Metabarcoding of unfractionated water samples relates phyto-, zoo- and bacterioplankton dynamics and reveals a single taxon bacterial bloom

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Summary

Most studies of aquatic plankton focus on either macroscopic or microbial communities, and on either eukaryotes or prokaryotes. This approach overlooks potential interactions among groups. We tested whether universal DNA-metabarcoding of unfractionated water samples could be used to qualitatively and quantitatively study the temporal dynamics of the total plankton community in a shallow temperate lake. We found significant changes in the relative proportions of normalized sequence reads of eukaryotic and prokaryotic plankton communities over a three-month period in spring. Patterns followed the same trend as plankton estimates using traditional microscopic methods. We characterized the bloom of a conditionally rare bacterial taxon belonging to *Arcicella*, which rapidly came to dominate the whole lake ecosystem and would have remained unnoticed without metabarcoding. Our data demonstrate the potential of universal DNA-metabarcoding applied to unfractionated samples for providing a more holistic view of plankton communities.

Introduction

Microbial communities are an integral component of total biodiversity (Barberán et al. 2014) and play key roles in all ecosystems. An understanding of their composition and dynamics is critical for studying ecosystem functions and services. Plankton communities in freshwater and marine ecosystems are comprised of both microbial and macroscopic organisms from all three domains of life (archaea, prokaryotes, and eukaryotes). Traditionally, plankton is classified into functional groups such as phytoplankton, zooplankton, and bacterioplankton; or into size classes such as picoplankton, nanoplankton, and

57 microplankton. This classification has resulted in the emergence of
58 independent fields of inquiry for many of the above groups. This is particularly
59 true for the separation of prokaryotic and eukaryotic groups.
60
61 A consequence of this separation is that simultaneous studies of all members
62 of the plankton community are rare, being thus far restricted to marine studies
63 in contemporary environmental surveys (Steele *et al.,* 2011, Lima-Mendez *et*
64 *al.,* 2015). This is despite the tremendous potential of integrated studies for
65 providing an interdisciplinary view of plankton communities (Fuhrman *et al.,*
66 2015). Most plankton studies employ size pre-selection steps (i.e. size
67 fractionation by selective filtration) and marker genes targeting bacteria,
68 archaea, or eukaryotes. Less than 1% of studies target all three, SI1). This
69 tradition impairs a full integration of microbial communities into ecological
70 concepts.
71
72 We used universal, cross-domain (*Bacteria, Archaea, Eukarya*) DNA-
73 metabarcoding of unfractionated water samples to study the entire plankton
74 community of the eutrophic, shallow, temperate Lake Gollin (*Kleiner Gollinsee*)
75 in northeastern Germany. We extracted total DNA from direct-filtered (0.2 µm)
76 lake water (0.5 to 1 L), enabling us to screen all organisms from what is
77 traditionally size-classified as pico- to mesoplankton (per definition 0.2 µm - 20
78 mm). Our aim was to characterize the whole plankton community and its
79 temporal dynamics in relation to algal biomass over a three-month period in
80 spring (April – June) 2010. This is the period with the highest dynamics of
81 plankton in most temperate eutrophic lakes. Our sampling (see SI1 for
82 parameters and experimental procedures) was part of a larger more traditional

whole-lake survey of bacteria, phytoplankton and zooplankton from April 2010 to December 2011 (Brothers *et al.,* 2013) that we used for comparison.

Results & Discussion

Prokaryotic- and eukaryotic population dynamics

The universal DNA-metabarcoding of unfractionated water samples successfully amplified across all three domains of life, yielding a total of 1307 bacterial, 388 eukaryal and 190 archaeal OTUs in the dataset. We recovered dominant organisms from nano- to mesoplankton size classes (see SI2 for taxa browsing) including typical freshwater bacteria (e.g. *Polynucleobacter*), phytoplankton (e.g. Cryptophyceae), and zooplankton (e.g., Maxillopoda). One field sample contained a small swimming fish larva, which was detected (as 1% of the total community, classified as Teleostei, see SI2) using our approach. Archaeal sequences were not abundant in Lake Gollin during the study period. This was unlikely to be caused by a primer bias, because the primer pairs have been successfully used to detect a dominance of *Archaea* in a meromictic lake (Gies *et al.,* 2014). The sum parameters for the three domains revealed a pronounced shift from a dominance of eukaryotes in April to a dominance of prokaryotes in June for all sampled water compartments (i.e. littoral, pelagic and sediment zones; Fig. 1a). This was accompanied by an increasing ratio of heterotrophs : phototrophs (SI3).

