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RESEARCH ARTICLE

Covariation patterns of phytoplankton and bacterioplankton in hypertrophic shallow lakes

M.R. Schiaffino[1,2,*], P. Huber[3], M. Sagua[1,2], C.A. Sabio y García[4,5] and M. Reissig[6]

[1]Departamento de Ciencias Básicas y Experimentales, Universidad Nacional del Noroeste de la Provincia de Buenos Aires, Roque Sáenz Peña 456, 6000, Junín, Argentina, [2]Centro de Investigaciones y Transferencia del Noroeste de la Provincia de Buenos Aires (CITNOBA) – UNNOBA-UNSAdA-CONICET, Jorge Newbery 355, 6000, Junín, Argentina, [3]Instituto Nacional de Limnología (INALI, CONICET-UNL), Colectora Ruta Nac. 168, Paraje El Pozo, 3000, Santa Fe, Argentina, [4]CONICET – Universidad de Buenos Aires, Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA), Intendente Güiraldes 2160, Ciudad Universitaria - C1428EGA, Buenos Aires, Argentina, [5]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Depto. Ecología, Genética y Evolución, Intendente Güiraldes 2160, Ciudad Universitaria - C1428EGA, Buenos Aires, Argentina and [6]Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), Universidad Nacional del Comahue - CONICET , Quintral 1250, 8400, San Carlos de Bariloche, Argentina

*Corresponding author: Centro de Investigaciones y Transferencia del Noroeste de la Provincia de Buenos Aires (CITNOBA) – UNNOBA-UNSAdA-CONICET, Jorge Newbery 355, 6000, Junín, Argentina. Tel: +54 236 440 7750 extension 12405; E-mail: rschiaffino@conicet.gov.ar

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ABSTRACT

The aim of this work was to assess the temporal patterns in the community composition of phytoplankton (PCC) and bacterioplankton (BCC) in two interconnected and hypertrophic Pampean shallow lakes in Argentina. Factors shaping their community dynamics and community temporal covariations were also analysed. We performed 4 years of seasonal samplings (2012–2016) and communities were studied by the Utermöhl approach (PCC) and Illumina MiSeq sequencing (BCC). We found marked seasonal variations in both communities and inter-annual variations with decreasing microbial community similarities during the study. We also observed covariation in community-level dynamics among PCC and BCC within and between shallow lakes. The within-lake covariations remained positive and significant, while controlling for the effects of intrinsic (environmental) and extrinsic (temporal and meteorological) factors, suggesting a community coupling mediated by intrinsic biotic interactions. Algal-bacterial associations between different taxa of phytoplankton and bacterioplankton within each lake were also found. PCC was mainly explained by pure regional extrinsic (17–21%) and intrinsic environmental (8–9%) factors, while BCC was explained by environmental (8–10%) and biotic interactions with phytoplankton (7–8%). Our results reveal that the influence of extrinsic regional factors can be channeled to bacterioplankton through both environmental (i.e. water temperature) and phytoplankton effects.

Keywords: microbial communities; community-level covariations; algal-bacteria associations; temporal patterns; shallow lakes; Pampa Plain

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INTRODUCTION

Microorganisms support the existence of all higher trophic life forms (Cavicchioli *et al.* 2019). The ecological and biotechnological services that they provide to the planet and humans highlight the need to preserve their diversity (Vitorino and Bessa 2018) and comprehend their ecological patterns, processes and mechanisms (Marco 2019). In particular, phytoplankton and bacterioplankton are major components of the aquatic microbial food web, which interact continuously and play essential roles in aquatic ecosystem functioning (Jones and Reynolds 1984; Fuhrman 2009; Sarmento and Gasol 2012). To date, comprehensive studies simultaneously evaluating the temporal patterns of phytoplankton and bacterioplankton have been performed mainly in oligo- and mesotrophic lakes, but are less frequent in eutrophic and hypertrophic systems (Su *et al.* 2017a and citations therein).

Temporal patterns and community dynamics are influenced by drivers acting from within and from outside an ecosystem. These forces can be separated into intrinsic or system-specific drivers (such as biotic interactions, nutrient availability, physical and chemical variables) and extrinsic (such as climate, temperature, solar irradiation and dry-wet periods) factors (Liebhold, Koenig and Bjørnstad 2004). Extrinsic drivers operating at a regional scale can impose synchrony and covariations on the dynamics of different ecosystem parameters and biological communities, while intrinsic drivers often lead to distinctive behaviour (e.g. Liebhold, Koenig and Bjørnstad 2004; Kent *et al.* 2007). However, how phytoplankton and bacterioplankton communities respond synchronously to changing intrinsic and extrinsic conditions remains poorly explored (Kent *et al.* 2007; Su *et al.* 2017a).

Temporal patterns of microbial communities show seasonal trends and synchrony between communities in different aquatic ecosystems, such as rivers, reservoirs and lakes (e.g. Crump and Hobbie 2005; Liu *et al.* 2015; Bock *et al.* 2018). Reports based on high frequency multiyear datasets of site-specific studies have shown that seasonal patterns in bacterioplankton community composition (BCC) recur in freshwater ecosystems, indicating that some microbial communities change directionally according to intrinsic environmental conditions (e.g. Rösel, Allgaier and Grossart 2012; Kara *et al.* 2013; Tammert *et al.* 2015). However, given bacterial inherent diversity, rapid generation times and the wide array of factors that can affect their abundance and activity, predicting population dynamics can be challenging for aquatic bacterial populations (Yannarell *et al.* 2003; Kent *et al.* 2004). Seasonal succession patterns have also been described for phytoplankton community composition (PCC) (e.g. Anneville *et al.* 2002; Rasconi, Winter and Kainz 2017) and its predictability varies according to intrinsic environmental (Baines *et al.* 2000; Bronmark and Hansson 2005) and extrinsic regional meteorological factors (e.g. Kent *et al.* 2007).

In recent years, a growing number of published works have investigated how BCC associates with PCC (Zhu *et al.* 2016; Mikhailov *et al.* 2019a). Many of these studies have found a pronounced metabolic covariation or couplings between bacteria and phytoplankton (e.g. Bouvy *et al.* 1998; Morán, Ducklow and Erickson 2013; Milici *et al.* 2016; Mikhailov *et al.* 2019a), as well as among different community structures (Bock *et al.* 2018; Mikhailov *et al.* 2019b; Jeong, Choi and Kim 2020). BCC is influenced by different kinds of biota, including the PCC, which were found to play key roles in shaping their composition (Niu *et al.* 2011; Liu *et al.* 2014; Su *et al.* 2017b). Succession in the PCC likely affects the concentration and biochemical composition of autochthonous organic matter available to bacteria (van Hannen *et al.* 1999; Arrieta and Herndl 2002; Pinhassi *et al.* 2004). However, the relationship between phytoplankton and bacterioplankton strongly depends on the specific characteristics of each aquatic system and therefore it is difficult to predict (Pérez *et al.* 2014).

An increasing number of studies have found a direct or indirect interaction between Cyanobacteria and heterotrophic bacteria. The structural diversity of BCC has been associated with bloom-forming freshwater Cyanobacteria genus, their diversity apparently being driven by the metabolic capacity to degrade cyanobacterial exudates and detrital materials (e.g. Zhu *et al.* 2014; Louati *et al.* 2015; Woodhouse *et al.* 2016). In this sense, significant changes in BCC were observed during the outbreak and decline of cyanobacterial blooms (Zhang *et al.* 2018, Wang, Razzano and Mou 2020).

Understanding how microbial communities respond to forces that shape their community structure and dynamics provides valuable information to help infer the underlying mechanisms that regulate microbial diversity and community assembly (e.g. Green, Bohannan and Whitaker 2008). The aims of this study were (i) to evaluate the temporal patterns of phytoplankton and bacterioplankton assemblages in two near (∼14 km) interconnected and highly hypertrophic Pampean shallow lakes, (ii) to study the temporal covariation between PCC and BCC, as well as the correlation network patterns between different taxa of phytoplankton and bacterioplankton, and (iii) to assess the effect of intrinsic (environmental and biotic) and extrinsic (temporal and meteorological) factors on PCC and BCC structures and dynamics. We performed 16S rRNA gene high-throughput sequencing (Illumina MiSeq) to study BCC and inverted microscopy counts (Utermöhl) to study PCC seasonally during 4 consecutive years (from 2012 to 2016). To the best of our knowledge, this work is the first study that analyses the temporal dynamic of BCC using molecular methods, together with their association with PCC in temperate hypertrophic shallow lakes.

MATERIALS AND METHODS

Study area

The two studied shallow lakes are located at Pampa Plain, Argentina. The climate in this region is warm temperate, with mean air temperature ranges of 10°C to 22°C in winter and summer, respectively. Annual precipitation averages 935 mm, most of which occurs during the spring-summer months (Sierra, Hurtado and Spescha 1994). In addition, there is a regional climatic phenomenon of inter-annual hydrologic cycles consisting of several consecutive wet years followed by dry years in an irregular pattern (Iriondo and Kröhling 2007). The Pampean lakes are polymictic due to their shallowness and the persistence of winds. Vertical and horizontal homogeneity of the water column is commonly observed for most limnological parameters (e.g. Rennella and Quirós 2006; Torremorell *et al.* 2007). These lakes contain very high nutrient concentrations and phytoplankton abundances (Quirós and Drago 1999; Quirós 1988). In addition, the presence of four distinct seasons in this mid-latitude temperate climatic zone can lead to a dynamic change of aquatic biological communities.

