Contrasting seasonal and interannual environmental drivers in bacterial communities within a large shallow lake: evidence from a seven year survey

Helen Tammert[1,2], Natalja Tšertova[2], Jelena Kiprovskaja[2], Florent Baty[3], Tiina Nõges[1], Veljo Kisand[1,2],*

[1]Centre for Limnology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Rannu 61117, Tartumaa, Estonia
[2]Institute of Technology, University of Tartu, Nooruse 1, Tartu 51411, Estonia
[3]Pneumology, Cantonal Hospital, Rorschacher Strasse 95, St. Gallen 9007, Switzerland

ABSTRACT: Bacterial populations experience rapid turnover in both time and space; however, the composition and diversity of their communities in plankton and the dynamics of community changes have not been well investigated over longer time scales. We explored the dynamics of bacterial community composition (BCC) monthly over a 7 yr period from 2004 to 2010, within a large, shallow, eutrophic lake. Seasonal and year-to-year changes in BCC were assessed using a PCR-based 16S rDNA fingerprinting technique based on denaturing gradient gel electrophoresis (DGGE). The bacterial operational taxonomic units differed between ice-free and ice-covered periods, and many recurred in specific seasons in consecutive years. The most significant variables related to within-year variation were temperature and the concentration of nutrients. Year-to-year variability was larger than within-year variability. Therefore, interannual variability tended to mask seasonal changes in BCC. The interannual differences in BCC were strongly related to abiotic conditions such as water level fluctuations that generally lead to water column mixing and sediment resuspension, and the qualitative composition of dissolved humic material. All highly related biotic variables that describe the total plankton community (in addition to bacteria, including phyto-, protozoo- and metazooplankton) were found to be modulated primarily by changes in the abiotic environment.

KEY WORDS: Bacterioplankton · Long-term temporal dynamics · Water level fluctuations · Eutrophic freshwater lake · Sediment re-suspension

INTRODUCTION

Understanding the patterns and processes that shape biodiversity is one of the central roles of ecological research (Preston 1960). Biodiversity itself is of significant value because it directly supports the function of ecosystems; however, knowledge regarding the community structure of aquatic bacterioplankton assemblies is relatively underdeveloped compared to assemblies of many other planktonic organisms (Sommer et al. 2011). A good description and understanding of characteristic variations in bacterial community composition (BCC) can provide basic knowledge regarding the processes that drive community assembly, and may also help predict how bacterial communities respond to perturbations, i.e. physical disturbances. Bacterial taxon distribution patterns have been successfully related to various drivers, such as predation (Pernthaler 2005), interactions with phytoplankton (Kent et al. 2007, Niu et al. 2011, Paver et al. 2013), chemical or resource gradients (Newton et al. 2006, Jones et al. 2012) and physical disturbances (Shade et al. 2012).

*Corresponding author: kisand@ut.ee

One phenomenon of plankton species dynamics, seasonal succession, is an annually repeated process of community assembly during which all major external factors and internal interactions shape changes in the community in a similar way each year. Seasonal succession is relatively well understood for phyto- and zooplankton (Sommer et al. 2011). Similar central patterns of seasonal cycles of aquatic bacterial communities have been suggested to occur in rivers, lakes and marine systems (Crump & Hobbie 2005, Fuhrman et al. 2006, Gilbert et al. 2012). However, an understanding of bacterioplankton succession, comparable to that which exists for phyto- and zooplankton, is lacking because most bacterioplankton studies are based on relatively short time periods, typically 2 or 3 consecutive years (e.g. Boucher et al. 2006, Berdjeb et al. 2011). Short time-series studies are helpful, but more confidence can be placed in the results of longer sampling campaigns (>5 yr), although these are often not feasible to conduct. Only a few studies have analysed the relationships of yearly repeated BCC dynamics (i.e. seasonal succession) with interannual variability over longer time periods (e.g. Rösel et al. 2012, Kara et al. 2013, Shade et al. 2013).

Determining the environmental factors that correlate with changes in BCC and potentially shape its dynamics requires detailed knowledge of the specific ecosystem. Population dynamics are driven by abiotic and biotic factors, most probably in combination; in many systems, however, one factor typically dominates. Extrinsic forces operating over broad spatial scales can impart synchronous behaviour to separate populations, while internal system-specific drivers often lead to idiosyncratic behaviour (Kent et al. 2007). As proposed by Yannarell et al. (2003), knowledge of water temperature and stratification dynamics in lakes can provide a good first approximation of the consecutive events that shape bacterial communities in many lakes, although the impact of specific biological and environmental factors requires more thorough investigation. Many previous efforts to determine the factors that might affect variability in bacterial communities have focused on a range of variables, such as water retention time (Lindström et al. 2006), water chemistry, including pH, alkalinity, dissolved organic carbon and nutrient concentration (Methé & Zehr 1999, Crump et al. 2003), and food web structure, including phytoplankton community composition (Niu et al. 2011), bloom formation (Eiler & Bertilsson 2004), grazing rate (Šimek et al. 2002, Yannarell et al. 2003) and viral abundance (Šimek et al. 2002, Maurice et al. 2010). The functional traits of bacteria (e.g. their central role in biogeochemical cycles and in food webs) are probably products of multiple interacting populations within these communities rather than those of single populations (Strom 2008). However, aquatic microbial ecologists are still a long way away from developing a conceptual framework for bacterial community assembly that can account for the relative importance of, and interactions among, these potentially hierarchical factors.