Comparison with microscopical observations

Abundance patterns based on DNA-metabarcoding data followed the trend of the sum parameters of phyto-, zoo- and bacterioplankton obtained from traditional microscopical counting data (SI3). Counting and sequence data were

not taken on the same day and are thus not directly comparable. Nevertheless, our data describe very well the previously reported dynamics of this lake: In the winter prior to our study period the lake underwent a significant fish-kill event caused by a prolonged ice-cover that led to anoxia. Thus, this event had an important impact on the whole lake ecosystem. There was a bloom of herbivorous ciliates in April 2010 (Lischke *et al.,* 2016) that was clearly visible in our data (approx. 30%, Fig. 1d). The ciliates likely exerted a very high grazing pressure upon the small plankton (<5 µm; Lischke *et al.,* 2016), which was mitigated when the ciliate population crashed in May-June (Fig. 1d) and replaced by increasing crustacean abundances (Hilt *et al.,* 2015; in our data especially in the pelagic zone, SI3). The replacement of ciliates by crustaceans may have then opened a niche for the observed bacterial dominance in June, through reduced grazing pressure and increased substrate supply via sloppy feeding of the copepods (Fig. 1).

Bloom-forming OTUs

Curiously, the bacterial dominance in June was attributed to operational taxonomic units (OTU) classified as the genera *Arcicella* and *Variovorax.* A single *Arcicella* OTU was most abundant in the pelagic, open water and potentially colonized the water from the surface (sampling depth = 1 m), where it reached highest proportions (Fig. 1b). *Variovorax* was more prevalent above the sediment, suggesting colonization from the sediment (Fig. 1c). In contrast to *Variovorax*, which exhibited already stable proportions at the other two sampling dates, *Arcicella* was present at very low abundances in April and May (<0.2%) and can thus be classified as a conditionally rare taxon (Lynch and Neufeld, 2015). There are few reports of blooms of rare bacterial taxa (e.g.,

Gilbert et al., 2012 described a *Vibrio* sp. bloom in the English channel; and Bizic-Ionescu et al. 2014 described the genera Flavobacterium and Undibacterium associated with a phytoplankton breakdown event in a lake) that were correlated to algae blooms. In order to test whether *Arcicella* was a reoccurring taxon in Lake Gollin or if this was a unique appearance related to the fish-kill disturbance, we screened additional samples (two size-fractions in this case: 0.2-5 µm & > 5 µm) that were available from Lake Gollin (Brothers *et al.,* 2013). A*rcicella* re-occurred in the following year (Fig. 2) and its appearance appeared negatively related to chlorophyll a concentrations and positively to crustacean biomass (SI1). Thus, this bacterial bloom appears to be rather different than previously described bacterial peak abundances coupled to phytoplankton. The presence of such bloom-forming conditionally rare taxa support the previously described food web dynamics of Lake Gollin (Lischke *et al.,* 2016), and extend it to the microbes. Conditionally rare taxa can have a disproportional significance for the overall community dynamics by shifting the total community structure (Shade *et al.,* 2014), however, knowledge of the ecological and metabolic potential of the rare species is required to draw conclusions about their ecosystem-wide consequences.

What is Arcicella?

Searching through existing freshwater and marine data sets, we found virtually nothing on *Arcicella* with a few exceptions (often in lotic ecosystems). Although not further discussed in their article, *Arcicella* was reported as the second most abundant OTU (6.8%) in all large circumpolar streams (Crump *et al.,* 2009), which instantly raised our attention. In other studies, we had to explicitly ask the authors for *Arcicella.* For example, a single *Arcicella* OTU was among the 10

most dominant OTUs in the large Danube River (however in total it comprised only moderate 2% ± 2%; Savio pers. comm.; Savio *et al.*, 2015) and again a single *Arcicella* OTU reached 12 ± 4% in a small turbid glacial lake (Peter pers. comm.; Peter and Sommaruga, 2016). It seems that this genus has a rather low diversity, but hosts single, occasionally very dominant OTUs. Despite those previous appearances, their autecology has never been investigated.

Study limitations

There was a large standard deviation among our replicates for crustaceans (S3). These organisms belong to the mesoplankton size class (0.2 to 20 mm) and it might be necessary to increase the water volume to better quantify their occurrence in future studies. Sequences belonging to Metazoa could only be classified to order level (e.g. Maxillopoda, Teleostei), because the small ribosomal subunit does not provide sufficient phylogenetically informative characters for many Metazoa groups (Tang et al. 2012). This low taxonomic resolution for certain organismic groups is one of the drawbacks of a universal marker that has to be considered in future studies of these groups. Moreover, DNA based methods are not error free. PCR-based approaches include taxonomic biases introduced by DNA extraction, primer choice, amplification, library preparation and sequencing (Gilbert et al. 2012; Singer et al. 2016). Thus, quantitative estimates can be only seen as semi-quantitative. Universal metabarcoding has the same limitation but at least comes with the advantage, that it provides a balance between all organisms, since most of the DNA template will be derived from the target groups (only excluding virus in this case). To date, it has produced conclusive results for the general trends in plankton communities (our study, see also Gies et al. 2014, Parada et al. 2015,

Needham and Fuhrman 2016).

Conclusions

Using unfractionated water samples with universal DNA metabarcoding allowed us to document major changes in almost the entire size- and functional spectrum of freshwater plankton with a single water sample analysis. Changes in the relative abundance of OTUs closely matched the seasonal dynamics of phyto-, zoo-, and bacterioplankton reported for this lake based on microscopy, indicating that relative abundance data based on read counts are ecologically meaningful. The discovery of a bloom of a largely overlooked freshwater bacteria genus was remarkable, with potential implications for the whole lake ecosystem. Our results highlight the potential of simultaneously studying both microbial and macrobial communities for an understanding of whole ecosystem changes. Integrative and interdisciplinary analyses may help to answer broad ecological questions in freshwater systems related to the role of keystone species; ecosystem resilience and resistance; and cross-domain interactions of species. Unfractionated sampling coupled to a universal metabarcoding represents a valuable means of studying plankton dynamics in aquatic systems and shows promise for long-term whole community monitoring.