The studied shallow lakes, Gómez (34.67°S; 61.04°W) and Carpincho (34.57°S; 60.89°W), are located at the headwaters of the Salado River. They are both connected by the aforementioned river, Gómez being located upstream and Carpincho downstream. Both lakes have floodgates to regulate the water level (Rennella 2007) and are used for recreational purposes. Carpincho is near Junín city (34.55°S; 60.93°W), Buenos Aires Province, Argentina) and receives waste discharges from it (e.g. Rennella and Quirós 2006). A complete description of the study area and main characteristics of the studied shallow lakes are given in Rennella and Quirós (2006), Rennella *et al.* (2019) and Schiaffino *et al.* (2019).

Samplings, physical and chemical variables

Integrated water samples were collected seasonally (January, April, July and October) from October 2012 to October 2016 (n = 17 for each shallow lake) in acid-washed polycarbonate bottles from the pelagic zone at 30–40 cm below the surface. Samples were transported in the dark and at 4°C immediately to the laboratory. Determinations of nutrients [ammonium ($N\text{-}NH_3$), soluble reactive phosphorus (SRP), total phosphorus (TP) and organic nitrogen (N org.)], chlorophyll a (Chl-a), dissolved organic carbon (DOC), total suspended solids (TSS), ash-free dry weight (AFDW) and inorganic weight were performed following the methods described in Schiaffino *et al.* (2019). Water samples for phytoplankton analysis were preserved with 1% acidified Lugol´s solution until further analysis. Around 200 ml of water samples were prefiltered *in situ* through a 55 μm net to remove zooplankton and then filtered through 0.22 μm pore-size polycarbonate filters (47 mm, Millipore) for bacterioplankton analysis. These filters were frozen (-80°C) until DNA extraction.

Temperature, pH, conductivity, dissolved oxygen (DO) and nephelometric turbidity were measured *in situ* with portable meters (Hanna HI991301; Hanna HI9146; Lutron TU-2016). Secchi depths (SD) measures were also performed *in situ*.

Monthly mean air temperature, daily rainfall and storm day data were provided by the National Meteorological Service (Argentina). The Oceanic Niño Index (ONI) for each sampling month was obtained from https://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php

PCC

Prefixed water samples were decanted in 2- and 5-ml chambers for at least 24 h before counting. Phytoplankton quantifications were performed with an inverted microscope (Olympus CKX41) at 400x magnification, following the Utermöhl (1958) approach. Counting errors were estimated according to Venrick (1978). These organisms were identified to the lowest possible taxonomic level (genus and, when possible, species level).

BCC

Genomic DNA from stored filters was extracted using a CTAB protocol (Fernandez Zenoff, Siñeriz and Farias 2006). The partial 16S rRNA gene was sequenced by Illumina MiSeq 2 × 300 paired-end sequencing (Macrogen, Corea), using the primers 341F/805R (Herlemann *et al.* 2011), which cover the hypervariable regions V3–V4 of 16S rRNA gene.

MiSeq data were analysed using a modified version of the pipeline proposed by Logares (2017) (https://github.com/ramalok). The reads were first analysed for error correction using the algorithms based on Hamming graphs and Bayesian subclustering (BAYES HAMMER tool) (Nikolenko, Korobeynikov and Alekseyev 2013) implemented in SPAdes v. 3.5.0 (Nurk *et al.* 2013). The forward and reverse reads of each sequence were paired using the function *fastq_mergepairs* from USEARCH-v. 10 (Edgar and Flyvbjerg 2015). The minimum overlap length was set to 20 bp and those assemblage sequences with less than 100 nucleotides were discarded and the rest of the parameters were used as the default. Quality control of the sequences took place by *fastq_filter* in USEARCH-v. 10 (Edgar and Flyvbjerg 2015). Low-quality reads were removed using the filter values fastq_fastq_minlen = 100 and fastq_maxee = 0.5. Reads that passed the quality control were then analysed using UNOISE2 (Edgar 2016) to define operational taxonomic units (OTUs) with no clustering (zero-radius OTUs [zOTUs]). zOTUs provide a higher accuracy than OTUs by achieving single-nucleotide resolution after correcting for Illumina sequencing errors and chimeras (Callahan *et al.* 2016; Edgar 2018). Finally, taxonomy assignment of zOTUs was done by BLAST (Altschul *et al.* 1990), using the SILVA database (SSURef 132 Nr99) as a reference, and the zOTU table was created with the function *otutab* in USEARCH-v10 (Edgar and Flyvbjerg 2015).

To reduce noise and thus false-positive predictions, we restricted our analysis to taxa with an abundance higher than 10 reads. zOTUs assigned to Archaea, chloroplasts and Cyanobacteria were omitted from the analysis unless specified otherwise. After this filtering, the number of total reads varied from 42 476 (spring 2012, Carpincho) to 18 108 (summer 2016, Gómez). With the aim of comparing the different samples, we normalised the BCC matrix. For this purpose, we randomly selected the same number of Illumina reads from each lake and sampling date based on the smallest sample size (matching the sample with the lowest number of reads, i.e. 18 108 sequences).

Sequences were deposited at the European Nucleotide Archive public database under the accession numbers ERR4439170-ERR4439203 (Project accession number PRJEB37379).

Data handling and statistical analyses

The integrated trophic state index (TSI) was calculated according to Adamovich *et al.* (2016), considering SD, Chl-a and TP (Carlson 1977). Index ranges between 40–50 indicate mesotrophic conditions, between 50–60 eutrophic and >70 hypertrophic systems (Adamovich *et al.* 2016).

All statistical analyses were performed in the R environment (R version 3.4.4; R Core Team 2018).

To study the association between environmental variables we performed pairwise correlations using Spearman´s rho tests. Environmental variables were first standardised (mean subtracted and divided by the standard deviation) using the vegan package (Oksanen *et al.* 2017). The relevance of the environmental variables and their overall trends was explored using principal components analysis (PCA) (ter Braak and Smilauer 2002) on the standardised environmental matrix using the Ade4 package (Dray and Dufour 2007).

To analyse if samplings were deep enough to get a reasonable estimation of OTU richness, rarefaction curves were performed on the zOTU matrix without normalising and using the vegan package. PCC and normalised BCC matrices, constructed with the highest taxonomic resolution obtained, were both Hellinger-transformed prior to the statistical analyses (Legendre and Gallagher 2001; Ramette 2007). PCC and BCC matrices were explored for each lake using non-metric multidimensional scaling (NMDS) and Bray-Curtis index to examine how the

microbial communities varied on a temporal scale in each shallow lake. To further study the influence of individual environmental variables on PCC and BCC, we used the function *envfit* (vegan package) to check for the correlations between the main ordination axes and the environmental variables.

In order to statistically assess the degree of resemblance of these microbial communities between seasons, analyses of similarity (ANOSIM; Clarke 1993) using the Bray-Curtis distance matrices were performed in each lake using the vegan package.

To study the phytoplankton and bacterioplankton alpha diversity patterns, we calculated the Shannon-Weaver index and the total richness for each sample date and shallow lake using the vegan package. Significant differences ($P < 0.05$) among seasons were evaluated with Kruskal-Wallis analyses and Mann-Whitney U post-hoc test.

To describe the temporal structure of microbial communities and to explore the relationship between dissimilarity data and temporal distances we performed Mantel correlograms (Borcard and Legendre 2012). To explore temporal patterns and to test for linear temporal trends in community dynamics (PCC and BCC dissimilarities) and the environmental variables (Euclidean dissimilarity matrices and single variables), we regressed the data (after meeting the assumptions) against the square root of the time-lags (Collins, Micheli and Hartt 2000; Liu *et al.* 2015) using the lm function in R. This approach can produce three theoretical patterns: a significant linear regression with positive slopes (implying a directional change); a regression that is not significant or the slope is not significantly different from zero (implying fluctuation or stochastic variation over time); and a negative significant and linear slope (implying convergence to an earlier sample period). The slope of the regression line indicates the rate and direction of change, while the regression coefficient (R^2) serves as a measure of signal versus noise (low values indicate high stochastic variation between sampling intervals, while high values indicate a stronger signal of change) (Collins, Micheli and Hartt 2000).

Variation partitioning using partial redundancy analyses (pRDA) was performed to estimate the unique and combined contributions of four factors or explanatory variables (extrinsic: temporal and meteorological; intrinsic: environmental and biotic) in shaping microbial communities (PCC and BCC) using the vegan package. The temporal explanatory matrix was constructed with 16 asymmetric eigenvector maps (AEM) following Legendre and Gauthier (2014). This AEM matrix was performed for a greater resolution of temporal trends using the 17 sampling dates and the functions *aem.weight.time* from the AEM (Blanchet, Legendre and Gauthier 2007) and adespatial (Dray *et al.* 2020) packages. The meteorological matrix was comprised of monthly air temperature, monthly rainfall, monthly storm days and ONI. The environmental explanatory matrix was composed of water level, SD, temperature, pH, conductivity, turbidity, TP, N org., N-NH_3 and DOC. The biotic explanatory matrix was constructed with the abundances of Chlorophyceae, Cyanobacteria, Bacillariophyceae, Zygnematophyceae, Cryptophyceae, Euglenophyceae, Chrysophyceae, Eustigmatophyceae and Dinophyceae (as explanatory variables of BCC) and with the sequencing reads of Actinobacteria, Bacteroidetes, Verrucomicrobia, Planctomycetes, Alphaproteobacteria, Gammaproteobacteria and other Proteobacteria (as explanatory variables of PCC). In each pRDA the forward selection was used for adding explanatory factors ($P < 0.05$) to the model. The explanatory power of the model was analysed using adjusted R^2 as coefficient of determination. The function *forward.sel* of the packfor package (Dray, Legendre and Blanchet 2007) was used for stepwise model-building for each level of explanatory variables.