We completed a 7 yr survey of BCC dynamics in a naturally highly eutrophic and shallow freshwater lake. This lake has a detailed database of both abiotic and biotic variables for over 50 yr. Our general aim was to identify and distinguish the seasonal and interannual patterns in BCC using a longer-term (>5 yr) data set. To achieve this, we analysed monthly samples and studied these using various statistical methods to discriminate between repeated seasonal variability and interannual variability in BCC. Because different environmental variables may drive the community variability on different time scales, we statistically analysed the relationship between BCC and environmental parameters on both seasonal and interannual time scales. We addressed 2 specific questions: (1) Does BCC show well defined and repeated seasonal succession in a large shallow lake with strong natural water level (WL) fluctuation where the latter determines the intensity of wind-induced water column mixing and sediment resuspension? (2) Do different abiotic and biotic factors influence BCC dynamics on a seasonal or interannual time scale?

MATERIALS AND METHODS

Study site and sampling

Lake Võrtsjärv is a shallow lake in Estonia (58° 05′–58° 25′ N, 25° 55′–26° 10′ E) with an area of 270 km^2 and mean and maximum depths of 2.8 and 6 m. The lake is highly eutrophic with a mean concentration of ~2 mg l^{-1} total nitrogen (N_{TOT}) and ~50 µg l^{-1} total phosphorus (P_{TOT}). The water is alkaline (pH 7.5–8.5), with a high buffering capacity and a high content of suspended solids. During the ice-free period (~230 d), the Secchi depth does not exceed 1 m. The absolute long-term range level fluctuation is 3.20 m, which corresponds to a 93 km^2 difference in the lake area (~35% of average area) and a 0.874 km^3 difference in its volume (~85% of average volume). Because this lake is shallow, the water temperature can change rapidly, especially in the

spring and fall. The total number of bacteria varies between 1×10^6 and 6×10^6 cells ml^{-1}. The chlorophyll *a* (chl *a*) concentration ranges up to 84 mg m^{-3}.

Water samples for interannual analysis of the plankton community and water chemistry were collected from the deepest (5–6 m) area of the lake at monthly intervals from January 2004 to December 2010. The lake's physical characteristics, including temperature, were also recorded. A depth-integrated sample was obtained by mixing the lake water taken with a 2 l Ruttner sampler at intervals of 1 m from the lake surface to the near-bottom layer. Water was mixed in a 20 l sterile plastic tank and transported to an onshore laboratory within 30 min for immediate subsampling. The WL was measured at the outflow by the Estonian Institute of Hydrology and Meteorology within the framework of a national monitoring programme.

Bacterial enumeration, DNA extraction and PCR-DGGE

Subsamples of 20 ml were collected and fixed in 4% formaldehyde (final concentration) to assess bacterial abundance. Abundance was determined by epifluorescence microscopy (Leica DM RB) at 1000-fold magnification after staining with 10 µg ml^{-1} DAPI (PolySciences). Samples were filtered onto black 0.22 µm pore-size polycarbonate filters (Osmonics) and stored at −21°C until analysis.

Bacteria were recovered by filtration from 150 ml water onto 0.22 µm filters (Poretics, 47 mm diameter). Nucleic acids were extracted from these samples using an SDS-polyphosphate buffer for lysis, and zirconia beads for bead-beating (Stevens et al. 2005). Both primers GM5F (341F, with GC-clamp) and 907RM, specific for *Bacteria,* were used to amplify ~550 bp fragments using PCR. The amplification reaction was conducted in an Eppendorf Mastercycler, using RedTaq™ (Sigma). Denaturing gradient gel electrophoresis (DGGE) was used with the D-Code System (Bio-Rad) or DGGEK-1001-220 (CBS Scientific) according to the method provided by Brinkhoff & Muyzer (1997). We used a denaturing gradient of urea/formamide between 20 and 70% on a polyacrylamide gel with an electrophoresis time of 20 h at a constant voltage of 100 V. Gels were stained with SYBR Gold (Molecular Probes) and visualized by Typhoon Trio (Amersham). Each band (DNA fragment travelling to a particular position) was assumed to represent a single operational taxonomic unit (OTU). Every gel contained an additional 2 lanes for

samples that contained a large number of OTUs and functioned as standards to fit and normalize different gel runs. Banding patterns were compared using the position of the bands with a binary coding system (1: band present, 0: no band) to create a Jaccard similarity matrix.

Sequencing of PCR-DGGE bands

DNA bands were excised from DGGE gels using sterile pipette tips. Small pieces of acrylamide gel were placed in 20 µl of sterile water and subjected to passive diffusion overnight at 4°C to allow gel fragments to dissolve in water. They were used as a template in a reamplification reaction with the primers GM5F and DS907RM. PCR products were purified with Quantum Prep PCR Kleen spin columns (Bio-Rad) and cloned into the pGEM Easy-T vector system (Promega). Clone identity was confirmed by a re-run of the DGGE gel to determine their positions according to the bands from which they were excised. The PCR products were purified with Quantum Prep PCR Kleen spin columns (Bio-Rad) and directly sequenced using an Applied Biosystem 3730XL according to the manufacturer's directions.

Accession numbers for all sequenced DGGE bands can be found in GenBank (KC815470 to KC815511). The preliminary phylogenetic identity of cloned DGGE band sequences was assessed using BLAST against NCBI GenBank.

