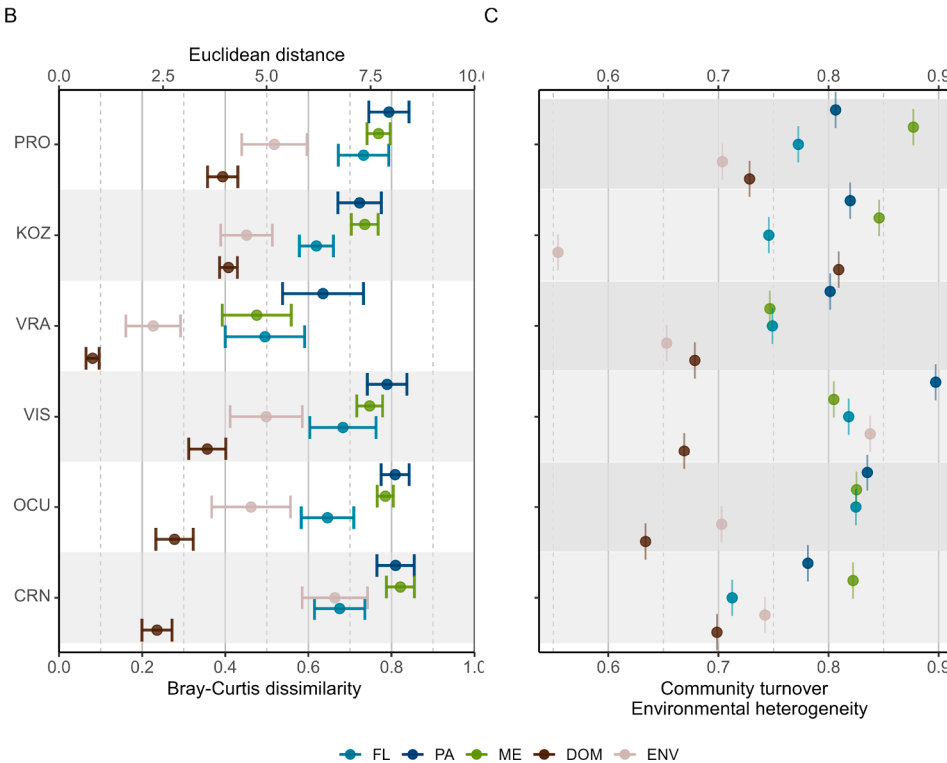
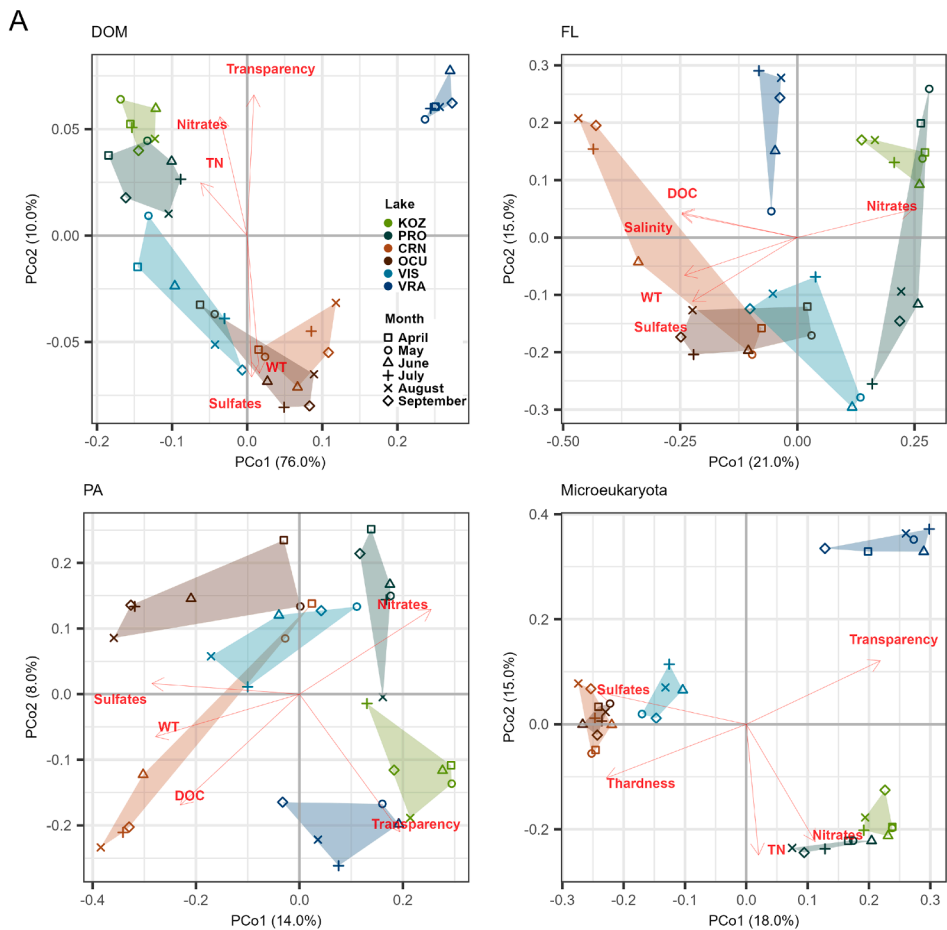
The number of unique microbial OTUs in the Croatian continental and Mediterranean regions accounted for a considerable proportion, although most of the microbial OTUs in the two habitats were shared, representing the core microbiome (Fig. S10). Shared OTUs of microbial communities highly contributed to the total relative abundance (96% FL OTUs, 90% PA OTUs, 90% ME OTUs) with taxonomically diverse taxa (240 taxa FL, 466 taxa PA, 383 taxa ME). Most abundant core OTUs persistent in the free-living bacterial community were assigned to

Sporichthyaceae hgcI clade (Actinobacteriota), while in the particle-attached bacterial community to Saprospiraceae candidatus Aquirestis (Bacteroidota) and in microeukaryotic community to Polar-centric-Mediophyceae (Ochrophyta). Microbes in the Mediterranean lakes contributed to the large number of unique OTUs, particularly in particle-attached bacterial communities (42.2% FL OTUs, 54.1% PA OTUs, 48.4% ME OTUs). There were 772 taxa unique to the Mediterranean particle-attached community, and these were composed mainly of Actinobacteriota (908 OTUs), Verrucomicrobiota (270 OTUs) and Cyanobacteria (231 OTUs). The dominating taxon of the Mediterranean microeukaryotic community was Gymnodinium from the Dinophyceae family (2.8%). Notably, taxa unique to the Croatian continental region were relatively rare with low relative abundance.