To study the degree of covariation between the PCC and BCC within each shallow lake, as well as between shallow lakes, we performed a simple Mantel test using Spearman correlations (Mantel and Valand 1970) and a permutation test based on Procrustes analyses (Peres-Neto and Jackson 2001; Kent *et al.* 2007). Procrustean matrix superimposition was achieved using the axes from ordination methods (Legendre and Legendre 1998; Peres-Neto and Jackson 2001) and the vegan package. The sum of squared residuals is used as a metric of association (m^2) and varies between 0 and 1. Smaller values of m^2 indicate stronger covariation between data sets (Peres-Neto and Jackson 2001). Besides, in order to disentangle the effect of abiotic variables (environmental, temporal and meteorological) on the PCC and BCC associations, we performed partial Mantel tests between PCC and BCC similarity matrices (Bray-Curtis index) while controlling for the effect of each abiotic similarity matrix (1–Euclidean distance). Simple and partial Mantel tests were performed using ecodist package (Goslee and Urban 2007).

To infer inter-taxa associations, we performed a correlation network analysis using the CoNet software v. 1.1.1.beta (Faust and Raes 2016) implemented in Cytoscape v. 3.7.1 (Shannon *et al.* 2003). Four measures were calculated: Bray–Curtis and Kullback–Leibler nonparametric dissimilarity indices, and Pearson and Spearman rank correlations. The combination of their results allows the appropriateness of scoring measures to determine the statistical significance of correlations (Faust and Raes 2016). The initial edge selection was set to include the 2000 positive and 2000 negative edges consistent across all four correlation measures. The significance of the edges was calculated using the ReBoot method (Faust *et al.* 2012) based on 1000 permutations with renormalisation and 1000 bootstrap iterations. Only edges supported by at least two methods were considered. Then edge-specific *P*-values were merged using Brown's method (Volterra 1926), followed by Benjamini–Hochberg for false discovery rate correction; edges with merged *P*-values below 0.05 were kept (Faust and Raes 2016). We constructed one network for each lake considering both BCC and PCC communities. For BCC we selected zOTU with abundance >50 reads and present in at least three samples; regarding PCC, only taxa present in at least three samples were selected. To explore indirect associations driven by environmental factors (Faust and Raes 2016), we included environmental information in an additional matrix containing the following variables: water temperature, DO, pH, turbidity, conductivity, TSS, AFWD, SRP, TP, N org., N-NH_3, DOC, inorganic weight. No indirect edges between environmental and taxa nodes were detected. The networks were visualised in Cytoscape v. 3.7.1 (Shannon *et al.* 2003) and the Network Analyser tool (Assenov *et al.* 2008) was used to calculate four network topology properties: number of nodes (number of BCC and PCC taxa), number of edges (number of associations), average number of neighbours and clustering coefficient. The average number of neighbours, as well as the clustering coefficients, were used to evaluate the connectedness of the correlation networks. The average number of neighbour measures, the average connectivity of a node in the network and the clustering coefficients indicate how nodes are embedded in their neighbourhood. Thus, higher values of both metrics are expected with an increase in the network connectivity (Newman 2003; Barabási and Oltvai 2004; Faust *et al.* 2015). Additionally, for each network we identified highly connected taxa (commonly referred as hub taxa) as those nodes with a high degree (>10), closeness cen-

trality (>0.26) and low betweenness centrality (<0.02) (Berry and Widder 2014; Agler et al. 2016; Layeghifard, Hwang and Guttman 2017; Banerjee, Schlaeppi and van der Heijden 2018) using the Network Analyser tool in Cytoscape. Closeness centrality measures the length of the shortest path between two nodes and thus reflects the importance of a node in disseminating information. Betweenness centrality quantifies how many steps away a particular node is from all the others in the web, denoting the amount of influence a node has over the flow of information in the network (Banerjee, Schlaeppi and van der Heijden 2018).

RESULTS

Environmental variables

In general, both shallow lakes presented similar ranges and dynamics of all measured environmental variables (Table 1, Supplementary Fig. S1). Nutrients and Chl-a concentrations were within the hypertrophic range (Table 1), with significant increasing TSI values throughout the 4-year study (Supplementary Fig. S1a; Gómez $R^2 = 0.25$, slope = 0.15 and Carpincho $R^2 = 0.23$, slope = 0.15; both $P < 0.05$). Contrarily, SD showed a significant decreasing temporal trend (Supplementary Fig. S1e; Gómez $R^2 = 0.37$, slope = -0.29 and Carpincho $R^2 = 0.50$, slope = -0.25; both $P < 0.01$). The lowest water levels were observed from July 2013 (winter) to January 2014 (summer) (1.0 ± 0.1 m) constituting a dry period (Supplementary Fig. S1b, grey area), in concordance with lower mean monthly precipitations (88 ± 77 mm). Conductivity presented an opposite pattern to and TP the same pattern as water level (Supplementary Fig. S1d and h, respectively). DOC concentrations were relatively constant throughout the study, except for a peak in summer 2014 in both lakes (Supplementary Fig. S1g). Slight significant linear temporal trends (linear regressions) were observed in the dissimilarity of environmental variables over time (Gómez $R^2 = 0.03$, slope = 0.020 and Carpincho $R^2 = 0.04$ slope = 0.027; both $P < 0.05$), indicating a smooth directional change.

The first two axes of the PCA explained around 69% of the variability in both lakes (Supplementary Fig. S2a and b). The first axis of each biplot was mainly defined by hydrological variables (such as conductivity and water level) and trophic state variables (such as TP, Chl-a), whereas the second axis was mainly correlated with seasonal variables (such as DO, water temperature). Thus, PCA showed that the environmental variables both presented a seasonal pattern (intra-annual), with differences mainly between summer and winter, and an inter-annual pattern shaped by hydrological (dry-wet periods) and trophic variables.

Phytoplankton richness, diversity and composition

Phytoplankton richness did not differ between seasons (Supplementary Fig. S3a and b). However, its richness positively correlated with TSI in both lakes, while its diversity did not show any significant correlation (Table 2).

On average, phytoplankton were dominated by Chlorophyceae (60%), followed by Cyanobacteria (23%) and Bacillariophyceae (13%), except during the conspicuous cyanobacterial bloom observed during summer 2014 (Fig. 1A and B), when the relative abundance of Cyanobacteria (*Coelosphaerium* sp., *Aphanothece* sp./*Aphanocapsa* sp., cf. *Sphaerospermopsis aphanizomenoides* and *Raphidiopsis mediterranea*) dominated in both lakes. In particular, the absolute abundance of Cyanobacteria during summer 2014 reached abundances 4-fold higher (1.1×10^5 ind. mL^{-1}) than the abundance registered in other months (mean value 2.7×10^4 ind. mL^{-1}) in both shallow lakes (Fig. 1C and D).

The NMDS analysis performed with the PCC of both shallow lakes also showed a seasonal ordination of the samples (Fig. 2A and B). The similarity test showed differences between seasons (ANOSIM Gómez R = 0.20, $P = 0.018$ and Carpincho R = 0.17, $P = 0.0373$), the composition being significantly different between summer and spring (*P*-value, sequential Bonferroni significance <0.0155) and summer and winter (*P*-value, sequential Bonferroni significance <0.0312) in both lakes. We further investigated the influence of individual environmental variables on PCC by correlating the scores derived from the ordination of sites (NMDS 1 and 2) shown in Fig. 2 with each environmental variable. Patterns in PCC were related to water temperature (parameter related to seasonality), as well as to TSI and SD (variables related to trophic state).

Heterotrophic bacteria richness, diversity and composition

Rarefaction curves performed for every sample showed that zOTU richness tended to reach a plateau, suggesting that sampling depth and sequencing coverage were satisfactory (Supplementary Fig. S4). The average zOTU richness per sampling date in Gómez was 2730 ± 400 (1687–3273) and in Carpincho it was 2806 ± 335 (1996–3306). Both connected lakes showed a high percentage of shared OTUs (94.1%) and low percentages of exclusive zOTUs (Gomez 2.5% and Carpincho 3.4%). Bacterial richness was significantly lower during summers compared with winters in both lakes (Supplementary Fig. S3c and d). The lowest bacterial richness (Supplementary Fig. S5a) and diversity (Supplementary Fig. S5b) were observed in summer 2014, coinciding with the beginning of an important cyanobacterial bloom and a lower water level being registered (Supplementary Fig. S1b). In addition, both bacterial richness and diversity were negatively correlated with water temperature, conductivity and DOC, while bacterial richness was positively correlated with water level and TSI in both shallow lakes (Table 2).

On average, Actinobacteria accounted for around 24% of total bacterial reads, followed by Proteobacteria (~22%), Bacteroidetes (~18%), Verrucomicrobia (~12%) and Planctomycetes (~10%) in both shallow lakes (Fig. 3A and B). Among Proteobacteria, Betaproteobacteria was the best represented (~42%) class, while Alphaproteobacteria accounted for around 29%, Gammaproteobacteria for around 19% and other Proteobacteria for around 10% in both lakes. These major groups were well represented in all seasons and showed small changes during the study period (Fig. 3). The best represented zOTU was *Sporichthyaceae* hgcl clade (Actinobacteria) in both lakes, except in summer 2014 during the cyanobacterial bloom (Fig. 1). Accordingly, Illumina reads of Cyanobacteria dominated widely over the other groups during the bloom (Fig. 3C and D).