Physicochemical variables and plankton abundance, biomass and taxonomic composition

Standard limnological variables were measured according to standard protocols; for details, see Table S1 in the Supplement at www.int-res.com/articles/suppl/a075p043_supp.pdf. All of these variables were considered in our statistical analyses.

Phytoplankton and protozooplankton (mostly ciliates) samples were preserved in acidified Lugol's solution (0.5% final concentration), and examined using the technique of Utermöhl (1958) for species composition, abundance and biomass. Ciliates were divided into 4 categories according to food preference: bacterivores, bacteri-herbivores, herbivores and predators. Metazooplankton samples were fixed with acidified Lugol's solution (0.5% final concentration) and counted under a binocular microscope in a Bogorov chamber at 32- to 56-fold magnification. For biomass calculations, we determined the average

body length of >20 individuals of each taxon. Meta-zooplankton were divided into bacterivorous and non-bacterivorous based on literature reports.

Data analysis

Descriptive statistical methods, such as linear re-gression and principal component analysis (PCA), were used to describe the variability of, presumably, abundant OTUs using a simple fingerprinting ap-proach (DGGE). A series of multivariate exploratory statistical methods was used to determine various factors that relate to changes in OTU abundance. These included between-group PCA (BGA; Doledec & Chessel 1987) for the analysis of inter-seasonal and interannual variations of the bacterial community composition. To explore the seasonal effects inde-pendently from interannual effects, the first step of partialling-out was accomplished using within-group analysis (WGA; Doledec & Chessel 1991). In addition, we compared within year (seasonal) and year-to-year variations using a permutational multi-variate ANOVA (PERMANOVA; implemented as the adonis procedure within the 'vegan' software pack-age). The synchrony of temporal variations between bacterial OTUs and the species composition of phyto, protozoo- and metazooplankton was investigated by multiple co-inertia analysis (MCOA; Chessel & Hanafi 1996, Bady et al. 2004). An additional step of partialling-out was applied to MCOA to subtract the effect of abiotic environmental variables. MCOA aims to simultaneously analyse *K* tables correspon-ding to *K* sets of variables that describe the same ob-servations. This analysis optimizes within-table vari-ance while maximizing the correlation between the scores of each individual table (data set). A synthetic score provides a reference structure for the analysis. MCOA is well adapted for the analysis of structures that vary over time and enables one to separate the stable part of the structure versus its fluctuating part. External information can be integrated within MCOA using linear constraints that work to improve the interpretability of this approach. Restricted MCOA can be used to remove unwanted effects from the analysis. Following this, we determined which variables significantly relate to the seasonal patterns using a vector-fitting procedure (vegan package tutorial: http://cc.oulu.fi/~jarioksa/opetus/metodi/ vegantutor.pdf). All analyses were performed using the R statistical software package that included the extension packages ade4 (Chessel et al. 2004, Dray et al. 2007) and vegan (Oksanen et al. 2012).

RESULTS

Bacterioplankton composition and phylogenetic identity

The BCC was analysed using 84 samples collected monthly, with an overall sampling period of 7 yr from January 2004 to December 2010. A total of 89 non-chloroplast bands were discovered out of 103 bands. To further analyse the BCC, cyanobacterial/plastid bands were removed. The number of OTUs per sam-ple ranged from 7 to 44, with a median value of 19. Twenty-one OTUs (23.6%) were transient and were observed in <10 samples. Fifteen of the 84 samples possessed 20 to 30 persistent OTUs, and only 4 sam-ples had >30 OTUs.

The 29 most prominent (i.e. dominating, encoun-tered in >20 samples) DGGE bands were excised and sequenced (≤5 bands per individual OTU). Over 50% of these OTUs belonged to 2 classes of *Proteobacteria* (10 *Alphaproteobacteria* and 8 *Beta-proteobacteria*). *Actinobacteria* and *Bacteriodetes* were equally represented by 5 phylotypes, and, additionally, 1 phylotype was classified as *Gamma-proteobacteria*. DNA was extracted from the total community samples with a high abundance of filamentous algae; therefore, 9 of these prominent bands were affiliated with *Cyanobacteria* and 4 with chloroplasts. An overview of the phylotypes identified by sequencing is given in Table S2 in the Supplement.

Trends in plankton abundance, biomass and taxonomic composition

The phytoplankton community was dominated by cyanobacteria and diatoms. Among many of them, an association of filamentous algae occurred in summer and autumn: cyanobacteria *Limnothrix planktonica* (Wołosz.), *L. redekei* (van Goor) accompanied by *Planktolyngbya limnetica* (Lemm.) Kom.-Legn. and *Aphanizomenon skujae* Kom.-Legn. et Cronb. Long cylindrical species of the diatom genus *Aulacoseira* dominated in spring (more details are provided in Nõges et al. 2010a).

Bacterial abundance and biomass had a pro-nounced reoccurring seasonal fluctuation, which usually peaked from July to September and was low-est during the ice-covered period (*t*-test, $t = 9.409$, p < 0.01). Bacterial biomass decreased during the inves-tigation towards a lower maximum number in the vegetation period (Fig. S1 in the Supplement).

Over the study period, the species composition of ciliates changed slightly towards bacterivores and predators, whereas other feeding groups decreased slightly in species numbers (Zingel & Nõges 2010). The overall number of species remained constant throughout the study. The total biomass of ciliates decreased during this time, mainly due to the remarkable constant downward trend of bacterivore abundance (Fig. S1). The spring peak was usually dominated by large herbivorous oligotrichs. The annual maximum abundance was recorded among small bacterivorous scuticociliates and oligotrich species, and usually occurred in late July or early August each year.