3.4. Diversity of DOM and microbial communities and causal relationship with environmental parameters

Given that the most significant differences occurred between the lakes and the climatic regions, seasonal changes played a lesser role in shaping DOM compositional variability (PERMANOVA, lakes: $R^2 = 0.83$, climate: $R^2 = 0.38$, $p = 0.001$; Fig. 3A). Based on the ordination, three distinct groups were identified: Croatian continental lakes (KOZ and PRO), Mediterranean lakes (VIS, OCU and CRN), and the isolated Mediterranean lake on Cres island (VRA). Most of the variation in DOM composition among lakes was explained by two dimensions (86%). To evaluate the correlations with environmental factors and the significance of the results of PCoA, the function *envfit* was applied. The overall molecular-level DOM composition of the Mediterranean lakes (CRN, OCU and VIS) was related to elevated WT ($R^2 = 0.50$, $p < 0.05$), concentrations of sulfates ($R^2 = 0.78$, $p < 0.05$), and lower transparency ($R^2 = 0.59$, $p < 0.05$), while in the Croatian continental lakes (KOZ and PRO) positively related to higher concentrations of nitrates ($R^2 = 0.86$, $p < 0.05$) and TN ($R^2 = 0.52$, $p < 0.05$). The free-living (FL) and particle-attached (PA) bacterial communities showed lower site-specificity (PERMANOVA, FL: $R^2 = 0.41$; PA: $R^2 = 0.33$, $p < 0.05$), in contrast to the microeukaryota (ME) community (PERMANOVA, ME $R^2 = 0.50$; Fig. 3A). The free-living and particle-attached bacterial communities of the Mediterranean lake CRN were linked to higher DOC (FL: $R^2 = 0.71$; PA: $R^2 = 0.66$, $p < 0.05$), sulfates ($R^2 = 0.77$; $R^2 = 0.67$, $p < 0.05$), WT ($R^2 = 0.51$; $R^2 = 0.67$, $p < 0.05$), and salinity within the free-living community ($R^2 = 0.57$, $p < 0.05$). Conversely, Croatian continental lakes were associated with higher concentrations of nitrates ($R^2 = 0.54$, $R^2 = 0.64$, $p = 0.01$). Sulfate concentrations and total hardness were significantly related to the microeukaryotic community of Mediterranean lakes (CRN, OC, and VIS; $R^2 = 0.55$, $R^2 = 0.86$, $p < 0.05$), whereas nitrate and TN concentrations were significantly related to Croatian continental lakes (KOZ and PRO; $R^2 = 0.86$, $R^2 = 0.65$, $p < 0.05$). In comparison with other lakes, the microbial diversity of the oligotrophic Mediterranean lake VRA was characterized by a lower average Bray-Curtis dissimilarity (Fig. 3B). In Croatian continental lakes, seasonal changes of DOM and microeukaryotes were significantly higher based on climatic region (ANOVA, $p = 0.01$). However, the turnover of DOM and microeukaryotes showed only modest specificity with lake trophic status. Based on the average seasonal environment dissimilarity between months per lake, Croatian continental lakes had significantly lower environmental homogenization (ANOVA, $p < 0.05$; Fig. 3C).

Analysis of the covariance between molecular-level DOM diversity and the diversity of individual microbial communities revealed the highest correlation to microeukaryotes and free-living bacteria diversity ($p < 0.001$; Fig. 4A, Fig. S12). At a climatic region level, Croatian continental lakes showed a higher correlation between microbial and DOM diversity (Table S3). Overall, microbial community diversities were more interconnected compared to DOM, with particle-attached bacteria showing the closest association with free-living bacteria and microeukaryotes ($p < 0.001$). The covariance between environmental parameters and microbial community diversity exhibited a higher covariance



(caption on next page)

Fig. 3. (A) Principal coordinates analysis (PCoA) of molecular DOM composition and microbial communities (FL, PA, ME) through the season in lakes with correlated environmental parameters. Symbols are color-coded by the lake and shape-coded by the month. Results of PERMANOVA indicate a significant effect of the sample origin on the separation of all microbial communities and DOM composition. Color-coded polygons group samples by lake. Vectors represent the significant correlation ($r^2 \geq 0.5$, $p \leq 0.05$) of microbial communities and DOM composition with physical and chemical parameters, including water temperature (WT), transparency, total hardness (Thardness), dissolved organic carbon (DOC), nitrates, sulfates and salinity. (B) Average Bray-Curtis dissimilarity in comparison to VRA of microbial communities (FL, PA, ME) and DOM composition, together with average Euclidean distance of environmental parameters (ENV). (C) Seasonal turnover of DOM and microbial community and environmental heterogeneity in lakes. Points represent the average dissimilarity and error bars indicate standard deviations from the mean.

compared to the covariance observed between DOM diversity and environmental parameters ($p < 0.001$; Fig. 4A, Fig. S12).

3.5. Driving processes behind molecular DOM and microbial community assembly

In order to assess the potential impact of stochastic and deterministic processes on DOM and microbial community assembly, we quantified DOM and phylogenetic turnover using null model and β NTI analyses. In light of the fact that the diversity of DOM on molecular level and microbial community differed most between lakes based on PERMANOVA results, we applied the null model to each lake separately and to the whole dataset to reveal among lake processes. As a result of applying the null model to the molecular DOM composition, we found that the deterministic process strongly drives temporal variation in metabolite assemblages for all three dendrograms (Fig. 4B). Based on metabolite properties and biochemical relationships, there was a consistent pattern of deterministic variable selection dominating with a significant positive correlation of β NTI values (Pearson's correlation $r = 0.88$, $r = 0.86$, $r = 0.87$, $p < 0.001$; Fig. S13, S18). Further, the β NTI values of MCD, TD, and TWCD demonstrated significant differences among lakes (ANOSIM, $R = 0.76$, $R = 0.77$, $R = 0.70$, $p = 0.001$), as well as significant differences among climatic regions (ANOSIM, $R = 0.42$, $R = 0.59$, $R = 0.49$, $p = 0.001$).

To determine the influence of ecological processes on the level of molecular reactivity and activity of DOM, a null model was applied to four different DOM fractions based on the MCD dendrogram. Active DOM fractions were strongly influenced by deterministic variable selection with their higher significant contribution in labile active fraction of Mediterranean lakes (ANOVA, $p < 0.001$; Fig. S16, S17). β NTI values of both active fractions significantly differed among lakes (ANOSIM, LA: $R = 0.52$, RA: 0.73 , $p = 0.001$), while recalcitrant active fraction also differed among climatic regions (ANOSIM, $R = 0.47$, $p = 0.001$). On the contrary, fractions of inactive DOM were influenced mainly by stochastic homogenizing dispersal.

The relative contributions of microbial assembly processes differed among the lakes and microbial community fraction (Fig. 4B, Fig. S13). This study identified variable selection as well as homogenizing dispersal and dispersal limitation as the main ecological processes contributing to turnover in free-living bacterial communities, where strong variable selection dominated the free-living community of lake VIS. Mediterranean particle-attached communities were predominantly controlled by the variable selection, whereas Croatian continental particle-attached communities were additionally influenced by dispersal limitation (PRO) and homogenizing dispersal (KOZ). Likewise, stochastic processes and dispersal limitation dominated among assembly processes in microeukaryotes. β NTI values of particle-attached and microeukaryote communities showed low significant differences between lakes (ANOSIM, PA: $R = 0.12$, $p = 0.02$; ME: $R = 0.22$, $p = 0.001$), while particle-attached community also between the climatic regions (ANOSIM, PA: $R = 0.17$, $p = 0.006$). The null model among the lake microbial communities revealed a higher contribution of stochastic processes governing the microbial community composition (Fig. S14, S15).