The NMDS analysis performed with BCC showed a seasonal ordination of the samples (Fig. 4A and B). The similarity test showed differences between seasons (ANOSIM Gómez R = 0.43 and Carpincho R = 0.49, both $P = 0.0001$), being the composition significantly different between summer and spring (*P*-value, sequential Bonferroni significance <0.0155), summer and winter ($P < 0.0312$), summer and autumn ($P < 0.0265$) and winter and autumn ($P < 0.0259$) in both lakes. We further studied the influence of individual environmental variables on BCC, correlating the scores derived from the ordination of sites (NMDS 1

Table 1. Average values (AVG), standard deviation (STDEV), maximum (MAX) and minimum (MIN) values of the main physical, chemical and biological parameters from the shallow lakes Gómez and Carpincho during the 4-year study (n = 17).

	Gómez				Carpincho			
	AVG	STDEV	MIN	MAX	AVG	STDEV	MIN	MAX
Water level (m)	1.36	0.19	0.95	1.60	1.31	0.15	1.00	1.52
SD (cm)	14.62	4.95	8.00	23.00	13.85	3.72	8.00	20.00
Water temperature (°C)	18.01	6.41	7.80	28.50	17.86	6.14	8.70	27.90
DO (mg L^{-1})	8.88	1.71	6.06	12.20	8.44	2.04	5.48	13.22
pH	8.87	0.24	8.33	9.27	8.88	0.20	8.54	9.18
Conductivity (μS cm^{-1})	4506	1407	2630	7960	4340	1133	2670	7210
Salinity (g L^{-1})	2.88	0.90	1.68	5.09	2.78	0.72	1.71	4.61
Turbidity (NTU)	141.82	67.96	53.00	310.00	139.76	57.95	60.00	244.00
TSS (mg L^{-1})	139.82	62.61	71.00	257.00	144.94	53.93	65.00	255.00
AFDW (mg L^{-1})	48.88	16.45	28.00	82.00	50.88	16.71	21.00	86.00
Inorganic weight (mg L^{-1})	90.94	47.95	38.00	182.00	94.06	39.52	44.00	169.00
TP (mg L^{-1})	0.86	0.21	0.56	1.17	0.89	0.24	0.43	1.30
SRP (mg L^{-1})	0.55	0.29	0.07	0.96	0.49	0.25	0.14	0.94
N org. (mg L^{-1})	4.03	0.55	3.14	5.20	3.98	0.92	2.59	6.68
Chl-a (μg L^{-1})	147	79	24	278	163	91	62	338
N-NH3 (mg L^{-1})	0.09	0.15	0.00	0.56	0.06	0.10	0.00	0.31
DOC (mg L^{-1})	29.02	5.58	21.40	43.23	26.40	7.43	11.48	49.47

SD, Secchi depth; DO, dissolved oxygen; TSS, total suspended solid; AFDW, ash-free dry weight; TP, total phosphorous; SRP, soluble reactive phosphorous; N org., Kjeldahl nitrogen; Chl-a, chlorophyll-a; DOC, dissolved organic carbon; NTU, nephelometric turbidity units.

and 2) shown in Fig. 4 with each environmental variable. Patterns in BCC were related to water temperature and DO (parameters related to seasonality) and water level, conductivity and TSI (variables related to hydrology and trophic state).

Heterotrophic bacteria and phytoplankton community patterns

We found inter-annual variations of microbial communities throughout the study. The dissimilarity of PCC and BCC increased with increasing temporal distance (Table 3). Furthermore, a significant linear temporal trend was present in PCC (Gómez: $R^2 = 0.15$, slope = 0.0043 and Carpincho: $R^2 = 0.23$, slope = 0.0053; both $P < 0.0001$) and BCC dissimilarities (Gómez: $R^2 = 0.029$, slope = 0.0015 and Carpincho: $R^2 = 0.030$, slope = 0.0014; both $P < 0.05$) over time, but BCC showed much lower rate of change (slopes) and regression coefficients (R^2).

The Mantel correlograms showed covariation patterns of microbial communities with similar temporal structure throughout the study in both lakes (Fig. 5). The correlograms for both communities and shallow lakes showed almost similar shapes and were globally significant at the Bonferroni-corrected α level (0.05/17 = 0.00294). Coincidently, PCC showed a more marked increasing dissimilarity with increasing time distance class (Fig. 5A and B) than BCC (Fig. 5C and D). There were significant positive temporal correlations at the beginning of the study, in the first and fourth temporal distance classes, suggesting that the community similarity among samples was higher (or community dissimilarity lower) than expected by chance. Besides, there was a significant negative autocorrelation between observations in the 11th temporal distance class, suggesting that dissimilarity was higher than expected by chance.

Microbial community covariations between shallow lakes were supported by positive correlations (Table 3). To further study these associations, we also performed a Procrustes analysis, showing significant temporal covariation between BCC (Procrustes sum of squares, $m^2 = 0.010$, $P < 0.001$) and between PCC (Procrustes sum of squares, $m^2 = 0.09$, $P < 0.001$) from both lakes.

Microbial community covariations within each shallow lake were also found and supported by positive correlations (Table 3). In coincidence, the Procrustes analysis also showed significant temporal associations between BCC and PCC in Gómez ($m^2 = 0.144$, $P = 0.001$) and Carpincho ($m^2 = 0.140$, $P = 0.001$). Besides, the covariation between BCC and PCC similarities within each shallow lake remained positive and significant, while controlling for the effects of environmental (intrinsic), temporal and meteorological (extrinsic) explanatory variables or all together (Table 3).

The variation partitioning analysis performed on PCC in Gómez (Fig. 6A) and Carpincho (Fig. 6B) showed that pure extrinsic temporal factors (forward selection: AEM1, AEM2, AEM8 modelling inter-annual variability and AEM9 modelling intra-annual variability) explained between 16–18% of the PCC variation and pure intrinsic environmental variables (forward selection: water temperature, SD, N-NH_3 and TP) explained between 8–9%. Meteorological factors (1–3%, forward selection: mean air temperature) and bacterioplankton classes (1–2%, forward selection: Alphaproteobacteria in Gómez, Planctomycetes in Carpincho) did not explain much of the PCC variation. Similarly, the combined contributions of factors explained low percentages (<6%) of PCC variation (Fig. 6A and B). The variation partitioning analysis performed on BCC in Gómez (Fig. 6C) and Carpincho (Fig. 6D) indicated that pure intrinsic environmental variables (forward selection: water temperature, SD, conductivity and pH) explained between 8–10% of the BCC variation and pure phytoplankton classes (forward selection: Chlorophyceae and Cyanobacteria) explained between 7–8%. Pure extrinsic meteorological factors (2–5%, forward selection: mean air temperature) and temporal factors (forward selection: AEM1, AEM7, AEM9) did not explain much of the BCC variation (0–1%). The combined contributions of factors explained between 1 and 8% of BCC variation in each shallow lake (Fig. 6C and D).

Overall, both correlation networks (Supplementary Fig. S6) showed similar topological features. The number of nodes was almost similar in both shallow lakes (573 in Gómez and 763 in Carpincho), whereas the number of connections (i.e. number

Table 2. Spearman´s rho correlation coefficients (below diagonal) and P-values (above diagonal) of bacterial and phytoplankton richness and Shannon-Weaver diversity versus environmental variables in Gómez and Carpincho.

	Bacterial richness	Bacterial diversity	Phytoplankton richness	Phytoplankton diversity	Water level (m)	Water temperature (°C)	Conductivity ($\mu S\ cm^{-1}$)	DOC ($mg\ L^{-1}$)	TSI
Gómez									
Bacterial richness		<0.0001	0.4281	0.1809	0.0106	0.0055	0.0004	0.0002	0.0483
Bacterial diversity	**0.87**		0.2747	0.0483	0.1114	0.0038	0.0093	0.0172	0.0161
Phytoplankton richness	0.21	0.28		0.0269	0.3802	0.1797	0.4866	0.5423	0.0483
Phytoplankton diversity	0.34	**0.49**	**0.53**		0.8580	0.2256	0.7012	0.4003	0.4003
Water level	**0.60**	0.40	−0.23	-0.05		0.3629	<0.0001	0.0269	0.5997
Water temperature	**−0.64**	**−0.66**	−0.34	-0.31	−0.24		0.0917	0.1218	0.1014
Conductivity	**−0.76**	**−0.61**	0.18	-0.10	**−0.88**	0.42		0.0010	0.3235
DOC	**−0.79**	**−0.57**	0.16	-0.22	**−0.53**	0.39	**0.72**		0.3737
TSI	**0.49**	**0.57**	**0.49**	0.22	0.14	−0.41	−0.25	−0.23	
Carpincho									
Bacterial richness		<0.0001	0.2467	0.3139	0.0157	0.0015	0.0009	0.0029	0.0279
Bacterial diversity	**0.91**		0.3551	0.3187	0.0658	0.0015	0.0172	0.0032	0.1037
Phytoplankton richness	0.30	0.24		0.0398	0.4618	0.3195	0.4713	0.9252	0.0225
Phytoplankton diversity	0.26	0.26	**0.50**		0.5820	0.7006	0.9777	0.5477	0.7468
Water level	**0.58**	0.46	−0.19	−0.14		0.0809	<0.0001	0.3350	0.8724
Water temperature	**−0.71**	**−0.71**	−0.26	−0.10	−0.44		0.0294	0.0999	0.2105
Conductivity	**−0.73**	**−0.57**	0.19	−0.01	**−0.85**	**0.53**		0.0398	0.6224
DOC	**−0.68**	**−0.67**	−0.02	−0.16	−0.25	0.41	**0.50**		0.5634
TSI	**0.53**	0.41	**0.55**	−0.08	0.04	−0.32	−0.13	−0.15	

DOC, dissolved organic carbon; TSI, trophic state index. N = 17. Bold values $P < 0.05$.