The maximum abundance of bacterivorous zooplankton and coinciding grazing pressure to bacteria, which can be assumed from the increase in biomass of the grazers, peaked throughout July when rotifers were at their maximum (H. Agasild unpublished data). Bacterivores contributed an average of 29 to 93.7% of the total zooplankton biomass without long-term trends over the years; however, we observed a decreasing trend in the total share of the biomass. The main contributors to bacterivorous feeding zooplankters were rotifers (70% of total abundance) and cladocerans (30%).

Seasonal succession versus interannual variation in BCC and its relationship to their environment

The BCC varied to a great degree between different years as shown by a BGA on PCA and PERM-ANOVA (details in 'Materials and methods'). The data used in this analysis consisted of the presence/absence of OTUs. Yearly differences in BCC were highly significant in residual variations after extracting the within-year (seasonal) variation (Fig. 1A, BGA on PCA, 1000 replicates of the Monte Carlo permutation test, p < 0.001, proportion of explained inertia, i.e. variation 0.7994). To properly decompose the interannual variation from the seasonal effect, we first applied WGA using year as a grouping factor, and thereafter tested the effect of 4 seasons (winter, spring, summer, autumn) on the residual variation of the presence/absence of OTUs (Fig. 1B). Only winter, defined as the ice-covered period, was significantly different (p = 0.021, proportion of explained inertia 0.0331) from the other seasons, which showed no significant differences between each other. The importance of an ice-covered versus an ice-free period was verified by BGA using ice/open as a 2-level factor (1000 replicates, p =

0.001). The most influential OTUs in this grouping, i.e. the 5 most highly ranked signature OTUs (Fig. 1B) were analysed in more detail along temporal scales, using absence/presence of these OTUs (Fig. 1C). All of these selected OTUs were characteristic to the winter season (i.e. ice-covered lake), but occasionally occurred during other seasons. Similarly, separation of within-year (seasonal) and year-to-year (interannual) variation by permutational MANOVA indicated that between-year variability was more significant than seasonal variability (PERMANOVA F. Model = 3.4923, p < 0.01 for yearly variation versus F. Model = 1.1403, p = 0.023 for seasonal variation).

To study possible synchrony in the temporal variation of bacterial OTUs with the species composition of phyto-, protozoo- and zooplankton, a covariation between 4 datasets was analysed by MCOA. In addition, a fifth dataset that included abiotic environmental variables was added to MCOA as a co-varying dataset. The covariation of plankton organisms with physicochemical limnological variables (i.e. abiotic conditions: temperature, WL, concentrations of nutrients etc., see details in Table S1) was analysed in 2 ways: with and without a partialling-out effect. Partialling-out the covariation with abiotic variables did substantially change the relationships between bacterio-, phyto-, protozoo- and zooplankton (Fig. 2). This indicates that an abiotic environment has a strong collinear influence on all plankton groups, and interactions between plankton groups were overruled by an abiotic environment. 'Residual' interactions between plankton groups themselves can be analysed only after partialling-out the covariation of an abiotic environment. The composition of bacterioplankton species was positively correlated with zooplankton community composition and did not correlate with phytoplankton community composition (Fig. 2B).