Correlation of DOM and microbial community assemblies was relatively low and overall, not significant (Fig. S18, S19). The contribution of environmental parameters to β NTI values of total and active DOM

fractions, and microbial communities was evaluated by the Mantel correlation test. MCD dendrogram based β NTI showed that total environmental parameters were the main predictors of DOM, together with nitrate and sulfate concentration, and transparency ($p = 0.001$; Table S4). β NTI relationships of microbial communities with environmental variables were significant but relatively weak ($p < 0.05$). Free-living communities correlated only to concentrations of Chl *a* ($p = 0.03$), while particle-attached community to DP ($p = 0.001$) and microeukaryotic community to WT and DP ($p = 0.001$; $p = 0.002$; Table S4).

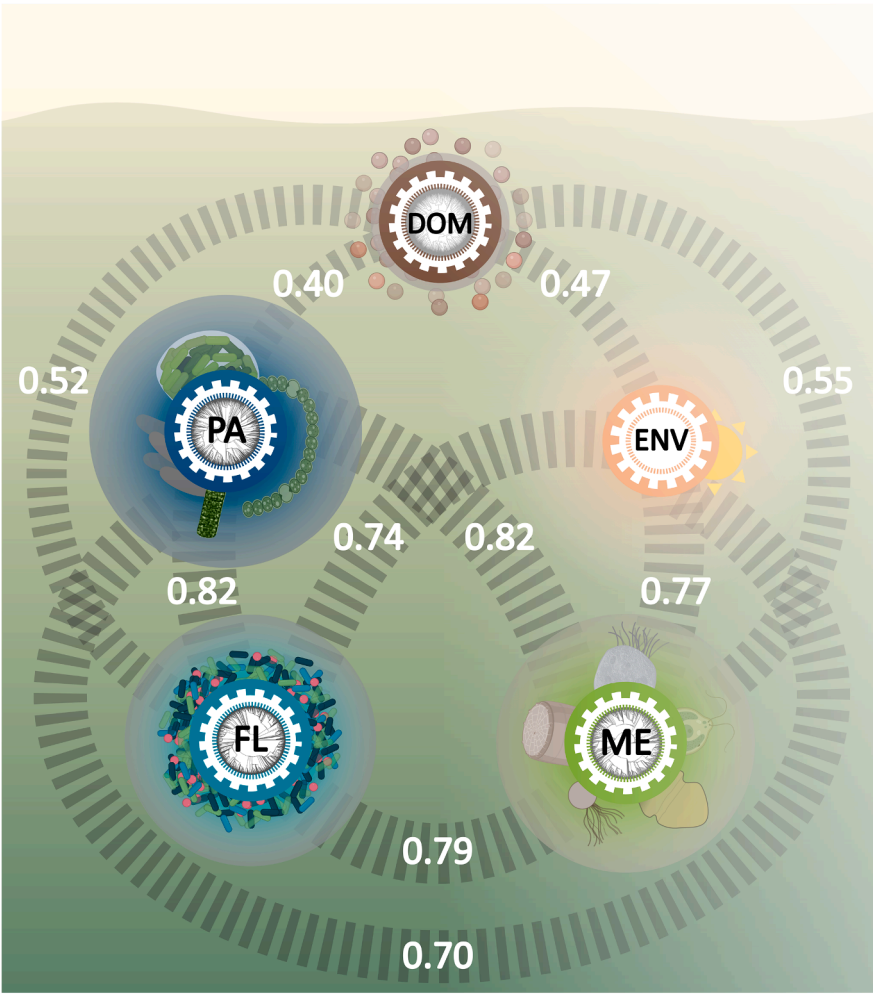
4. Discussion

In this study, we reveal the diversity and underlying ecological processes controlling the turnover of DOM and microbial communities in freshwater temperate lakes during a productive season throughout the Croatian continental and Mediterranean region. Significant differences were found between dimictic and monomictic lakes, reflecting the environmental characteristics of the Croatian continental and Mediterranean regions. These findings suggest that environmental filtering constitutes a significant mechanism for the generation of spatial distribution patterns of DOM and microbial communities in the studied systems. We were able to confirm that the molecular DOM compositions of Croatian continental and Mediterranean lakes differed significantly in terms of their molecular composition. The cooler Croatian continental lakes were dominated by more aromatic, carbon-rich DOM (low S:C and N:C ratios) of higher molecular weight, originating from the forested catchment with hardwood and coniferous vegetation (Thieme et al., 2019). On the other hand, the Mediterranean lakes exhibited higher intra-lake chemodiversity in season, likely due to the greater importance of autochthonous production compared to allochthonous input from sparsely vegetated catchments dominated by deciduous trees and shrubs. The higher temperatures in Mediterranean lakes can also promote the production of transparent exopolymers and directly influence the release of DOM by phytoplankton (Thornton, 2014) together with higher rates of photochemical transformations which may occur in clearer waters (Bertilsson and Tranvik, 2000). The deepest sampled Mediterranean lake VRA displayed distinct DOM characteristics with an enrichment of bioavailable compound classes, including lipid-, amino sugar- and carbohydrate-like compounds. This can be attributed to its location on a remote island, long water retention time and reliance on atmospheric precipitation as the main water source, while terrestrial sources are sparse due to poor vegetation in the catchment area which makes autochthonous input the main source of DOM in this lake.

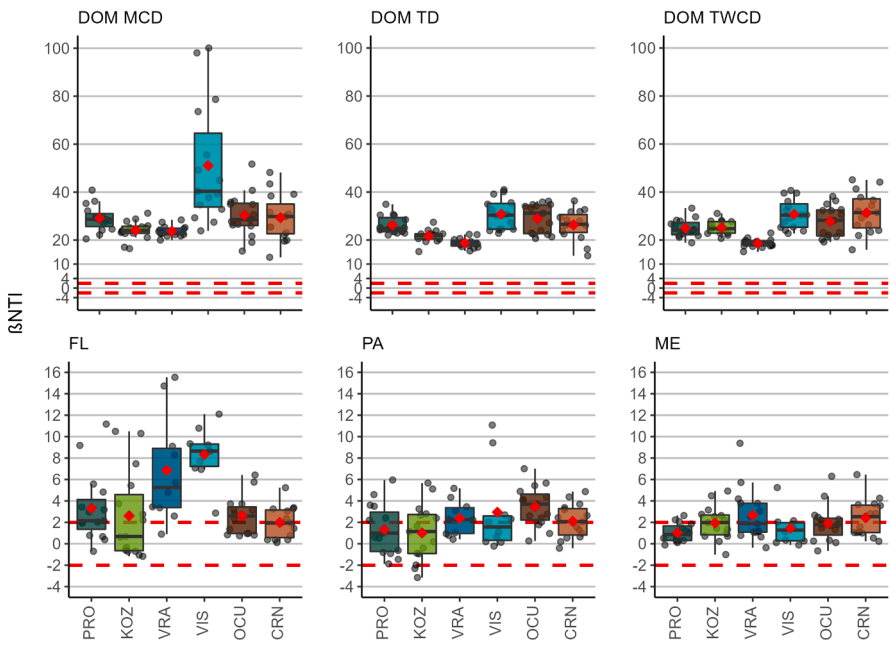
During the study period, the concentrations of DOC, Chl *a* and nitrate in the lakes were low, indicating a lower trophic status and reduced primary production intensity. However, our findings only partially confirmed the relative importance of allochthonous sources increasing with decreasing lake trophic status (Grey et al., 2000). The trophic status of a lake strongly regulates whether water bodies act as net DOC sources or sinks, and that rates of both DOC production and consumption can be predicted from water retention time (Evans et al., 2017). In our study, lake morphometry, including depth and water retention time, strongly influenced the alpha diversity of DOM, with lakes of greater depth and shorter retention time exhibiting the lowest number of MFs (Kothawala et al., 2021; Zhang et al., 2022).