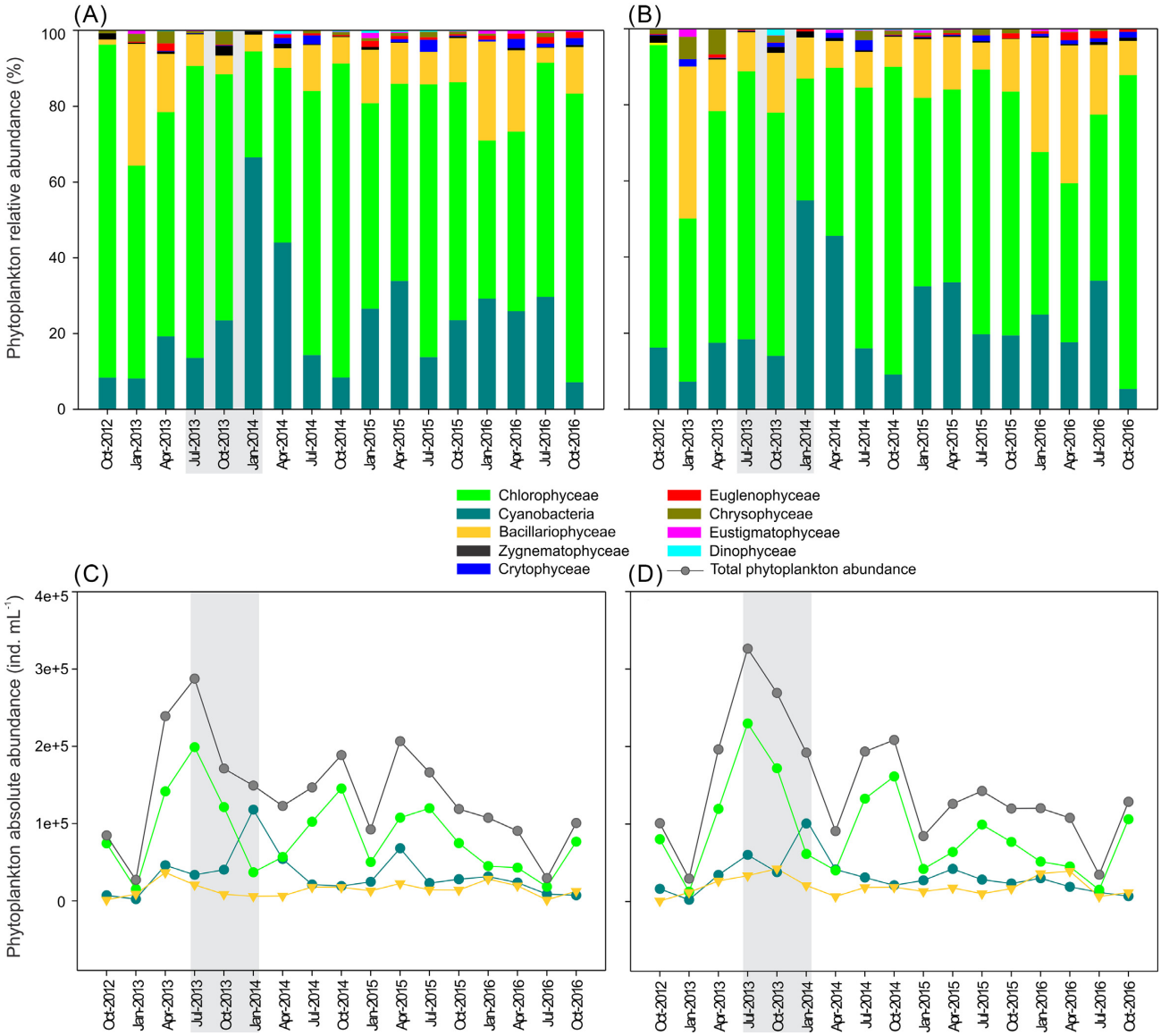

Figure 1. Relative abundance of the phytoplankton classes found during the 4-year study in **(A)** Gómez and **(B)** Carpincho shallow lakes. Absolute abundance dynamics of the dominant phytoplankton classes in **(C)** Gómez and **(D)** Carpincho. Grey areas denote the dry periods during the study.

of edges) was lower in Gómez than in Carpincho (812 and 1252, respectively). Additionally, the number of positive correlations was higher than the number of negative correlations in both lakes, being higher in Gómez (positive edges in Gómez 84.5% and in Carpincho 59.1%). The connectivity of the networks was also similar in both lakes as reflected by the average number of neighbouring metrics (Gómez = 2.8, Carpincho = 3.3) and the number of clustering coefficients (Gómez = 0.16, Carpincho = 0.14). Most of the BCC taxa were more related to each other than with PCC species, being Actinobacteria and Bacteroidetes the phyla with the major contribution to the total correlation numbers (Gómez = 37.9% and 31.7% and Carpincho = 37.9% and 31.6%, respectively). Regarding the PCC, Chlorophyta, Cyanobacteria, Bacillariophyta and Zygnematophyta showed significant interactions with heterotrophic bacteria in both Gómez and Carpincho shallow lakes (Supplementary Tables S1 and S2, respectively). Besides, Euglenophyta and Eustigmatophyta were also significantly correlated with heterotrophic bacteria in Carpincho (Supplementary Table S2). We detected 37 different highly connected taxa (11 taxa in Gómez and 26 taxa in Carpincho), all belonging to the BCC. The majority were exclusive of a single lake, being only three shared by both shallow lakes (Supplementary Table S3).

DISCUSSION

This work shows significant temporal patterns in PCC and BCC throughout the 4-year study, with both intra-annual (seasonality) and inter-annual variations. The marked seasonal patterns shown by both communities had generally higher differences between summer and winter (Figs 2 and 4, Supplementary Fig. S3). Seasonal changes have strong influences on microbial community structure, shaping its diversity and dynamics (e.g. Crump and Hobbie 2005; Bock *et al.* 2014). Similar seasonal patterns of BCC were reported in different aquatic environments, such as temperate eutrophic shallow lakes (De Figueiredo, Pereira and Correia 2010), temperate meso-eutrophic estuarine systems (Morán, Ducklow and Erickson 2013), temperate rivers

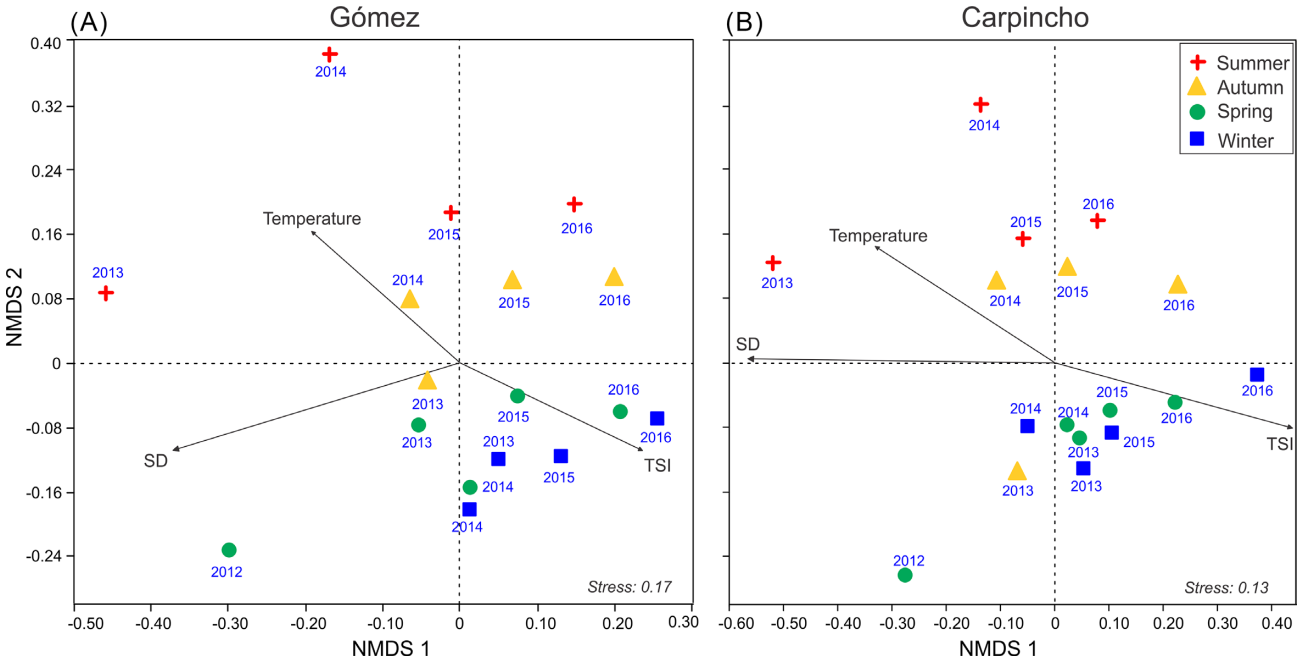

Figure 2. Biplots of the non-metric multidimensional scaling (NMDS) performed on Hellinger-transformed phytoplankton community composition using Bray-Curtis similarity index in **(A)** Gómez and **(B)** Carpincho. The arrows indicate the significant correlations ($P < 0.05$) between the environmental variables and the ordination axes and were calculated passively after NMDS by the *envfit* function. SD: Secchi depth. TSI: trophic state index.

Table 3. Results of the simple (between two matrices) and partial Mantel test (between two matrices while controlling for the effect of a third matrix) performed with bacterioplankton (BCC) and phytoplankton (PCC) community composition similarity matrices (Bray-Curtis). Partial Mantel test performed while controlling for the effect of environmental—env (Gómez: water temperature, SD, conductivity; Carpincho: water temperature, SD, conductivity, pH, TP, N-NH_3), temporal—temp (AEM1, AEM2, AEM7, AEM8, AEM9) and meteorological—met (mean air temperature) similarity matrices (1- Euclidean distance). PCC_d and BCC_d dissimilarities (1-Bray-Curtis similarity) against time-lag (pairwise Euclidean distances in sample times).

