Interplay between stochastic and deterministic processes in the maintenance of alternative community states in Verrucomicrobia-dominated shallow lakes

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Keywords: *bacterioplankton; verrucomicrobia; alternative states; shallow lakes; community structuring processes*

Abstract

We analyzed the interplay between neutral and deterministic processes in maintaining contrasting alternative bacterioplankton communities through time in highly productive shallow lakes and we evaluated the relevance of these processes when a regime shift from a clear to a turbid state occurred. We observed that local assembly is ruled primary deterministically, via local habitat filtering, with a secondary role of stochastic processes. We also found a hierarchy in the environmental sorting: while an unusual Verrucomicrobia dominance characterizes the three systems, local conditions limit within-bacterial community membership to closely phylogenetically related and ecologically similar taxa. These results indicate that bacterial abilities to establish in these lakes are strongly determined by their traits, and point toward special physiological adaptations to persist when these systems undergo a regime shift. Altogether, these results hint to a divergence in function among these alternative communities, mediated by major shifts in bacterial community trait structure, particularly regarding carbon use.

INTRODUCTION:

Bacterial activities drive most of Earth's biogeochemical cycles and, thus, much effort has been devoted to understanding the mechanisms governing their community assembly in nature. Traditionally, the prevailing view within microbial ecology has been that deterministic processes (*i.e.* niche-related), which include selection imposed by the abiotic environment as well as interespecies interactions, shape microbial communities (Baas-Becking, 1934; Fenchel & Finlay, 2004). More recently, the neutral theory (Hubbell, 2001) has challenged this perspective by suggesting that all species are ecologically equivalent and that stochastic processes such as birth, death, colonization, extinction, and speciation (Hubbell *op. cit*; Chave, 2004), govern the diversity and species composition of local communities, independent of their traits and niches.

Nowadays, it is recognized that these mechanisms are not mutually exclusive, but interact in the assembly of microbial communities to determine how species diversity and composition vary along environmental and/or spatio-temporal gradients (reviewed in Lindström and Langenheder, 2012; Hanson *et al*, 2012).

Within this context, models of multiple stable states or alternative community states (Lewontin, 1969; Holling, 1973; May, 1977) are often used to explain why assemblages of different species can occur side by side in apparently the same environment (Petraitis & Latham, 1999 and references therein). Theoretical and experimental approaches have focused the attention on the mechanisms that give raise to the occurrence of alternative communities and proposed priority effects as well as stochasticity as prevailing mechanisms generating these contrasting regimes (*e.g.* Chase, 2003; Beisner *et al*, 2003; Chase, 2007; Jiang & Patel, 2008; Price & Morin, 2009; Zhou *et al*, 2013). However, the processes underpinning the coexistence and maintenance of multiple stable states through time and the relevance of those processes driving a shift of regime have been understudied.

Pampean shallow lakes provides a valuable natural experiment to analyze the relative relevance of local niche-based processes over neutral ones in shaping bacterial communities in eutrophic-hypertrophic systems that exhibit alternative stable states. The Pampa plain of Argentina comprises a mosaic of shallow lakes interconnected by fluvial networks, some of which are stabilized in either a turbid or a clear alternative state (*sensu* Scheffer *et al*, 1993), while others recurrently shift between different regimes (Quirós *et al*, 2002; Allende *et al*, 2009). Moreover, when confronted with other lakes worldwide, Pampean shallow lakes depart from most of them as they stand as extremes of the trophic-state continuum (Diovislavi *et al*, 2015). Because of their occurrence in fertile lands, these already eutrophic lakes (Quirós & Drago, 1999) have experienced increased levels of eutrophication in recent times, and many of them have switched to a turbid state, which represents the extreme of the environmental variation gradient (Scheffer *et al*, 1993; Scheffer *et al*, 2001).

Using high-throughput sequencing, we explored over an annual cycle the underlying processes structuring bacterial communities in three neighboring hypertrophic shallow lakes with contrasting alternative states: one stabilized in a clear-vegetated state ("clear"), one stabilized in a turbid state ("turbid") and one that alternates between phytoplankton-turbid and clear vegetated states and that during the study period shifted from a clear state at the beginning, to a predominantly turbid state towards the end of the study ("alternating"). The bacterial communities of these systems belong to a regional metacommunity, which is expressed differently under the various regimes (Llames *et al* 2013).