Higher sulfate concentrations in the Mediterranean lakes at lower

A



B



(caption on next page)

Fig. 4. (A) Conceptual figure of molecular DOM composition, microbial communities (FL, PA and ME), and environmental parameters (ENV) interconnections through the vegetation period in the epilimnion of freshwater lakes. Line widths are proportional to RV values of CIA (all $p < 0.001$). The size of a circle area represents the average intra-compartment Bray-Curtis dissimilarity of molecular DOM composition and microbial communities (free-living bacteria, particle-attached bacteria and microeukaryota). (B) Patterns of β NTI per lake based on the DOM composition (MCD, TD and TWCD), free-living bacteria (FL), particle-attached bacteria (PA) and microeukaryota (ME) community. Horizontal dashed red lines indicate upper and lower significance thresholds at β NTI = +2 and -2, respectively. Boxplots represent the 1st and 3rd quartiles, the line represents the median, and the points are the established data. The red rectangle denotes the average value per lake.

latitudes in warmer and dryer region, were positively associated to higher water temperatures, chemodiversity and lower transparency. Sulfate concentration was also associated to diversity of microbial community and DOM in Mediterranean lakes at lower latitude, especially to higher abundance of S-containing organic molecules which can derive from phytoplankton proliferation (Wen et al., 2022) and sediments (Chen et al., 2021; Luo et al., 2022; Valle et al., 2020; Zak et al., 2021). Their higher abundance was previously recorded in lakes with higher trophic status (Wen et al., 2022). Furthermore, previous studies have confirmed that rapidly increasing sulfate concentration are hidden promoter of eutrophication in shallow lakes, which may be connected to cyanobacterial blooms (Zhou et al., 2022). During our studied period relative abundance of Cyanobacteria did not show difference in lakes, which we can confirm with previously published results of phytoplankton diversity where no significant bloom of Cyanobacteria was recorded (Hanžek et al., 2021). Although DOM mineralization rates within lakes may increase with increasing temperatures, high temperatures may also enhance DOM release from soils into lakes (Luo et al., 2022). This can be also confirmed with the prediction of the study where highest organic carbon burials are appearing at lower latitudes in warmer and dryer regions (Mendonça et al., 2017).

Our results have shown high covariance between molecular-level DOM diversity and the diversity of individual microbial communities especially with diversity of microeukaryotes and free-living bacteria indicating their dynamic feedback. The observed spatial differentiation in DOM composition and microeukaryotic community is likely a response to a combination of their covariance, climate and land vegetation (Grossmann et al., 2016; Kothawala et al., 2021). Additionally, the seasonality-related abiotic factors strongly contributed to shaping microbial community patterns and correlated more strongly than molecular DOM composition. Considerable seasonal differences in bacterial community compositions were indicating the fast and pronounced response of aquatic systems to climatic shift on a microbial level. A stronger environmental influence was also recorded in estuaries ecosystem where abiotic factors had more impact on the bacterial diversity than on the DOM composition (Chen et al., 2021). The release of DOM by microeukaryotes was only partially reflected in the composition of the lake DOM, as the method does not efficiently capture amino acids, carbohydrates and colloidal organic matter, which make up a large proportion of the fresh DOM derived from algae (Toming et al., 2013). DOM in the studied lakes comprised a considerable amount of molecules with presumably relatively low bioavailability which might not be involved in further microbial transformations (Jiao et al., 2010), with the aromatic-type DOM which is less accessible to bacteria than the freshly produced DOM derived from phytoplankton (Patriarca et al., 2021; Pérez and Sommaruga, 2006). Furthermore, the high synchrony between microeukaryotic and bacterial communities can be due to the multiple mechanisms directly caused by biotic interaction (such as mutualism, antagonism, parasitism, and competition), the common response to the same environmental drivers, or due to both interactions and responses, that influence the microbial structure and their ecological functions (Bock et al., 2020; Schweitzer-Natan et al., 2019).

On the contrary to high diversity covariance, different processes influenced the assembly of DOM, the bacterial community, and the microeukaryotic community. The null model revealed that the molecular DOM composition was governed exclusively by deterministic variable selection, whereas the microbial community was governed by deterministic and stochastic processes. This indicates that spatial

processes and random events have essentially no impact on DOM composition in the studied lakes, which contrasts with previous work in river corridor systems, where both deterministic and stochastic processes influenced DOM assembly (Danczak et al., 2021, 2020; Garayburu-Caruso et al., 2020).

In accordance to the results of our study, we did not indicate a significant correlation between assembly processes of microbial communities and DOM they produce and consume. Furthermore, the molecular properties of DOM assemblage were governed by variable selection and had higher average β NTI values from microbial community. We infer that deterministic processes have caused divergence in metabolome composition while a given pair of DOM assemblages were significantly more different from each other than would be expected under stochastic assembly. While the lack of association of assembly processes between microbial communities and DOM is intriguing, it highlights the complex factors influencing both. In freshwater lakes, other factors such as vegetation, photodegradation, mineralogy, etc., likely have an impact on DOM assemblages independent of microbial communities. The following processes likely alter DOM assemblages in a way that is not reflected in microbial community composition. Additionally, DOM assemblages may change faster than microbial community composition, which may further indicate a closer association between DOM assemblages and expressed metabolisms or relative changes in activity. Overall, the lack of coordination between the processes driving the DOMs and the microbial assemblages does not indicate that there is no interaction between microbial communities and DOM assemblages, rather it suggests that distinct factors may be influencing microbial communities and DOM assemblages separately. Based on the given results, we emphasize the need for further investigation to understand better the underlying mechanisms driving DOM dynamics and their relationship with microbial communities in freshwater lake ecosystems.