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Figure Legends

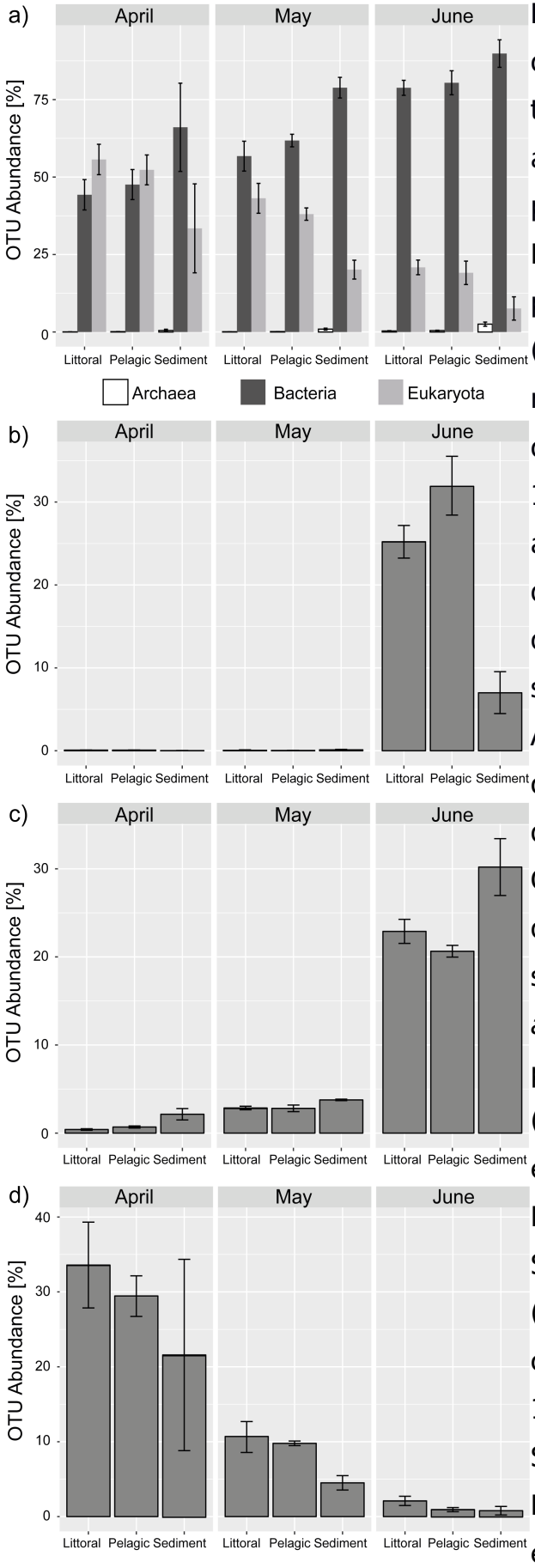

Figure 1. Spatial and temporal changes of microorganisms in Lake Gollin from three locations (littoral, pelagic, and above sediment; sampled as triplicates) per sampling event (21. April 2010, 19. May 2010 and 17. June 2010). The panels display sequence abundance (mean ± standard deviation) based on a rarefied OTU matrix (1744 reads/sample) of (a) all three domains with a total of 1307 bacterial, 388 eukaryal, and 190 archaeal OTUs in the dataset; (b) the dominant *Arcicella* OTU; (c) the dominant *Variovorax* OTU and (d) the sum of all ciliate OTUs (70 OTUs). Amplicons were based on the V9 region of the ribosomal small subunit for taxa detection (Engelbrektson *et al.,* 2010, Gies *et al.*, 2014). Methodological discussions on a related cross-domain single marker can be found in Parada *et al.* (2015). Sequencing followed the procedures described by Hölker *et al.* (2015) with the modification that we employed the AccuPrime High Fidelity Polymerase (Invitrogen, Carlsbad, USA). Sequences were processed in Mothur (version 1.24.1; Schloss *et al.,* 2009) and classified with SINA aligner (version 1.2.11; Pruesse *et al.,* 2012) against the SILVA SSU reference database (115 Ref NR 99, www.arb-silva.de). For details on experimental procedures see SI.

320

321 **Figure 2.** Seasonal appearance of *Arcicella* exhibited pronounced maxima and
322 minima over the course of the 2 years and appeared in the particle-attached (>
323 5 µm) and free-living fraction (0.2 - 5 µm). *Arcicella* was detected using a PCR
324 assay (see experimental procedures S1) and evaluated based on gel
325 electrophoresis band intensity where 0 = no PCR product, 1 = very weak
326 product, 2 = weak product, 3 = medium product, 4 = strong product.