To determine which variables significantly relate to the formation of a seasonal pattern in BCC, the physicochemical limnological variables were linearly fitted on a PCA space of BCC (using the envfit function in the R extension vegan). To analyse seasonal, i.e. within 1 yr, dynamics, the yearly difference of BCC was decomposed before fitting (WGA on PCA of BCC using year as a 'within groups' factor). To find the variables that significantly correlate to interannual variation, the monthly variation in BCC was decomposed in a similar way before (WGA on PCA of BCC using month as a 'within groups' factor), both results are presented in Table 1. This analysis shows that variables related to BCC
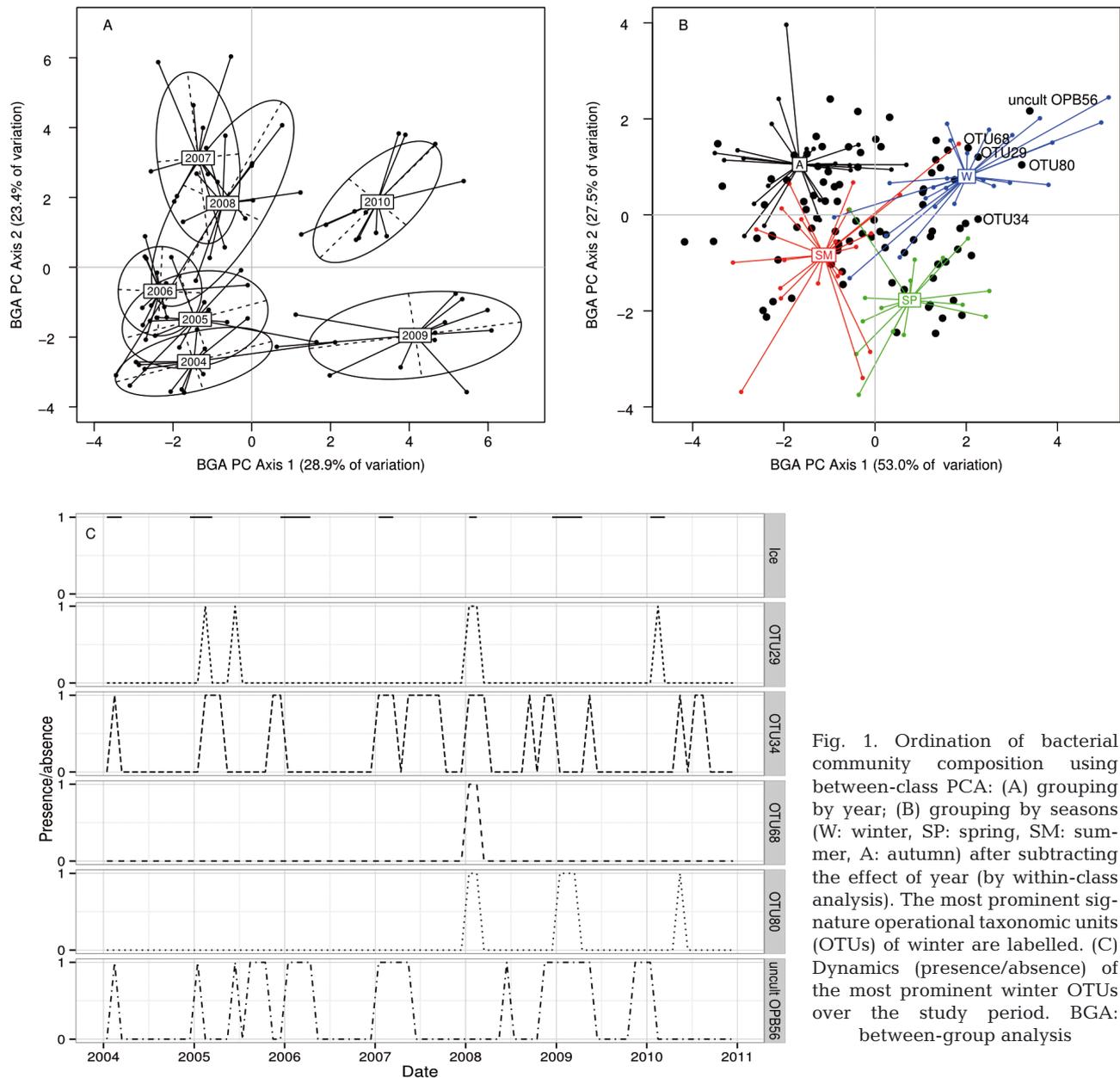
Fig. 1. Ordination of bacterial community composition using between-class PCA: (A) grouping by year; (B) grouping by seasons (W: winter, SP: spring, SM: summer, A: autumn) after subtracting the effect of year (by within-class analysis). The most prominent signature operational taxonomic units (OTUs) of winter are labelled. (C) Dynamics (presence/absence) of the most prominent winter OTUs over the study period. BGA: between-group analysis

at different time scales were different. Seasonal succession was closely related to temperature, water transparency (measured as Secchi depth), concentration of various nutrients, N_{TOT}:P_{TOT} ratio, pH etc. Variation between years was strongly correlated with changes in WL, to variables indirectly characterizing the composition of dissolved organic matter (coloured dissolved organic matter calculated from specific absorption at wavelength 380 nm, CDOM; chemical oxygen demand by permanganate oxidation, COD_{Mn}) and to the concentration of certain micro-nutrients (e.g. [K], [Cl]).