5. Conclusion

Concluding this study, a null model used here based on the DOM and different microbial fractions revealed that different processes were involved in their structuring during the investigated period despite their high covariance. We found that the differences between lakes and climatic regions were mainly reflected in the diversity of DOM at the molecular formula-level and the microeukaryota community. The free-living and the particle-attached fraction of the bacterial community showed lower site-specificity in contrast to the DOM and microeukaryotic community. Overall DOM assembly was governed by deterministic variable selection, whereas active DOM fractions were governed deterministic and inactive were governed mainly by stochastic processes. On the contrary, community assembly was shaped dependently of the fraction that was analyzed. The increase in the size of the microbial fraction resulted in the decreases of the deterministic influence. Difference in the processes governing DOM and microbial composition does not indicate weak coupling between these components, rather it suggests that distinct factors may be influencing microbial communities and DOM assemblages separately. In this context, the identification of ecological processes shaping different microbial community and DOM in the long term is necessary in our understanding of the response of lakes ecosystems to environmental perturbations.

Data availability

The sequences generated for this study were deposited in the European Nucleotide Archive under project numbers PRJEB44080 and PRJEB52138. All final datasets of DOM molecular composition and microbial communities from this study are available at <https://figshare.com/s/578a7ddf25422380bef4>.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We extend our sincere gratitude to dr. Igor Stanković and Nikola Hanžek for sample collection and management, Prof. Dr. Thorsten Dittmar for providing FT-ICR-MS instrument time, Ina Ulber for technical assistance with the laboratory work, Lorena Selak for help with the laboratory work, Prof. Dr. Thorsten Stoeck and Prof. Dr. Sabine Filker for their help and support with NGS data analysis. Fig. 4a was created using BioRender.com.

This research was partially supported under the project STIM-REI, Contract Number: KK.01.1.1.01.0003, a project funded by the European Union through the European Regional Development Fund – the Operational Programme Competitiveness and Cohesion 2014–2020 (KK.01.1.1.01), partially by DNKVODA project Contract Number: KK.01.2.1.02.0335, by Croatian Science Foundation (HRZZ IP-2020–02–9021) and by the COST DNAqua-Net grant (CA15219) (to K. K.).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2023.120653](https://doi.org/10.1016/j.watres.2023.120653).

References

- Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A., Agatha, S., Berney, C., Brown, M.W., Burki, F., Cárdenas, P., Čepička, I., Chistyakova, L., del Campo, J., Dunthorn, M., Edvardsen, B., Eglit, Y., Guillou, L., Hampl, V., Heiss, A.A., Hoppenrath, M., James, T.Y., Karnkowska, A., Karpov, S., Kim, E., Kolisko, M., Kudryavtsev, A., Lahr, D.J.G., Lara, E., Le Gall, L., Lynn, D.H., Mann, D.G., Massana, R., Mitchell, E.A.D., Morrow, C., Park, J.S., Pawlowski, J.W., Powell, M.J., Richter, D.J., Rueckert, S., Shadwick, L., Shimano, S., Spiegel, F.W., Torruella, G., Youssef, N., Zlatogursky, V., Zhang, Q., 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. *J. Eukaryot. Microbiol.* 66, 4–119. <https://doi.org/10.1111/jeu.12691>.
- Aguilar, P., Sommaruga, R., 2020. The balance between deterministic and stochastic processes in structuring lake bacterioplankton community over time. *Mol. Ecol.* 29, 3117–3130. <https://doi.org/10.1111/mec.15538>.
- Antonić, O., Bukovec, D., Krizan, J., Marki, A., Hatic, D., 2000. Spatial distribution of major forest types in Croatia as a function of macroclimate. *Natura Croatica* 9, 1–13.
- Bertilsson, S., Jones, J.B., 2003. Supply of Dissolved Organic Matter to Aquatic Ecosystems. *Aquatic Ecosystems*. Elsevier, pp. 3–24. <https://doi.org/10.1016/B978-012526371-3/50002-0>.
- Bertilsson, S., Tranvik, L.J., 2000. Photochemical transformation of dissolved organic matter in lakes. *Limnol. Oceanogr.* 45, 753–762. <https://doi.org/10.4319/lo.2000.45.4.0753>.
- Bock, C., Jensen, M., Forster, D., Marks, S., Nuy, J., Psennner, R., Beisser, D., Boenigk, J., 2020. Factors shaping community patterns of protists and bacteria on a European scale. *Environ. Microbiol.* 22, 2243–2260. <https://doi.org/10.1111/1462-2920.14992>.
- Bramer, L.M., White, A.M., Stratton, K.G., Thompson, A.M., Claborne, D., Hofmoeckel, K., McCue, L.A., 2020. FtmsRanalysis: an R package for exploratory data analysis and interactive visualization of FT-MS data. *PLoS Comput. Biol.* 16, 1–12. <https://doi.org/10.1371/journal.pcbi.1007654>.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pêa, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*. <https://doi.org/10.1038/nmeth.f.303>.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* 108, 4516–4522. <https://doi.org/10.1073/pnas.100080107>.
- Chase, J.M., Myers, J.A., 2011. Disentangling the importance of ecological niches from stochastic processes across scales. *Philosoph. Trans. R. Soc. B* 366, 2351–2363. <https://doi.org/10.1098/rstb.2011.0063>.
- Chen, Q., Chen, F., Gonsior, M., Li, Y., Wang, Yu, He, C., Cai, R., Xu, J., Wang, Yimeng, Xu, D., Sun, J., Zhang, T., Shi, Q., Jiao, N., Zheng, Q., 2021. Correspondence between DOM molecules and microbial community in a subtropical coastal estuary on a spatiotemporal scale. *Environ. Int.* 154, 106558. <https://doi.org/10.1016/j.envint.2021.106558>.
- Danczak, R.E., Chu, R.K., Tfaily, M.M., Toyoda, J., Fansler, S.J., Goldman, A.E., Graham, E.B., Stegen, J.C., 2020. Using metacommunity ecology to understand environmental metabolomes. *Nat. Commun.* 11, 1–16. <https://doi.org/10.1038/s41467-020-19989-y>.
- Danczak, R.E., Goldman, A.E., Chu, R.K., Toyoda, J.G., Garayburu-Caruso, V.A., Tolić, N., Graham, E.B., Morad, J.W., Renteria, L., Wells, J.R., Herzog, S.P., Ward, A. S., Stegen, J.C., 2021. Ecological theory applied to environmental metabolomes reveals compositional divergence despite conserved molecular properties. *Sci. Total Environ.* 788. <https://doi.org/10.1101/2020.02.12.946459>.
- D'Andrilli, J., Cooper, W.T., Foreman, C.M., Marshall, A.G., 2015a. An ultrahigh-resolution mass spectrometry index to estimate natural organic matter lability. *Rapid Commun. Mass Spectrom.* 29, 2385–2401. <https://doi.org/10.1002/rcm.7400>.
- D'Andrilli, J., Cooper, W.T., Foreman, C.M., Marshall, A.G., 2015b. An ultrahigh-resolution mass spectrometry index to estimate natural organic matter lability. *Rapid Commun. Mass Spectrom.* 29, 2385–2401. <https://doi.org/10.1002/rcm.7400>.
- Dini-Andreote, F., Stegen, J.C., van Elsas, J.D., Salles, J.F., 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. *Proc. Natl. Acad. Sci. USA* 112, E1326–E1332. <https://doi.org/10.1073/pnas.1414261112>.
- Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnol. Oceanogr. Methods* 6, 230–235. <https://doi.org/10.4319/lom.2008.6.230>.
- Drake, T.W., Raymond, P.A., Spencer, R.G.M., 2018. Terrestrial carbon inputs to inland waters: a current synthesis of estimates and uncertainty. *Limnol. Oceanogr. Lett.* 3, 132–142. <https://doi.org/10.1002/lo.21005>.
- Dray, S., Dufour, A.B., 2007. The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Softw.* 22, 1–20. <https://doi.org/10.18637/jss.v022.i04>.
- Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194–2200. <https://doi.org/10.1093/bioinformatics/btr381>.
- Evans, C.D., Futter, M.N., Moldan, F., Valinia, S., Frogbrook, Z., Kothawala, D.N., 2017. Variability in organic carbon reactivity across lake residence time and trophic gradients. *Nat. Geosci.* 10, 832–835. <https://doi.org/10.1038/NGEO3051>.
- Garayburu-Caruso, V.A., Danczak, R.E., Stegen, J.C., Renteria, L., McCall, M., Goldman, A.E., Chu, R.K., Toyoda, J., Resch, C.T., Torgeson, J.M., Wells, J., Fansler, S., Kumar, S., Graham, E.B., 2020. Using community science to reveal the global chemogeography of river metabolomes. *Metabolites* 10, 1–20. <https://doi.org/10.3390/metabo10120518>.
- Grey, J., Jones, R.I., Sleep, D., 2000. Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state 232–240.
- Grossmann, L., Jensen, M., Heider, D., Jost, S., Glücksmann, E., Hartikainen, H., Mahamdallie, S., Gardner, M., Hoffmann, D., Bass, D., Boenigk, J., 2016. Protistan community analysis: key findings of a large-scale molecular sampling. *ISME J.* 10, 2269–2279. <https://doi.org/10.1038/ismej.2016.10>.
- Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., De Vargas, C., Decelle, J., Del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W.H.C.F., Lara, E., Le Bescot, N., Logares, R., Mahé, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., Christen, R., 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic. Acids. Res.* 41. <https://doi.org/10.1093/NAR/GKS1160>.
- Hanžek, N., Gligora Udovič, M., Kajan, K., Borics, G., Várbiro, G., Stoeck, T., Žutinić, P., Orlić, S., Stanković, I., 2021. Assessing ecological status in karstic lakes through the integration of phytoplankton functional groups, morphological approach and environmental DNA metabarcoding. *Ecol. Indic.* 131. <https://doi.org/10.1016/j.ecolind.2021.108166>.
- Hu, A., Jang, K.S., Meng, F., Stegen, J., Tanentzap, A.J., Choi, M., Lennon, J.T., Soininen, J., Wang, J., 2022. Microbial and environmental processes shape the link between organic matter functional traits and composition. *Environ. Sci. Technol.* 56, 10504–10516. <https://doi.org/10.1021/acs.est.2c01432>.
- Huber, P., Metz, S., Unrein, F., Mayora, G., Sarmiento, H., Devercelli, M., 2020a. Environmental heterogeneity determines the ecological processes that govern bacterial metacommunity assembly in a floodplain river system. *ISME J.* <https://doi.org/10.1038/s41396-020-0723-2>.