	Gómez				Carpincho				Between lakes	
	Simple Mantel		Partial Mantel		Simple Mantel		Partial Mantel		Simple Mantel	
Matrix type	r	p	r	p	r	p	r	p	r	p
PCC_d vs time-lag	**0.39**	<0.001			**0.46**	<0.001				
BCC_d vs time-lag	**0.21**	0.020			**0.20**	0.030				
PCC Gómez vs PCC Carpincho	-	-	-	-	-	-	-	-	**0.87**	<0.0001
BCC Gómez vs BCC Carpincho	-	-	-	-	-	-	-	-	**0.94**	<0.0001
BCC vs PCC	**0.67**	<0.0001	-	-	**0.63**	<0.0001	-	-		
BCC vs PCC, controlling env	-	-	**0.66**	<0.0001	-	-	**0.59**	<0.0001		
BCC vs PCC, controlling temp	-	-	**0.66**	<0.0001	-	-	**0.62**	<0.0001		
BCC vs PCC, controlling met	-	-	**0.67**	<0.0001	-	-	**0.63**	<0.0001		
BCC vs PCC, controlling all abiotic	-	-	**0.66**	<0.0001	-	-	**0.57**	<0.0001		

Bold values $P < 0.05$. Abiotic variables (intrinsic+extrinsic): environmental+temporal+meteorological.

(Crump and Hobbie 2005) and subtropical eutrophic shallow lakes (Su *et al.* 2017a). Long-term studies have also shown recurring seasonal patterns of BCC in freshwater systems, indicating that some microbial communities change directionally according to environmental conditions (e.g. Rösel, Allgaier and Grossart 2012; Kara *et al.* 2013), and that certain bacterial groups can be strongly repeatable and dependent on some environmental variables, such as temperature, nutrients, organic carbon and daylight hours (Gilbert *et al.* 2012). Seasonal succession patterns have also been described for PCC (e.g. Anneville *et al.* 2002; Rasconi, Winter and Kainz 2017), their community composition being strongly related to the annual temperature cycle in warm temperate reservoirs (Grover and Chrzanowski 2006). Accordingly, we found that water temperature was an important intrinsic variable shaping both PCC and BCC (Figs 2, 4 and 6), which is also modulated by regional extrinsic factors (i.e. regional climate). This intrinsic environmental variable has been described as a primary factor regulating the structure and temporal patterns of bacterioplankton and phytoplankton in many aquatic systems (e.g. Crump and Hobbie 2005; Scheibner *et al.* 2014; Su *et al.* 2017a; Mikhailov *et al.* 2019a).

Additionally, we found inter-annual variations with increasing PCC and BCC dissimilarities with temporal distance (Table 3). Similarly, the linear temporal trends (linear regressions) had significant and positive slopes, indicating that microbial communities were undergoing a directional change. Notably, the dissimilarity of PCC increased with higher slopes and R^2 values compared with BCC, indicating that phytoplankton had a faster

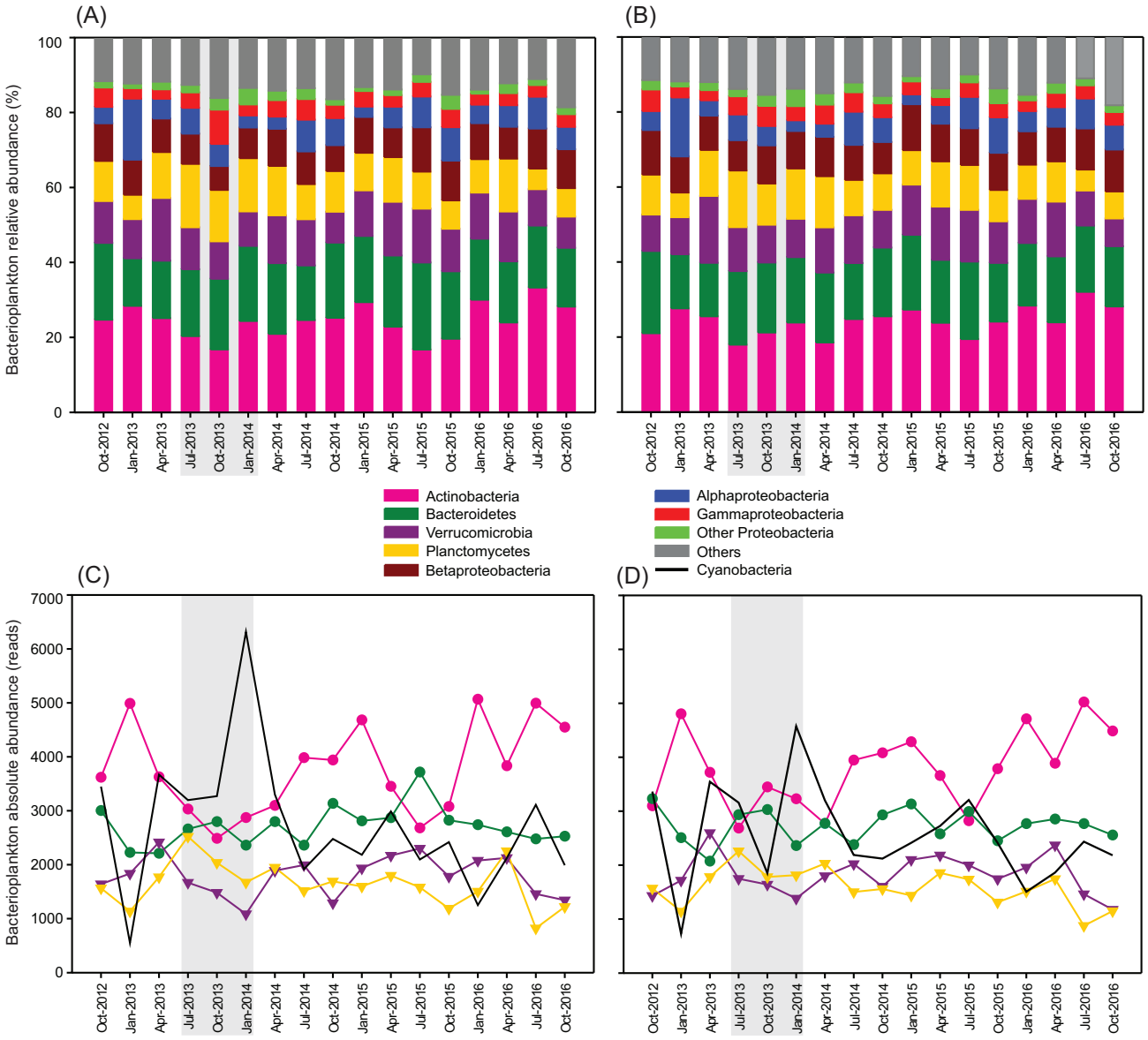

Figure 3. Relative number of reads of heterotrophic bacterial groups during the study period in **(A)** Gómez and **(B)** Carpincho shallow lakes. Dynamics of the absolute number of reads of major bacterial groups, including cyanobacterial reads (black line), in **(C)** Gómez and **(D)** Carpincho. Grey areas denote the dry periods during the study.

rate of change and less stochastic variation between sample intervals than bacterioplankton. Similar results were found by Liu *et al.* (2015) in subtropical reservoirs. On the other hand, Su *et al.* (2017a) reported that the dynamics of phyto- and bacterioplankton dissimilarities exhibited different temporal patterns in the eutrophic lake Taihu (China) depending on the taxonomic resolution. Bacterioplankton showed more stochastic variation than phytoplankton at the phylum level, although significant temporal directional change was found when examined at the genus level. Coincidently, in our study the correlograms for both communities and shallow lakes showed almost similar temporal structure and shapes throughout the study. However, PCC showed a more marked increasing dissimilarity with increasing time than BCC (Fig. 5). This could be associated to the bacterial traits, such as high abundance, high dispersion rates, rapid growth rates and small cell sizes, which may induce a different response of bacterioplankton to the environmental changes when compared with phytoplankton. In particular, the aforementioned bacterial traits could allow bacteria to quickly adapt to new environmental conditions, causing their lower rate and signal of change throughout the study period.