DISCUSSION

The BCC displayed significant interannual fluctuations in Lake Võrtsjärv, and typically underwent seasonal dynamic shifts between ice-covered and ice-free periods. Variation between years was larger compared with seasonal fluctuations in BCC. This clearly indicates that the environment is unstable between years and that the processes driving these changes differ from year to year. One of the most prominent factors that influences the lake environment on an interannual time scale was strong WL
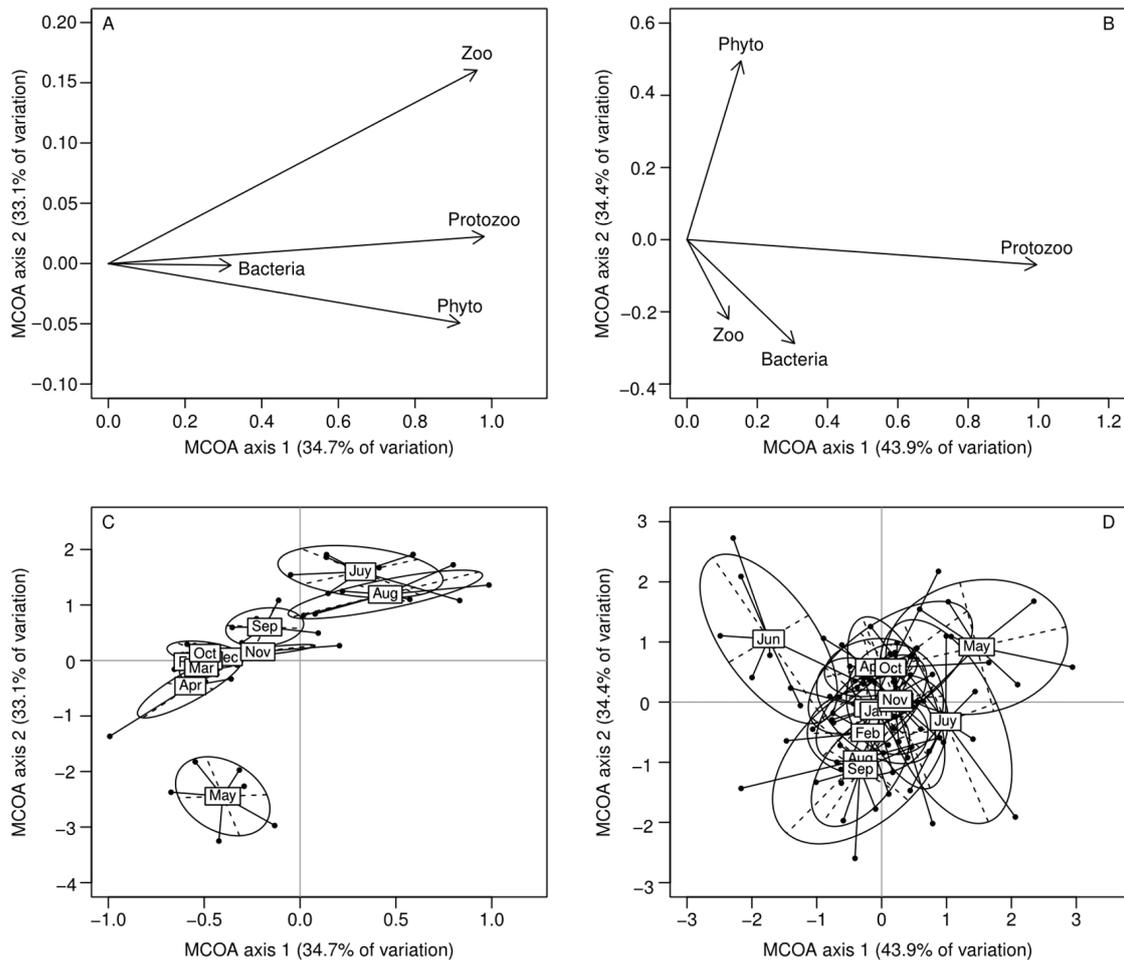
Fig. 2. Relationship between various plankton groups analysed using multiple co-inertia analysis (MCOA). (A) Firstly, the interaction of physicochemical variables of an abiotic environment is included and all 4 datasets are covarying similarly, or (B) covariation of physicochemical variables is partialled-out by partial MCOA and thereafter the groups of plankton organisms do not correlate. (C, D) Clustering of samples by season disappears when partialling-out the interaction of physicochemical variables with bacterio- phyto, protozoo- and metazooplankton. This suggests the primary influence of abiotic variables on all groups of plankton organisms

fluctuation, which is a good proxy for wind-driven physical disturbances such as mixing of the water column (Shade et al. 2011) and intensity of sediment re-suspension (Bloesch 1995 and references therein). Within-year seasonal succession was modulated mostly by ice cover. The only statistically significant repeated 'seasons' in the BCC were the under-ice community and the free-water community. In addition to previously described processes that drive bacterial community assembly (e.g. predation, interaction with phytoplankton, chemical and resource gradients), we demonstrate the importance of natural disturbance due to strong WL fluctuations that leads to more intensive re-suspension of the soft sediment and keeps the water column mixed at lower wind speed. However, such a central role of these abiotic processes applies only to shallow aquatic ecosys-

tems, viz. shallow lakes, estuaries and marine coastal areas.

What accounts for the interannual and seasonal variability of BCC?

In our analysis of the complex interactions over a longer time period (i.e. several years), it is important to decompose and compare the degree of variation on different time scales. This means that the dynamics and trends in seasonal fluctuations should be discriminated from each seasonal and presumably repeated dynamics. Even more importantly, collinear behaviour of several inter-related variables (abiotic versus food web interactions) should be decomposed and separated from each other in order to understand major

Table 1. Significance of the relationships of bacterial community composition (BCC) to physicochemical variables. 'Seasonal' refers to linear combinations of BCC with abiotic environment within a 1 yr period, 'year-to-year' refers to linear combinations of BCC over the entire study. P_{TOT}: total phosphorus, N_{TOT}: total nitrogen, COD_{Mn}: chemical oxygen demand by permanganate oxidation, CDOM: coloured dissolved organic matter calculated from specific absorption at 380 nm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Variable	Seasonal				Year-to-year			
	PC1	PC2	r^2	$p_r(>r)$	PC1	PC2	r^2	$p_r(>r)$
Secchi depth	0.777	−0.629	0.227	0.002**	−0.085	0.996	0.061	0.072
Temperature	−0.659	0.753	0.116	0.007**	−0.581	−0.814	0.001	0.967
Oxygen conc.	0.275	0.962	0.009	0.713	−0.851	−0.524	0.017	0.486
Water level	−0.109	−0.994	0.031	0.260	0.996	0.095	0.177	0.001***
pH	−0.675	0.738	0.172	0.001***	0.124	−0.992	0.005	0.817
[HCO_3]	0.349	−0.937	0.136	0.009**	−0.992	−0.122	0.001	0.980
P_{TOT}	−0.970	0.241	0.080	0.036*	−0.642	−0.767	0.077	0.044*
[PO_4]	−0.971	0.238	0.091	0.023*	0.765	−0.644	0.066	0.058
N_{TOT}	0.588	−0.809	0.143	0.005**	−0.687	−0.727	0.039	0.203
[NO_3]	0.662	−0.749	0.235	0.001***	−0.587	−0.810	0.001	0.971
[NH_4]	0.324	−0.946	0.044	0.169	−0.556	−0.831	0.042	0.192
[NO_2]	0.009	1.000	0.002	0.879	−0.798	−0.603	0.002	0.951
N_{TOT}:P_{TOT}	0.848	−0.530	0.171	0.004**	−0.853	0.522	0.009	0.685
COD_{Mn}	−0.988	0.157	0.039	0.197	0.934	−0.357	0.151	0.004**
[SO_4]	0.586	−0.810	0.054	0.098	−0.839	−0.544	0.192	0.002**
[Cl]	0.367	−0.930	0.044	0.152	−0.808	−0.589	0.172	0.002**
[Ca]	0.525	−0.851	0.184	0.001***	0.995	0.099	0.021	0.442
Alkalinity	0.937	−0.350	0.200	0.006**	−0.271	0.963	0.026	0.341
Colour	0.545	−0.839	0.008	0.727	0.999	−0.036	0.227	0.001***
Seston	−0.715	0.699	0.187	0.001***	−0.532	−0.847	0.052	0.100
[Fe]	−0.849	−0.528	0.031	0.295	0.964	−0.267	0.018	0.464
[Na]	0.342	−0.940	0.091	0.033*	−0.965	−0.260	0.138	0.003**
[K]	0.300	−0.954	0.085	0.035*	−0.998	−0.069	0.118	0.006**
[Mg]	0.177	−0.984	0.048	0.138	−0.894	−0.448	0.100	0.016*
Conductivity	0.501	−0.866	0.185	0.002**	−0.912	−0.410	0.001	0.971
[Si]	0.719	−0.695	0.184	0.001***	−0.828	−0.561	0.001	0.952
CDOM	−0.982	0.187	0.123	0.007**	0.403	−0.915	0.098	0.012*

