

Supplemental Information 1: Detailed experimental procedures

ISI web of knowledge search

In order to calculate the percentage of studies that have considered all three domains with the most common molecular ribosomal marker (SSU), we looked up studies which included prokaryotic and eukaryotic SSU genes (16S and 18S, respectively). We searched document type “articles” in the ISI Web of Science (<http://www.webofknowledge.com/>, accessed in February 2017) for topics within the object areas: “ENVIRONMENTAL SCIENCES”, “ECOLOGY”, and “MARINE FRESHWATER BIOLOGY” with “16S” (8087 hits) or “18S” (1529 hits) as search term compared to “16S 18S” (235 hits), which results in a ratio of 0.024 (2.4%). Since 16S may be composed of bacteria and archaea we expanded the search term specifying the target domain. When including the term archaea and bacteria as “16S 18S bacteria archaea” (15 hits) for referring to studies targeting all three domains vs. “16S bacteria” (4998 hits), “16S archaea” (584 hits), “16S bacteria archaea” (434 hits), and “18S” (1529 hits), the ratio dropped to 0.003 (0.3%), calculated as “16S 18S bacteria archaea”/((“16S archaea”-“16S archaea bacteria”)+ (“16S bacteria”-“16S archaea bacteria”)).

Detailed methods

Study site and sampling strategy

We conducted our study in Kleiner Gollinsee (53°01'N, 13°35'E, herein referred to as Lake Gollin) which is a eutrophic shallow lake located in a low-lying rural region of north eastern Germany. It is a turbid system with distinct open-water (pelagic) and littoral zone vegetated by floating-leaved water lilies (primarily *Nymphaea alba* L.) and shoreline reed belts (*Phragmites australis* Trin. ex Steud.). For detailed information please refer to Brothers et al. (2013). Samples for the 454 sequencing approach were taken in three consecutive months on 21. April 2010, 19. May 2010 and 17. June 2010. We took water samples from three lake zones: a) the pelagic and b) littoral zone with a water sampler (LIMNOS, Turku, Finland), and c) benthic water was taken above the sediment by gathering a sediment core (from the boat) and decanting the first 20 cm of the water column without disturbing the sediment. All samples were taken in triplicates from random locations in the lake. Subsequently, 500 - 1000 ml were filtered above 0.22 µm Sterivex filters (Millipore, Darmstadt, Germany)

with the help of a peristaltic pump. The filters were stored at -80°C until DNA extraction. Additional monthly samples were taken as part of another project (Brothers et al. 2013) from April 2010 to March 2011. Those samples (500 ml) were filtered through $5.0\ \mu\text{m}$ and the filtrate again through $0.2\ \mu\text{m}$ polycarbonate filter (Nucleopore, GE Healthcare Life Sciences, Freiburg, Germany) from each water sample at the lake site. They were immediately frozen in liquid nitrogen and stored at -80°C until DNA extraction. In parallel we took water samples for analyzing the physicochemical parameters (Table S1) as described in Rösel et al. (2012).

Pyrosequencing approach

The community composition was analyzed using 454 sequencing with universal primers targeting bacteria, archaea and eukaryotes. The DNA for the 454 sequencing approach was extracted with DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. To mechanically disrupt the cells, all samples were subjected to a bead-beating step (MMX400, 2x 2 min, $f=30\ \text{sec}^{-1}$, Retsch, Haan, Germany) before extraction. Target SSU sequences (16S/18S) were amplified using the universal primers 926F (5'-aaactYaaaKgaattgacgg-3') and 1392R (5'-acgggCGgtgtgtRc-3') after Engelbrektson et al. (2010). The primer system was already applied to several aquatic systems (Angly et al. 2014, Gies et al. 2014, Hölker et al. 2015). It shares one primer with the universal primer set discussed by Parada et al. (2015). The primers were modified with barcoded 5' sequencing adaptors, consisting of MIDs recommended by Roche and Lib-L adapters. This enabled the labeling of individual samples, in this study months, lake zones, and replicates. The DNA concentration after the extraction was measured using Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Carlsbad, USA) and ~20ng of DNA extract was used as template for the Polymerase Chain Reaction (PCR) with AccuPrime High Fidelity Polymerase (Invitrogen, Carlsbad, USA): initial denaturation at 95°C for 3 min followed by 31 cycles of 30 sec at 95°C , 45 sec at 55°C , 90 sec at 72°C and a final extension at 72°C for 10 min (modified from Engelbrektson et al., 2010). Amplicons were purified with Agencourt AMPure beads (Beckman Coulter, Brea, USA) following the Amplicon Library Preparation Method Manual (GS FLX Titanium Series). The single amplicons were pooled equimolar into three pools, each corresponding to one sampling date. The three

pools were subjected to emulsion PCR for pyrosequencing and each pool was sequenced on one/eight lane of the 454 benchtop sequencer (454 GS FLX Titanium Series, Roche, Basel, Switzerland).