- Huber, P., Metz, S., Unrein, F., Mayora, G., Sarmento, H., Devercelli, M., 2020b. Environmental heterogeneity determines the ecological processes that govern bacterial metacommunity assembly in a floodplain river system. *ISME J.* 14, 2951–2966. <https://doi.org/10.1038/s41396-020-0723-2>.
- Jerusalén-Lleó, E., Nieto-Cid, M., Fuentes-Santos, I., Dittmar, T., Álvarez-Salgado, X.A., 2023. Solid phase extraction of ocean dissolved organic matter with PPL cartridges: efficiency and selectivity. *Front. Mar. Sci.* 10, 1–17. <https://doi.org/10.3389/fmars.2023.1159762>.
- Jia, X., Dini-Andreote, F., Salles, J.F., 2022. Unravelling the interplay of ecological processes structuring the bacterial rare biosphere. *ISME Commun.* 2, 96. <https://doi.org/10.1038/s43705-022-00177-6>.
- Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., Kirchman, D.L., Weinbauer, M.G., Luo, T., Chen, F., Azam, F., 2010. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. *Nat. Rev. Micro.* 8, 593–599. <https://doi.org/10.1038/nrmicro2386>.
- Kemmel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: r tools for integrating phylogenies and ecology. *Bioinformatics* 26, 1463–1464. <https://doi.org/10.1093/bioinformatics/btq166>.
- Kim, S., Kramer, R.W., Hatcher, P.G., 2003. Graphical method for analysis of ultrahigh-resolution broadband mass spectra of natural organic matter, the Van Krevelen diagram. *Anal. Chem.* 75, 5336–5344. <https://doi.org/10.1021/ac034415p>.
- Koch, B.P., Dittmar, T., 2006. From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. *Rapid Commun. Mass Spectrom.* 20, 926–932. <https://doi.org/10.1002/rcm.2386>.
- Kosztra, B., Büttner, G., Hazeu, G., Arnold, S., 2017. Updated CLC illustrated nomenclature guidelines. Final Report European Environ. Agency.
- Kothawala, D.N., Kellerman, A.M., Catalán, N., Tranvik, L.J., 2021. Organic matter degradation across ecosystem boundaries: the need for a unified conceptualization. *Trends Ecol. Evol.* 36, 113–122. <https://doi.org/10.1016/j.tree.2020.10.006>.
- Kritzbach, E.S., Langenheder, S., Lindström, E.S., 2006. Influence of dissolved organic matter source on lake bacterioplankton structure and function - Implications for seasonal dynamics of community composition. *FEMS Microbiol. Ecol.* 56, 406–417. <https://doi.org/10.1111/j.1574-6941.2006.00084.x>.
- Kujawinski, E.B., 2011. The impact of microbial metabolism on marine dissolved organic matter. *Annu. Rev. Mar. Sci.* 3, 567–599. <https://doi.org/10.1146/annurev-marine-120308-081003>.
- Li, H.Y., Wang, H., Wang, H.T., Xin, P.Y., Xu, X.H., Ma, Y., Liu, W.P., Teng, C.Y., Jiang, C.L., Lou, L.P., Arnold, W., Cralle, L., Zhu, Y.G., Chu, J.F., Gilbert, J.A., Zhang, Z.J., 2018. The chemodiversity of paddy soil dissolved organic matter correlates with microbial community at continental scales. *Microbiome* 8, 1–16. <https://doi.org/10.1186/s40168-020-00945-3>.
- Li, Y., Shang, J., Zhang, C., Zhang, W., Niu, L., Wang, L., Zhang, H., 2021. The role of freshwater eutrophication in greenhouse gas emissions: a review. *Sci. Total Environ.* 768. <https://doi.org/10.1016/j.scitotenv.2020.144582>.
- Lin, Q., Dini-Andreote, F., Meador, T.B., Angel, R., Meszárosová, L., Hedénc, P., Li, L., Baldrian, P., Frouz, J., 2022. Microbial phylogenetic relatedness links to distinct successional patterns of bacterial and fungal communities. *Environ. Microbiol.* 24, 3985–4000. <https://doi.org/10.1111/1462-2920.15936>.
- Liu, S., He, Z., Tang, Z., Liu, L., Hou, J., Li, T., Zhang, Y., Shi, Q., Giesy, J.P., Wu, F., 2020. Linking the molecular composition of autochthonous dissolved organic matter to source identification for freshwater lake ecosystems by combination of optical spectroscopy and FT-ICR-MS analysis. *Sci. Total Environ.* 703, 134764. <https://doi.org/10.1016/j.scitotenv.2019.134764>.
- Llames, M.E., Huber, P., Metz, S., Unrein, F., 2017. Interplay between stochastic and deterministic processes in the maintenance of alternative community states in Verrucomicrobia-dominated shallow lakes. *FEMS Microbiol. Ecol.* 93, 1–10. <https://doi.org/10.1093/femsec/fix077>.
- Logares, R., Deutschmann, I.M., Junger, P.C., Giner, C.R., Krabberød, A.K., Schmidt, T.S.B., Rubinat-Ripoll, L., Mestre, M., Salazar, G., Ruiz-González, C., Sebastián, M., de Vargas, C., Acinas, S.G., Duarte, C.M., Gasol, J.M., Massana, R., 2020. Disentangling the mechanisms shaping the surface ocean microbiota. *Microbiome* 8, 1–17. <https://doi.org/10.1186/s40168-020-00827-8>.
- Luo, J., Zhou, Q., Hu, X., Zeng, H., Deng, P., He, C., Shi, Q., 2022. Lake chemodiversity driven by natural and anthropogenic factors. *Environ. Sci. Technol.* 56, 5910–5919. <https://doi.org/10.1021/acs.est.1c08148>.
- Mahé, F., Rognes, T., Quince, C., de Vargas, C., Dunthorn, M., 2015. Swarmv2: highly-scalable and high-resolution amplicon clustering. *PeerJ*. <https://doi.org/10.7717/peerj.1420>, 2015.
- Mendonça, R., Müller, R.A., Clow, D., Verpoorter, C., Raymond, P., Tranvik, L.J., Sobek, S., 2017. Organic carbon burial in global lakes and reservoirs. *Nat. Commun.* 8, 1–6. <https://doi.org/10.1038/s41467-017-01789-6>.
- Merder, J., Freund, J.A., Feudel, U., Hansen, C.T., Hawkes, J.A., Jacob, B., Klaproth, K., Niggemann, J., Noriega-Ortega, B.E., Osterholz, H., Rossel, P.E., Seidel, M., Singer, G., Stubbins, A., Waska, H., Dittmar, T., 2020. ICBM-OCEAN: processing ultrahigh-resolution mass spectrometry data of complex molecular mixtures. *Anal. Chem.* 92, 6832–6838. <https://doi.org/10.1021/acs.analchem.9b05659>.
- Messenger, M.L., Lehner, B., Grill, G., Nedeva, I., Schmitt, O., 2016. Estimating the volume and age of water stored in global lakes using a geo-statistical approach. *Nat. Commun.* 7, 13603. <https://doi.org/10.1038/ncomms13603>.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., Hara, R.B.O., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., 2020. *vegan: community ecology package*. R Package Version 2, 5–7.