We performed a combined approach based on the analysis of community taxonomic composition as well as metrics of community phylogenetic structure, with randomization procedures and estimating the deviations from null-model expectations (*e.g.* Stegen *et al*, 2012, 2013; Valverde *et al*, 2014; Dini-Andreote *et al*, 2015). Our main objective was to analyze the interplay between neutral and niche-related processes in maintaining contrasting alternative bacterioplankton communities through time, as well as to evaluate the relevance of these processes when a regime shift from a clear to a turbid state occurs.

MATERIALS AND METHODS

Study site

The three studied lakes are located in Buenos Aires province, Argentina, in the Salado River floodplain basin (**Supplementary Figure S1**). These lakes have a mean depth less than 2 m and range from eutrophic to hypereutrophic (**Supplementary Table S1**).

Following the classification proposed by Scheffer and co-workers (Scheffer *et al,* 1993), El Triunfo ("clear") (TRI; 35° 51'S, 57° 52'W) has stabilized in a clear-vegetated state, while Chascomús ("turbid") (CH; 35° 36'S, 58° 02'W) is characterized by low Secchi depth values (< 15 cm) and high phytoplankton abundances. In the case of Lacombe ("alternating") (LAC; 35° 49'S, 57° 49'W), previous studies have shown that this shallow lake alternates between phytoplankton-turbid and the clear vegetated states (Cano, 2008; Casco, 2009). Particularly, during the the study period, LAC shifted from a clear state at the beginning, to a predominantly turbid state towards the end of the study.

Field sampling

The study was conducted over a period of 13 months, from March 2008 to April 2009. Subsurface water samples (from about 30 cm) were taken from a central point in each lake using a 10 L-van Dorn bottle and poured into acid-washed and lake-rinsed polypropylene containers. The containers were kept in a cooler until samples were processed in the laboratory approximately 2 to 4 hours after collection.

Data was obtained every two months, resulting in a total of 20 sampling instances (7 for CH and TRI and 6 for LAC). Environmental data collected resulted in 16 quantitative environmental variables: Incident irradiance (I_0); water temperature (T°), pH, conductivity (Cond), alkalinity (Alk), nephelometric turbidity (Turb), dissolved oxygen (DO), diffuse attenuation coefficient (K_d PAR); total nitrogen concentration (TN), total phosphorus concentration (TP), total suspended solids (TSS, also referred as Seston), organic matter content (OM), inorganic matter content (IM), chlorophyll-*a* (Chl-a), chromophoric dissolved organic matter (CDOM) absorption coefficient at 440 nm (α_{440}) and the inverse index of dissolved organic matter (DOM) average molecular weight (ratio 250:365 nm).

Details on the physical-chemical characteristics of the studied lakes can be found in **Supplementary Material** section, **Supplementary Table S1** and in Llames *et al*, (2013).

Data analysis

DNA extraction, pyrosequencing analysis and processing of data generated from the 454-sequencing runs to obtain the final OTU table and the phylogenetic tree are explained in the **Supplementary Material** section. Briefly, samples were sequentially pre-filtered through 50µm, 20µm nylon mesh to remove larger protists and then through a 3µm and 0.2µm pore-size polycarbonate filter. Only DNA from material retained in the 0.2 µm pore-size filters was extracted and analyzed. Filters were extracted using a CTAB protocol (Fernández Zenoff *et al* 2006) and samples were submitted to INDEAR (Rosario, Santa Fe, Argentina) for tag-pyrosequencing using F515-R806 primers set (see details in **Supplemetary Material**). Pyrosequencing data generated from the 454-sequencing runs were processed using QIIME (Caporaso *et al*, 2010).In brief, sequences were demultiplexed and trimmed by quality using a split_libraries.py script from QIIME (minimum length =200 bp, reads size of quality score window= 50bp; qual. score minimum= 25), and clustered into Operational Taxonomic Units (OTUs) using the pick_otus.py script and Uclust (Edgar, 2010) at 97% similarity. The most abundant sequence from each cluster was chosen as a representative and the phylogenetic relationships of the 16S sequences obtained were determined by a BLAST search within the Ribosomal Database Project (RDP) database (http://rdp.cme.msu.edu/). All representative sequences were aligned with the PyNAST method (Caporaso et al., 2010) with a phylogenetic tree constructed using the maximum likelihood algorithm implemented in FastTree (Price et al., 2009). Singletons were removed from the OTU table obtained and a randomly subsample OTU table was generated by subsampling down to the lowest number of reads (726 reads, after singleton removal) in order to correct for possible biases introduced by unequal sequencing efforts (normalized OTU table). This normalization was performed in the *R* environment (www.r-project.org) using the package Phyloseq (Mac Murdie & Holmes, 2013) and the resulting normalized matrix was used for further analyses. On the other hand, a phylogenetic tree was constructed using the maximum likelihood algorithm implemented in FastTree (Price *et al*., 2009).All sequences generated in this study can be accessed through National Center for Biotechnology Information (NCBI) under the BioProject ID PRJNA324643, accession SRP076211.