Not only the microbial communities, but also the environmental variables, showed both seasonal and inter-annual patterns (Supplementary Fig. S2). Similarly, the environmental variables also evidenced seasonality with marked differences between summer and winter. The inter-annual pattern, marked by dry-wet periods, showed increasing TSI and decreasing SD linear trends (Supplementary Fig. S1a and e) throughout the study. Accordingly, in a previous study performed in these two hypertrophic shallow lakes, it was found that both lakes were strongly influenced by temporal factors, modulated by intra- and inter-annual variations, affecting not only the abundance of microbial components (bacterioplankton and microplankton

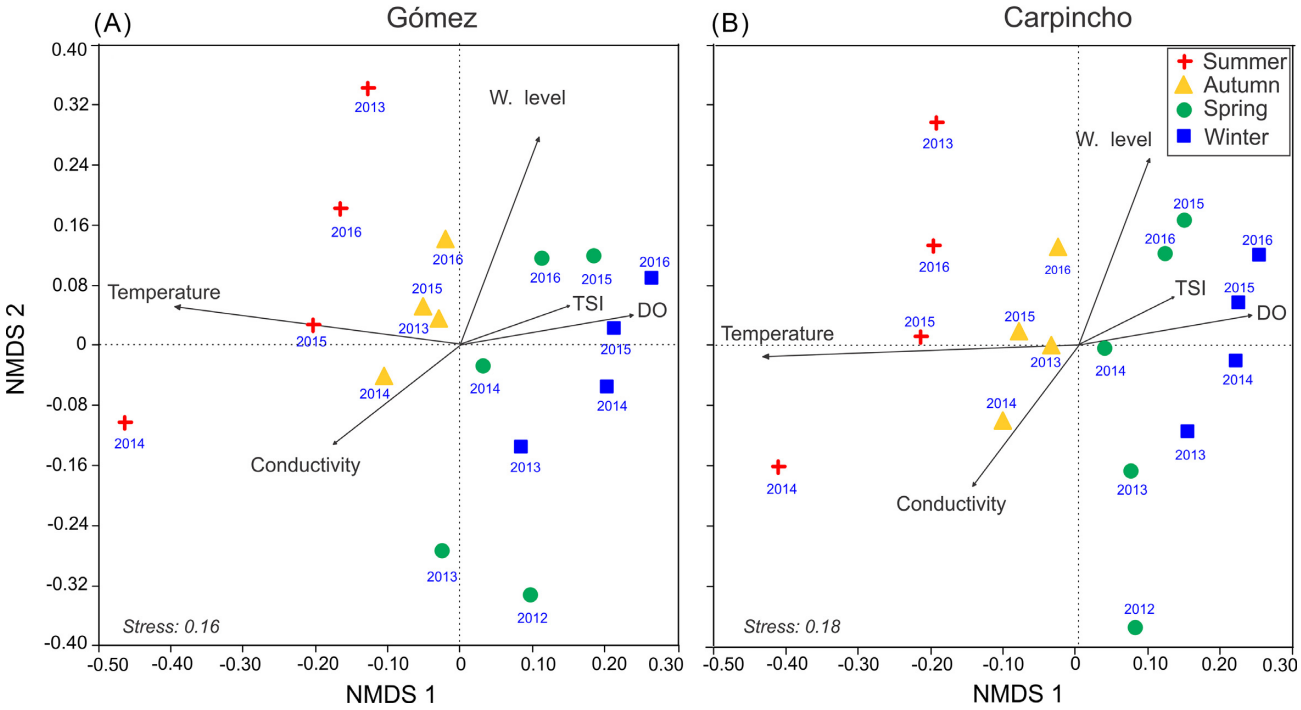

Figure 4. Biplots of the non-metric multidimensional scaling (NMDS) performed on Hellinger-transformed bacterioplankton community composition using Bray-Curtis similarity index in **(A)** Gómez and **(B)** Carpincho. The arrows indicate the significant correlations ($P < 0.05$) between the environmental variables and the ordination axes and were calculated passively after NMDS by the *envfit* function. W. level: water level, TSI: trophic state index, DO: dissolved oxygen.

abundances), but also the physical, chemical and biological variables (Schiaffino *et al.* 2019).

Although different temporal rates of change and signals of change of PCC and BCC similarities were observed, these communities showed within and between shallow lake temporal associations and synchronous shifts (Table 3 and Procrustes analyses). Besides, inter-taxa algal-bacterial connections were also found in each shallow lake (Supplementary Fig. S6). The covariation of microbial communities between shallow lakes is probably caused not only by the effect of extrinsic regional factors (i.e. temperature, climate), but also for the connection and closeness between both shallow lakes. These lakes are connected by the Salado River and accordingly they also presented a high percentage of shared bacterial zOTUs (94.1%) and similar phytoplankton composition. Likewise, temporal associations and linkages of phytoplankton and prokaryotes within and between aquatic environments have also been described for various types of freshwater systems (e.g. Kent *et al.* 2007; Liu *et al.* 2014; Mikhailov *et al.* 2019a).

The within-lake covariation between PCC and BCC was especially exemplified during the cyanobacterial bloom observed in summer 2014, which caused a strong sudden disruption in bacterioplankton richness and diversity (Supplementary Fig. S5). Several studies demonstrated that algal blooms are biological disturbances that affect BCC in freshwater systems (e.g. Berry *et al.* 2017; Scherer *et al.* 2017; Su *et al.* 2017b; Zhang *et al.* 2018) and marine environments (Teeling *et al.* 2012; Bunse *et al.* 2016). In particular, the structural diversity of BCC was associated with some bloom-forming freshwater Cyanobacteria genus (e.g. Wiedner *et al.* 2007; Zhu *et al.* 2014; Louati *et al.* 2015; Woodhouse *et al.* 2016). It was observed that Cyanobacteria had greater impacts on BCC than planktonic algae and zooplankton (Wang, Razzano and Mou 2020). Accordingly, during the cyanobacterial bloom registered in our study, the best represented zOTU *Sporichthyaceae* decreased its number of reads. On the other hand, during the bloom, a peak of DOC was observed in both lakes (Supplementary Fig. S1g). Similarly, Ye *et al.* (2012) found an accumulation of DOC during a spring-summer cyanobacterial bloom in a Chinese lake. This is in concordance with the production of organic matter that is consumed by specific groups of heterotrophic bacteria, leading to synchronisation of some planktonic bacterial groups with phytoplankton blooms (Buchan *et al.* 2014; Tan *et al.* 2015; Mikhailov *et al.* 2019a).

Algal-bacterial associations between different taxa of phytoplankton and bacterioplankton within each lake were also found (Supplementary Fig. S6). Analysis of the topological parameters of the two correlation networks revealed that the inter-taxa associations between BCC and PCC presented a general similar pattern in both lakes. Interestingly, in both shallow lakes the number of positive correlations was higher than the number of negative ones. Previous studies in terrestrial and marine bacterial communities have also revealed association patterns dominated by positive correlations (Barberán *et al.* 2012; Liu *et al.* 2015; Ma *et al.* 2016). These correlations may indicate a mutualistic interaction, while the negative correlations have a competitive relationship (Lupatini *et al.* 2014; Liu *et al.* 2019; Mikhailov *et al.* 2019a). However, microbes can also positively or negatively correlate for indirect reasons, due to their environmental preferences (Weiss *et al.* 2016). Therefore, these associations may be explained by the similar environmental preference, which enables them to coexist in the same ecological niche. Thus, taxa could be related due to a true ecological association or because of an abiotic or biotic environmental factor (Faust and Raes 2016). In our study, the network analyses showed true correlations between algal-bacterial taxa independently of the effect of environmental variables (Supplementary Fig. S6).

The nodes of the networks with more connections with others were considered as hub or highly connected taxa (Berry

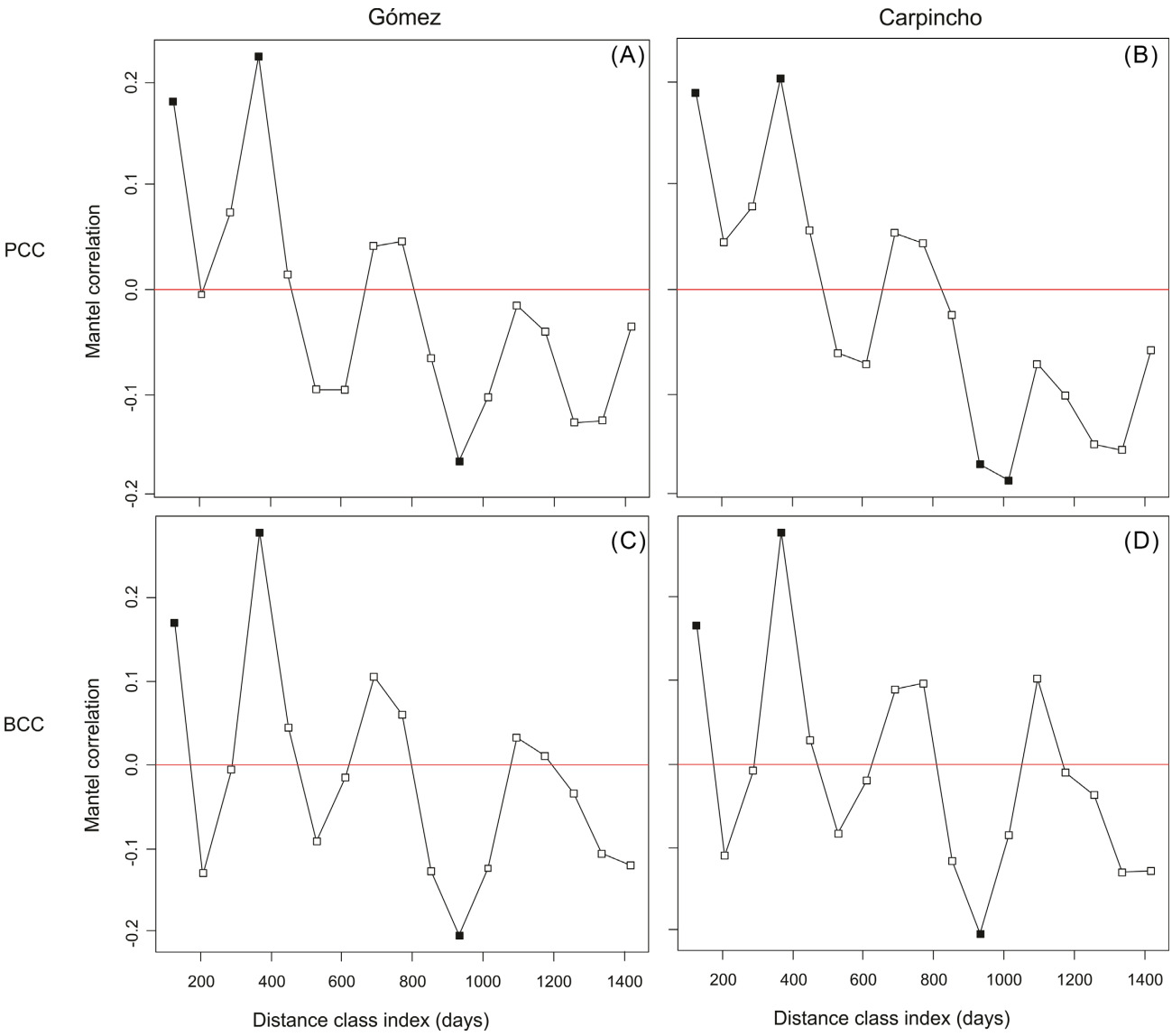

Figure 5. Multivariate Mantel correlograms of PCC and BCC dissimilarity. The ordinate indicates correlations (positive or negative) between PCC in a distance class in **(A)** Gómez and **(B)** Carpincho and between BCC in a distance class in **(C)** Gómez and **(D)** Carpincho, while the abscissa indicates temporal distance classes in days. Each point indicates the correlation for a temporal distance class, which represents about 100 days. Black squares indicate significant multivariate temporal correlation at $P < 0.05$ after Holm correction for multiple testing.