- Orland, C., Yakimovich, K.M., Mykityczuk, N.C.S., Basiliko, N., Tanentzap, A.J., 2020. Think global, act local: the small-scale environment mainly influences microbial community development and function in lake sediment. *Limnol. Oceanogr.* 65, S88–S100. <https://doi.org/10.1002/lno.11370>.
- Osterholz, H., Dittmar, T., Niggemann, J., 2014. Molecular evidence for rapid dissolved organic matter turnover in Arctic fjords. *Mar. Chem.* 160, 1–10. <https://doi.org/10.1016/j.marchem.2014.01.002>.
- Osterholz, H., Kirchman, D.L., Niggemann, J., Dittmar, T., 2018. Diversity of bacterial communities and dissolved organic matter in a temperate estuary. *FEMS Microbiol. Ecol.* 94, 1–11. <https://doi.org/10.1093/femsec/fiy119>.
- Osterholz, H., Kirchman, D.L., Niggemann, J., Dittmar, T., 2016. Environmental drivers of dissolved organic matter molecular composition in the Delaware estuary. *Front. Earth Sci. (Lausanne)* 4. <https://doi.org/10.3389/feart.2016.00095>.
- Patriarca, C., Sedano-Núñez, V.T., García, S.L., Bergquist, J., Bertilsson, S., Sjöberg, P.J.R., Tranvik, L.J., Hawkes, J.A., 2021. Character and environmental lability of cyanobacteria-derived dissolved organic matter. *Limnol. Oceanogr.* 66, 496–509. <https://doi.org/10.1002/lno.11619>.
- Pérez, M.T., Sommaruga, R., 2006. Differential effect of algal- and soil-derived dissolved organic matter on alpine lake bacterial community composition and activity. *Limnol. Oceanogr.* 51, 2527–2537. <https://doi.org/10.4319/lno.2006.51.6.2527>.
- Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 - approximately maximum-likelihood trees for large alignments. *PLoS ONE* 5. <https://doi.org/10.1371/journal.pone.0009490>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic. Acids. Res.* 41, D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- R Core Team, 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/> [WWW Document]. URL <https://www.eea.europa.eu/data-and-maps/indicators/oxygen-consuming-substances-in-rivers/r-development-core-team-2006> (accessed 11.5.20).
- Ranjard, L., Dequiedt, S., Chemidlin Prévost-Bouré, N., Thioulouse, J., Saby, N.P.A., Lelievre, M., Maron, P.A., Morin, F.E.R., Bispo, A., Jolivet, C., Arrouays, D., Lemanceau, P., 2013. Turnover of soil bacterial diversity driven by wide-scale environmental heterogeneity. *Nat. Commun.* 4. <https://doi.org/10.1038/ncomms2431>.
- Riedel, T., Dittmar, T., 2014. A method detection limit for the analysis of natural organic matter via fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* 86, 8376–8382. <https://doi.org/10.1021/ac501946m>.
- Santillan, E., Wuertz, S., 2022. Microbiome assembly predictably shapes diversity across a range of disturbance frequencies in experimental microcosms. *NPJ Biofilms Microbiomes* 8, 1–11. <https://doi.org/10.1038/s41522-022-00301-3>.
- Schweitzer-Natan, O., Ofek-Lalzar, M., Sher, D., Sukenik, A., 2019. Particle-associated microbial community in a subtropical lake during thermal mixing and phytoplankton succession. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.02142/FULL>.
- Shade, A., Stopnisek, N., 2019. Abundance-occupancy distributions to prioritize plant core microbiome membership. *Curr. Opin. Microbiol.* 49, 50–58. <https://doi.org/10.1016/j.mib.2019.09.008>.
- Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J.D., Higgins, D.G., 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* 7. <https://doi.org/10.1038/msb.2011.75>.
- Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L., Konopka, A., 2013. Quantifying community assembly processes and identifying features that impose them. *ISME J.* 7, 2069–2079. <https://doi.org/10.1038/ismej.2013.93>.
- Stegen, J.C., Lin, X., Fredrickson, J.K., Konopka, A.E., 2015. Estimating and mapping ecological processes influencing microbial community assembly. *Front. Microbiol.* 6, 1–15. <https://doi.org/10.3389/fmicb.2015.00370>.
- Stegen, J.C., Lin, X., Konopka, A.E., Fredrickson, J.K., 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* 6, 1653–1664. <https://doi.org/10.1038/ismej.2012.22>.
- Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H.W., Richards, T.A., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol. Ecol.* 19, 21–31. <https://doi.org/10.1111/j.1365-294X.2009.04480.x>.
- Strickland, J.D.H., Parsons, T.R., 1970. A Practical Handbook of Seawater Analysis, Fisheries Research Board of Canada. <https://doi.org/10.1002/iroh.19700550118>.
- Tanentzap, A.J., Fitch, A., Orland, C., Emilson, E.J.S., Yakimovich, K.M., Osterholz, H., Dittmar, T., 2019. Chemical and microbial diversity covary in fresh water to influence ecosystem functioning. *Proc. Natl. Acad. Sci. USA* 116, 24689–24695. <https://doi.org/10.1073/pnas.1904896116>.
- Tarigan, P.B., 2013. Standard Method For the examination for Water and Wastewater. *J. Chem. Inf. Model.* 53, 1689–1699.
- Thieme, L., Graeber, D., Hofmann, D., Bischoff, S., Schwarz, M.T., Steffen, B., Meyer, U.N., Kaupenjohann, M., Wilcke, W., Michalzik, B., Siemens, J., 2019. Dissolved organic matter characteristics of deciduous and coniferous forests with variable management: different at the source, aligned in the soil. *Biogeosciences* 16, 1411–1432. <https://doi.org/10.5194/bg-16-1411-2019>.
- Thornton, D.C.O., 2014. Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. *Eur. J. Phycol.* 49, 20–46. <https://doi.org/10.1080/09670262.2013.875596>.
- Toming, K., Tuvikene, L., Vilbaste, S., Agasild, H., Viik, M., Kisand, A., Feldmann, T., Martma, T., Jones, R.L., Nöges, T., 2013. Contributions of autochthonous and allochthonous sources to dissolved organic matter in a large, shallow, eutrophic lake