driving forces behind changes in BCC. This can be accomplished using well-designed experiments; how ever, carrying out long-term experiments over a decade or more is challenging. An alternative is to collect detailed data from observations, and decompose different levels of variation by carefully designing the data analysis. Multivariate statistical approaches, mostly used for preliminary description, are fully capable of providing this type of analysis. Their power over traditional statistics is their ability to describe a large number of variables, a feature typical of community composition data. To determine the arbitrary directions of the major variation in these datasets, we used well known ordination techniques such as principal component (PCA) or correspondence analysis (CA) and their supervised counterpart including between- (BGA) and within-group analyses (WGA) (Sabatier et al. 1989). A combination of these constrained analyses (BGA/WGA) allows one to decompose different levels of temporal variation and assess the remaining residual variation. BGA and WGA decompose the total variance into between- and within-group sub-parts, and the discrimination of predefined groups can be tested using permutation procedures (sometimes referred as a pseudo-F-statistic). Using BGA/WGA, between-class ratio of inertia (year-to-year variation in our analysis) and within-class inertia (seasonal variation) can be compared. To perform this comparison, we applied PERMANOVA, which is based on dissimilarity distances and from which a pseudo F-statistic can be calculated. This was done in order to validate ordination-based results for partitioning seasonal variation from year-to-year variation. Thereafter, we employed MCOA (Bady et al. 2004) and its more advanced extension, partial MCOA (Chessel & Hanafi 1996). This approach allowed us to analyse separate interactions between different plankton groups, and to estimate the effect of co-varying abiotic variables that potentially combine the entire ecosystem into a single analysis. Our results suggested that different sets of variables are important in shaping the dynamics of BCC over dif-

ferent time scales. Temperature and nutrient concentration were found to strongly modulate microbial composition dynamics on a seasonal scale (Table 1). This observation is in good concordance with previous studies (e.g. Degerman et al. 2013). It should be noted that this finding does not exclude their importance in changes that occur over longer time periods (i.e. climate change and/or eutrophication). However, the time frame of our study was too short and the seasonal variability was too large to reveal the influence of changing temperature and nutrient load that occurs over many decades. Interannually, the dynamics of BCC were more strongly related to generic climatic variables (e.g. North Atlantic Oscillation index). It should be noted that, for simplicity, we used linear models to relate BCC to environmental variables; at the same time, these environmental variables, which include climatic variables, might be cyclic in nature. However, the period of these cycles is usually longer compared to the time period over which this study was conducted. In a shallow and natural (an unregulated WL) freshwater lake, the most prominent variable associated with climate fluctuations is WL (Nõges et al. 2010b). It has been shown that WL affects both the abundance and biomass levels of bacterio- and phytoplankton (Kisand & Nõges 2004).

The most obvious influence of low WL conditions to the composition of lake plankton would be an increase in the probability of whole water column mixing at lower wind speeds compared with the higher WL condition. The WL varied by 2.4 m during this study period, and almost equals the mean depth of the lake (2.8 m). The mean annual WL amplitude was 1.12 m. The total recorded range of WL fluctuations is 3.2 m, with an annual mean amplitude of 1.38 m (Nõges & Nõges 1999). Homogeneous versus stratified water columns are obviously contrasting conditions for bacterial communities in any lake (Shade et al. 2011). During the ice-free period, short-term temporal stratification events can occur in Lake Võrtsjärv (Kisand et al. 1998); however, the influence of these events on the BCC was analysed using a sampling interval with a time resolution of 1 mo, which is too long to detect the influence of these events. Depletion of oxygen and reverse temperature stratification occur in most winters under the ice (Järvalt et al. 2004). Another direct influence of wind-driven waves in shallow lakes is re-suspension of sediment particles (Aalderink et al. 1985). This introduces sediment bacteria together with other microorganisms attached to re-suspended particles into the water column (Garstecki et al. 2000) and can be an important mechanism for the exchange of