and Widder 2014; Agler *et al.* 2016; Banerjee, Schlaeppi and van der Heijden 2018). These taxa play a fundamental role in determining the structure and function of microbiomes. However, as found in previous studies, most hub taxa were not the most abundant, contributing less than 9% to the total reads (e.g. Comte *et al.* 2016; Xue *et al.* 2018). Additionally, in our study the set of hub or highly connected taxa were different in both lakes (Supplementary Table S3). This result is surprising considering the huge similarity of the two lakes in their environmental conditions, taxonomic compositions, association patterns and between-lake covariations; characteristics probably given by their connection and closeness. These results emphasised the importance of studying highly connected taxa to fully understand and comprehend microbial communities (Agler *et al.* 2016; Banerjee, Schlaeppi and van der Heijden 2018).

The intrinsic environmental variables affected both PCC and BCC variations (8–10%), and in particular part of the variation of BCC (7–8%) was also affected by phytoplankton (i.e. Cyanobacteria and Chlorophyceae), but not the other way around (Fig. 6). Accordingly, Chlorophyta and Cyanobacteria showed the highest number of interactions with bacterioplankton taxa (Supplementary Fig. S6). Besides, we further found within-shallow lake association between BCC and PCC, even controlling for extrinsic and intrinsic factors, suggesting a covariation mediated by biotic interaction (Table 3). These results are in line with earlier studies, which suggested that a large part of the bacterial variability is influenced by phytoplankton (e.g. Kisand and Tammert 2000; Kent *et al.* 2007; Niu *et al.* 2011; Teeling *et al.* 2012). Experimental incubations provided evidence that phytoplankton assemblages shape bacterial community development (Sarmento and Gasol 2012; Paver *et al.* 2013) and algal exudates influence the structure of BCC (Paver and Kent 2010). However, it would be necessary to combine high-frequency environmental time series data and experimental manipulations to confirm the phytoplankton

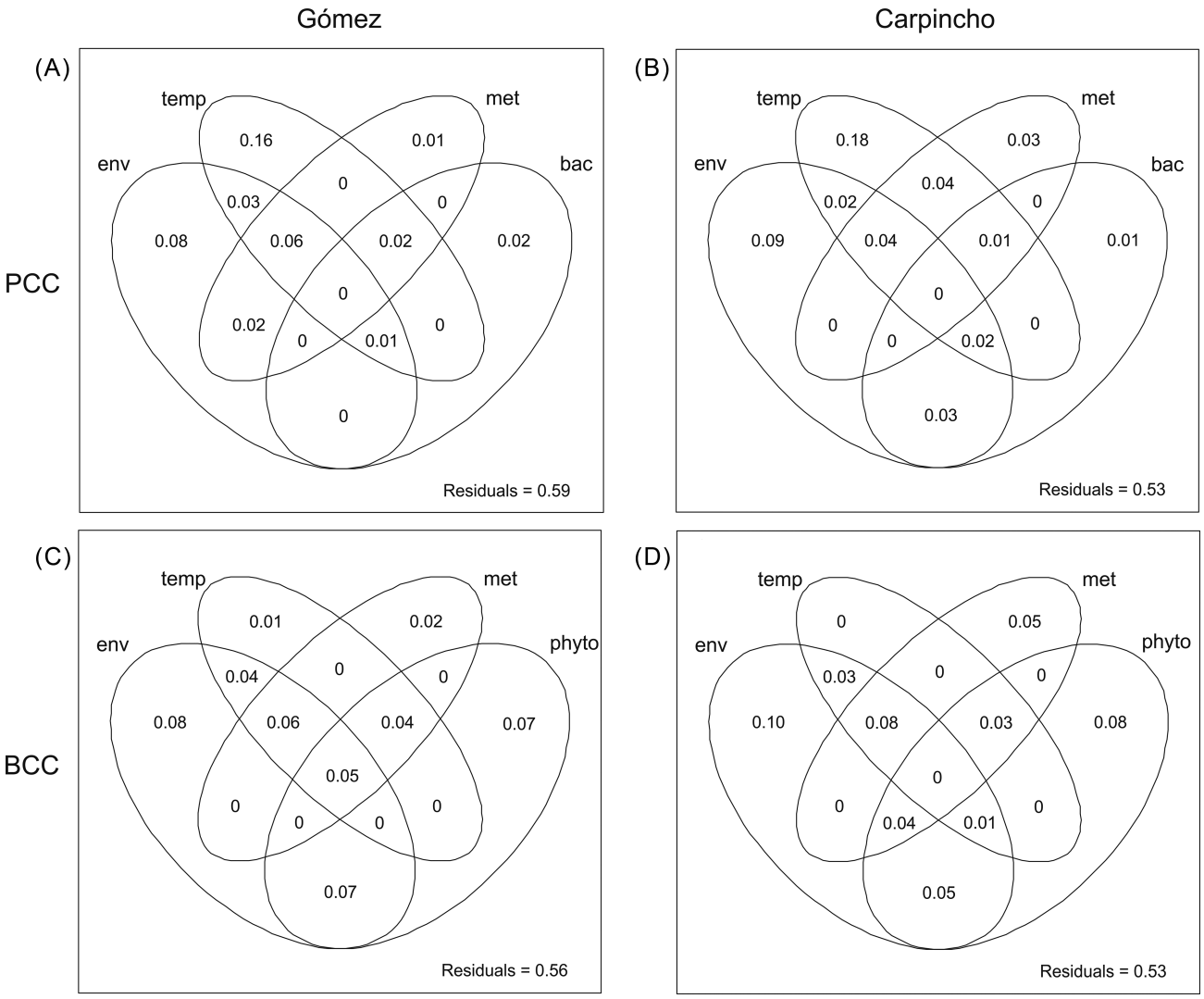

Figure 6. Variation partitioning performed with the PCC in **(A)** Gómez and **(B)** Carpincho and BCC in **(C)** Gómez and **(D)** Carpincho as response matrices versus environmental (env), temporal (temp), meteorological (met) and biotic (bacterioplankton- bac or phytoplankton-phyto) explanatory factors. On the PCC response matrix, the forward selection of explanatory variables in both lakes were: AEM 1, AEM 2, AEM 8 and AEM 9 (temporal explanatory matrix); monthly mean air temperature (meteorological explanatory matrix); water temperature, SD (Gómez) and water temperature, SD, N-NH_3, TP (Carpincho) (environmental explanatory matrix); Alphaproteobacteria (Gómez) and Planctomycetes (Carpincho) (bacterioplankton explanatory matrix). On the BCC response matrix, the forward selection of explanatory variables in both lakes were: AEM 1, AEM 7 and AEM 9 (temporal explanatory matrix); monthly mean air temperature (meteorological explanatory matrix); water temperature, SD, conductivity (Gómez) and water temperature, SD, conductivity, pH (Carpincho) (environmental explanatory matrix); Chlorophyceae and Cyanobacteria (phytoplankton explanatory matrix).

influence on BCC dynamics and to firmly establish cause and effect.

The extrinsic (temporal and meteorological) factors explained a significant part of PCC variation (17–21%), but did not explain much of the BCC variation (Fig. 6). However, we found a marked seasonal trend in BCC (Fig. 4), with water temperature as one of the main intrinsic variables. These results suggest that the influence of extrinsic regional factors can be channelled to bacterioplankton through both environmental (i.e. water temperature) and phytoplankton effects. Similarly, Kent *et al.* (2007) reported that bacteria dynamics were driven by intrinsic interactions with phytoplankton and demonstrated that these interactions transmitted the signal of the regional extrinsic factors to the bacterial communities. Besides, it was found that synchrony between phytoplankton and bacteria may be caused directly by biotic interactions (such as mutualisms, antagonisms, parasitism and competition) that affect the microbial structure and their ecological functions (e.g. Cole 1982; Kisand and Tammert 2000; Joint *et al.* 2002; Sarmento and Gasol 2012).

Overall, our results showed annual (seasonality) and interannual variations in microbial composition and environmental variables, and also revealed within- and between-shallow lakes synchronous shifts in PCC and BCC. The within-lake covariations between PCC and BCC persisted, while controlling for the effects of intrinsic and extrinsic factors, supporting a community coupling mediated by intrinsic biotic interactions. These results open new perspectives for future studies involving experiments and high-frequency samplings in order to further comprehend the algal-bacteria interactions, focusing on bloom-forming Cyanobacteria and heterotrophic bacteria from hypertrophic aquatic systems.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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Conflicts of interest. None declared.

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