- with a highly calcareous catchment. *Limnol. Oceanogr.* 58, 1259–1270. <https://doi.org/10.4319/lo.2013.58.4.1259>.
- Tranvik, L.J., Downing, J.A., Cotner, J.B., Loiselle, S.A., Striegl, R.G., Ballatore, T.J., Dillon, P., Finlay, K., Fortino, K., Knoll, L.B., Kortelainen, P.L., Kutser, T., Larsen, S., Laurion, I., Leech, D.M., Leigh McCallister, S., McKnight, D.M., Melack, J.M., Overholt, E., Porter, J.A., Prairie, Y., Renwick, W.H., Roland, F., Sherman, B.S., Schindler, D.W., Sobek, S., Tremblay, A., Vanni, M.J., Verschoor, A.M., von Wachenfeldt, E., Weyhenmeyer, G.A., 2009. Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol. Oceanogr.* 54, 2298–2314. <https://doi.org/10.4319/lo.2009.54.6.part.2.2298>.
- Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M., Lee, Y.K., 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. *ISME Journal* 12, 1072–1083. <https://doi.org/10.1038/s41396-018-0082-4>.
- Valle, J., Harir, M., Gonsior, M., Enrich-Prast, A., Schmitt-Kopplin, P., Bastviken, D., Hertkorn, N., 2020. Molecular differences between water column and sediment pore water SPE-DOM in ten Swedish boreal lakes. *Water Res.* 170, 115320 <https://doi.org/10.1016/j.watres.2019.115320>.
- Vass, M., Székely, A.J., Lindström, E.S., Langenheder, S., 2020. Using null models to compare bacterial and microeukaryotic metacommunity assembly under shifting environmental conditions. *Sci. Rep.* 10, 1–13. <https://doi.org/10.1038/s41598-020-59182-1>.
- Wen, Z., Shang, Y., Song, K., Liu, G., Hou, J., Lyu, L., Tao, H., Li, S., He, C., Shi, Q., He, D., 2022. Composition of dissolved organic matter (DOM) in lakes responds to the trophic state and phytoplankton community succession. *Water Res.* 224, 119073 <https://doi.org/10.1016/j.watres.2022.119073>.
- Yan, Q., Liu, Y., Hu, A., Wan, W., Zhang, Z., Liu, K., 2022. Distinct strategies of the habitat generalists and specialists in sediment of Tibetan lakes. *Environ. Microbiol.* 24, 4153–4166. <https://doi.org/10.1111/1462-2920.16044>.
- Yuan, H., Mei, R., Liao, J., Liu, W.T., 2019. Nexus of stochastic and deterministic processes on microbial community assembly in biological systems. *Front. Microbiol.* 10, 1–12. <https://doi.org/10.3389/fmicb.2019.01536>.
- Zak, D., Hupfer, M., Cabezas, A., Jurasinski, G., Audet, J., Kleeberg, A., McInnes, R., Kristiansen, S.M., Petersen, R.J., Liu, H., Goldhammer, T., 2021. Sulphate in freshwater ecosystems: a review of sources, biogeochemical cycles, ecotoxicological effects and bioremediation. *Earth Sci. Rev.* 212, 103446 <https://doi.org/10.1016/j.earscirev.2020.103446>.
- Zhang, Y., Wang, J., Tao, J., Zhou, Y., Yang, H., Yang, X., Li, Y., Zhou, Q., Jeppesen, E., 2022. Concentrations of dissolved organic matter and methane in lakes in Southwest China: different roles of external factors and in-lake biota. *Water Res.* 225, 119190 <https://doi.org/10.1016/j.watres.2022.119190>.
- Zhou, C., Peng, Y., Chen, L., Yu, M., Zhou, M., Xu, R., Zhang, Lanqing, Zhang, S., Xu, X., Zhang, Limin, Wang, G., 2022. Rapidly increasing sulfate concentration: a hidden promoter of eutrophication in shallow lakes. *Biogeosciences* 19, 4351–4360. <https://doi.org/10.5194/bg-19-4351-2022>.
- Zhou, J., Ning, D., 2017. Stochastic community assembly: does it matter in microbial ecology? *Microbiol. Mol. Biol. Rev.* 81, 1–32.