Sequence data analysis

Raw sequences were submitted to the European Nucleotide Archive (ENA) with the accession number PRJEB13126. Sequences were analyzed in Mothur (version 1.24.1; Schloss et al. 2009). Within Mothur, sequences were first demultiplexed and quality trimmed by sliding window (window size: 50, quality: 27). We exported all single reads (172k) and aligned them against the SILVA SSU reference database (115 Ref NR 99, www.arb-silva.de) with SINA aligner (version 1.2.11, Pruesse et al. 2012). The alignment was imported into Mothur, in which we followed the standard recommendations (http://www.mothur.org/wiki/454_SOP, accessed December 2013), which include trimming, pre-clustering, chimera removal and single linkage clustering. We exported the resulting operational taxonomic unit (OTU) table (clustered at a distance of 0.03, resulting in 2862 OTU) and reclassified the representative sequences using the least common ancestor option of SINA with the SILVA reference database (v112) and the SILVA taxonomy. The amount of sequences per sample ranged from 1744 to 17500 (total amount of remaining reads: 101522). This classified OTU table was imported into and further analyzed with R (<http://www.r-project.org/>, version 3.03). Plots were generated with ggplot2 on proportional OTU table. Ratios were calculated based on the classification. For instance, all phytoplankton species were summarized and related to the residual taxa to calculate the phototroph : heterotroph ratio; or all ciliate taxa were summarized to calculate the relative contribution of ciliates. The Krona chart (SI2) was based on the mean of the replicates, generated with Krona tools (Ondov et al. 2011). Statistical comparison between count and OTU data was achieved by summarizing all matching or nested classifications between the two datasets, resulting in 15 matching phytoplankton groups and 11 matching zooplankton groups. Finally, the count data of two sampling dates were averaged to account for the difference in sampling dates.

PCR assay

DNA from particle-associated (5.0 µm filters) and free-living microorganisms (0.2 µm filters) from monthly samples were extracted using the peqGOLD Tissue DNA Mini Kit (Peqlab, Erlangen, Germany) according to the manufacturer's instructions. Prior to that, zirconia beads (0.7 mm and 1.0 mm in diameter, Carl Roth, Karlsruhe, Germany) and 400 µl Lysis Buffer T (from the kit) were added to the cut filter pieces and subjected to a mechanical disruption step (MMX 400, f = 30 sec⁻¹, 2x2 min, Retsch, Haan, Germany). DNA concentrations were measured using the Quantus™ Fluorometer and a corresponding kit (QuantiFluor® dsDNA System, Promega, Fitchburg, USA). Since DNA yields from 0.2 µm filters were rather low, those samples were concentrated in a vacuum concentrator (Eppendorf® Concentrator plus, Eppendorf, Hamburg, Germany) to 30 µl. Based on the SILVA reference database we generated primers for the genus *Arcicella* with the help of Arb's (version 5.5) integrated probe design tool (Ludwig et al. 2004). The forward primer is specific to *Arcicella* and its sister genus *Pseudoarcicella* with more than 5 mismatches to the next relatives. And the reverse primer is highly specific for *Arcicella* with 5 mismatches to the sister genus *Pseudoarcicella*. The PCR product length is approx. 300 bp. After re-measuring DNA concentrations, 10 ng of extracted DNA per sample were used as a template for PCR. Each reaction contained further 1x MyTaq Reaction Buffer (Bioline, London, UK), 2 mM MgCl₂ (Bioline, London, UK), 1.2 µg µl⁻¹ BSA (Carl Roth, Karlsruhe, Germany), 200 nM Primer Arci-for (acggctcgtcaacgattgcag), 200 nM Primer Arci-rev (cttaacgatttctctgtaccactgac), 1.25 U MyTaq DNA Polymerase (Bioline, London, UK) and nuclease-free water added to a final volume of 50 µl. DNA samples were amplified after an initial denaturation step at 95°C for 3 min by 35 cycles of 95°C for 45 s, 63°C for 45 s, 72°C for 45 s and a final extension step at 72°C for 5 min (FlexCycler, Analytik Jena, Jena, Germany). 15 µl of each PCR product and 2 µl of EasyLadder I (Bioline, London, UK) as a DNA marker were loaded on a single 2% agarose gel and run for 25 min at 180 V. The results were documented under UV light (Alphamager™ System 2200, Alpha Innotech, Kasendorf, Germany) and the intensity of each sample was estimated based on the following system: 0 = no product, 1 = very weak product, 2 = weak product, 3 = medium product, 4 = strong product. For the final value we averaged the rating of littoral and pelagic sampling point as replicates. We further conducted a local similarity analysis with this discrete time series using

eLSA (Xia et al. 2011) based on Spearman correlations (see supplemental Table 2).