organic matter (including nutrients such as phosphorus). The effect of the latter on plankton communities has not been exhaustively studied (Hamilton & Mitchell 1997). In most cases, mircoorganisms are transferred from anoxic conditions in the sediment (oxygen was measured within a few millimetres in lake sediment; Tšertova et al. 2010) to lake water that was mostly oxic. It is not clear whether such a transfer can be considered as a negative disturbance event to the bacterioplankton. To assess this, the enhancement of growth must be observed in specific cases (Garstecki & Wickham 2001). An earlier study in Lake Võrtsjärv showed that higher WL favoured the production of more biomass and growth of bacterioplankton (Kisand & Nõges 2004), although the underlying causal mechanisms remain unknown.

Direct physical forcing of bacteria by waves can probably be neglected. This disturbance has been demonstrated to be important to various planktonic organisms such as phytoplankton (Padisák et al. 1988, Hamilton & Mitchell 1997), but there is no evidence that aquatic bacteria are disturbed by direct physical shear stress. Shearing of particles colonised by bacteria (Liu et al. 2012) and physically removing bacteria from particulates (Phillips et al. 2014) may play a role.

All of the mechanisms mentioned above can be included into 1 proxy variable, viz. WL, which was found to have a strong and significant relationship with BCC over the study period but not over a seasonal time scale (Table 1). Variables that indirectly characterize the composition of dissolved organic matter such as CDOM, COD_{Mn} and water colour were among the parameters that most significantly related to interannual changes in BCC. CDOM has been shown to be strongly related to allochthonous humic material in this lake (Toming et al. 2013). As a result, interannual changes in catchment load that arise due to variation in precipitation seem to be an important driver of changes in BCC. Interestingly, the total phosphorus concentration (P_{TOT}) is the only variable significantly (p = 0.036) related to BCC on both the seasonal and year-to-year time scales. This suggests that phosphorus could affect species composition via resource partitioning in different niches, which corroborates earlier observations on bacterioplankton abundance and biomass formation (Kisand et al. 2001). In addition, various food web interactions such as predation (estimated via the proxy variables biomass and abundance of protozooplankton species) and interactions with phytoplankton species were not found to be significant using the analyses we performed.

Persistent taxa of bacterioplankton and their potential ecological role

Frequently appearing bacterial taxa, i.e. persistent OTUs, were identified by collecting the material of common DGGE bands and by subsequently sequencing 16S rDNA fragments. These identified OTUs belonged to 3 major groups of bacteria, viz. *Proteobacteria*, *Actinobacteria* and *Bacteriodetes* (Table S2 in the Supplement), which are typically highly abundant in freshwater lakes (e.g. Glöckner et al. 2000, Newton et al. 2011). The most persistent taxon detected in 58% of all samples was an alpha-proteobacterium of the genus *Brevundimonas* (KC815500), which might be associated with *Cyanobacteria* (Berg et al. 2009, Heinrich et al. 2013). Three representatives of *Alpha*- and *Betaproteobacteria* could be methanotrophic: uncultured *Methylocystaceae* (KC815503 and KC815504) and *Methylophilus* sp. (KC815493). KC815504 was detected in 56% of the samples analysed, which suggests that methane might be an additional carbon source for bacterioplankton. Methane-derived carbon contributes to the food web of this lake (Agasild et al. 2014).

Succession of bacterial communities over annual cycles has been reported several times while examining the temporal dynamics of bacterioplankton communities in lakes using fingerprinting techniques, such as terminal restriction fragment length polymorphism, amplified ribosomal intergenic spacer analysis and DGGE separation of 16S rRNA genotypes (for examples, see Yannarell et al. 2003, Kent et al. 2004). More recent reports based on high-frequency, multi-year data sets have shown that seasonal patterns in bacterial community structure recur in freshwater ecosystems (Crump & Hobbie 2005, Kent et al. 2007, Shade et al. 2007, Crump et al. 2009, Kara et al. 2013). Although the interannual variability of BCC was strong, and only 2 seasons had statistically significant differences in clustering (ice-covered and ice-free period), more fine-grained seasonal changes do cluster into visibly distinct subsets (Fig. 1B, BGA p = 0.063). Fingerprinting methods, such as DGGE, are sub-optimal for the analysis of finer-grained variability of BCC and richness/diversity, and therefore presence/absence data of the most abundant OTUs might not be enough to fully characterize seasonal variability. On the other hand, as suggested by comparing next-generation sequencing-based 16S rDNA fragment deep sequencing and DGGE (Hanning & Ricke 2011), the latter can be considered a robust and adequate method to analyse interannual variability of the most prominent changes in BCC in a shallow lake with natural WL fluctuations.

Conclusions

Our study showed that BCC was highly dynamic over several years. The variability between years was higher compared to within-year variability, and our data analysis strongly suggests that changes in bacterioplankton community were mostly related and therefore probably driven by various abiotic factors. Different abiotic factors were related to these changes in different time scales. Seasonal dynamics were mostly related to 'seasons' of ice cover and ice absence, and the under-ice community could be discriminated from ice-free bacterioplankton. Differences in BCC between years were found to be related to WL fluctuation, which is believed to be a proxy variable for the intensive mixing of the water column and sediment resuspension.

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