Environmental parameters for Lake Gollin

Supplemental Table S1. Environmental parameters for the corresponding sampling dates of this study are given as mean (SD). Total organic carbon (TOC), dissolved organic carbon (DOC), total phosphorous (TP), soluble reactive phosphorous (SRP), total nitrogen (TN), ammonium (NH₄), nitrite (NO₂), nitrate (NO₃), dry weight (DW), and temperature (temp).

	TOC [mg l ⁻¹]	DOC [mg l ⁻¹]	TP [µg l ⁻¹]	SRP [µg l ⁻¹]	TN [mg l ⁻¹]	NH ₄ [µg l ⁻¹]	NO ₂ [µg l ⁻¹]	NO ₃ [µg l ⁻¹]	DW [mg l ⁻¹]	temp [°C]
April	18.5 (0.2)	16.1 (0.1)	55 (5)	7 (3)	1.34 (0.05)	23 (8)	1.97 (1.63)	24.12 (23.08)	4.02 (0.6)	11.7 (0.7)
May	17.9 (0.1)	16.4 (0.1)	27 (7)	4 (1)	1.29 (0.18)	17 (5)	0.12 (0.24)	6.33 (7.58)	2.93 (0.61)	13.0 (0.1)
June	16.8 (0.3)	16.0 (0.2)	32 (1)	2 (1)	1.09 (0.03)	23 (8)	0.27 (0.65)	2.93 (7.19)	4.6 (0.83)	19.8 (0.1)

Comparison of OTU data with count data

Supplemental Table S2. Comparison of microscopic count data with OTU data obtained from DNA-metabarcoding. Classification of Metazoa sequences could be improved by changing from SILVA taxonomy to original EMBL/NCBI taxonomy. OTU matches (%) to microscopic counts tests whether the taxonomic groups of the count data matches or is nested within the OTU data. The number in brackets gives the percentage of count groups that have perfect matches to the OTU data (identical classifications).

Taxonomic lineage	Estimated level of resolution (V9)	#OTUs in Lake Gollin dataset	Taxon-classes in microscopic dataset	OTU matches % to microscopic observations (perfect matches)
Archaea	Genus	315	NA	NA
Bacteria	Genus	1986	NA	NA
Ciliophora	Genus	89	16	100 (38)
Rotifera	Order*	9	15	100 (0)
Crustaceae	Family**	15	7	100 (0)
Teleostei	Family**	1	NA	NA
Fungi	Order***	60	NA	NA
Choanomonada	Family	10	NA	NA
Protalveolata	Genus	23	NA	NA
Bicosoecida	clade	27	NA	NA
Cercozoa	clade	28	NA	NA
Euglenozoa	Genus	5	2	0 (0)
Cyanobacteria	Genus	34	4	100 (> 25)****
Bacillariophyta	Genus	8	5	100 (> 0)****
Chlorophyta	Genus	22	5	100 (0)
Chrysophyceae	Genus	21	3	100 (> 33)****
Haptophyta	Genus	5	1	100 (100)
Cryptophyceae	Genus	41	2	100 (50)
Dinoflagellata	Genus	31	3	100 (66)

*with SILVA: Phylum level classification

**with SILVA: Class level classification

***within Chytridiomycota: Genus level classification

****Organism were partly classified on morphology as being coccoid, filamentous, large or small, and thus real values will be higher.

Correlation analysis of PCR assay results with environmental parameters and plankton dynamics

Supplemental Table S3. Local rank based Spearman correlations of PCR assay results and environmental parameters monitored in the TerraLac project (cf. Brothers et al. 2013 and Lischke et al. 2016) based on eLSA analysis with a maximum delay of 1 (Xia et al. 2011). +/- = positive or negative correlation, n.s. = not significant (either p and/or q value), n.a. = not applicable.

correlated parameter	<i>Arcicella</i> (> 5.0 μm)	<i>Arcicella</i> (0.2-5.0 μm)
<i>Arcicella</i> (5.0 μm)	n.a.	+
chlorophyll a [$\mu\text{g l}^{-1}$]	-	-
phytoplankton biomass [mg C m^{-3}]	n.s.	-
BPP (5.0 μm) [$\mu\text{g C l}^{-1} \text{d}^{-1}$]	+	n.s.
BPP (5.0 - 0.2 μm) [$\mu\text{g C l}^{-1} \text{d}^{-1}$]	+	n.s.
bacterial cells (5.0 μm) [10^6ml^{-1}]	-	n.s.
bacterial cells (5.0 - 0.2 μm) [10^6ml^{-1}]	-	n.s.
crustacean biomass [mg C m^{-3}]	+	+
rotifer biomass [mg C m^{-3}]	+	n.s.
ciliate biomass [mg C m^{-3}]	-	n.s.
DIC [mg l^{-1}]	n.s.	-
TDP [$\mu\text{g l}^{-1}$]	n.s.	-
TP [$\mu\text{g l}^{-1}$]	n.s.	-
NH4 [mg l^{-1}]	-	n.s.
Mg [mg l^{-1}]	n.s.	+
Ca [mg l^{-1}]	n.s.	+

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S15.