Similarity Percentage Analysis

Similarity Percentage Analysis (SIMPER) was performed in order to observe the relative contribution of main OTUs to the average dissimilarities among lakes (Clarke, 1993). Thisanalysis was carried out with the software PAST 2.0 (Hammer *et al*, 2001).

Definition of "core OTUs" and identification of indicator taxa

To describe the dominant taxa of each environment (i.e. "core OTUs", Magurran and Henderson, 2003; Pedrós-Alió, 2006), an *ad hoc* definition of locally abundant OTUs was implemented following previous studies in prokaryotes (Galand *et al*, 2009, Pedrós- Alió, 2012, Logares *et al*, 2013), and protists (Mangot *et al*, 2013). "Abundant" OTU were defined as those with relative abundances ≥1% in each sample.

We used the INDVAL analysis (Dufrene and Legendre, 1997) to identify indicator OTUs of each type of regime (*i.e.* turbid, alternating and clear system) based on OTU fidelity and relative abundance. Analyses were run using the package labdsv (http://ecology.msu.montana.edu/labdsv/R/) within the *R* environment. Only OTUs with significant ($p < 0.05$) INDVAL values that were > 0.3 were considered, as this latter values can be regarded as a good threshold for habitat specialization (Dufrene and Legendre *op. cit*.).

Turnover in OTUs composition

Patterns in taxonomic beta-diversity were explored using the modified Raup-Crick metric (Chase *et al*, 2011). This null model approach allowed us to compare the beta-diversity of the different habitat types independently of differences in alpha-diversity and provides indication of the possible underlying mechanisms of community assembly. This metric expresses the compositional dissimilarity, using presence/absence data, between the observed communities relative to those generated under the null model, by estimating the probability that any two null communities drawn randomly from the "regional" species pool have the same number or more species in common than the observed communities. If Raup-Crick dissimilarity values (β_{RC}) are not significantly different from 0 this indicates community assembly is stochastic. β_{RC} values approaching −1 indicate that communities are deterministically assembled and more similar than expected by chance due to strong habitat filtering, whereas β_{RC} values close to +1 indicate that deterministic factors (e.g., interspecies competition) favor dissimilar communities, or that dispersal between sites is very low (Chase *et al*, 2011.). This analysis was performed in the *R* environment (R Development Core Team 2013) and the null expectation was generated using 9999 randomizations. A permutational analysis of variance (PERMANOVA; Anderson, 2001) was used to test for differences in composition between habitats, whereas permutation dispersion (Anderson *et al*, 2006) was used to test for differences in their within-habitat dissimilarity. Both analyses were run in the *R* environment using the package Vegan (Oksanen *et al*, 2008).

Patterns in the similarity matrix obtained were explored using nonmetric multidimensional scaling (NMDS) (Clarke & Green, 1988).

Testing phylogenetic signal

To test for phylogenetic signal (Webb, 2000; Losos, 2008) we performed a Mantel correlogram (R function mantel.correlog in package Vegan) based on Pearson correlation coefficients between differences in environmental optima and phylogenetic distances. The significance of the correlations was evaluated using 1000 permutations, no distance class cutoff, and a progressive Bonferroni correction (Legendre & Legendre, 1998). A positive correlation coefficient indicates that more closely related OTUs are more similar ecologically (*i.e.* phylogenetic signal is supported).

Environmental-optimum for each OTU was estimated by means of a Canonical Correspondence Analysis (ter Braak, 1986; ter Braak &Verdonschot, 1995) following the two-step criterion proposed by Blanchet *et al.* (2008). The overall test of significance showed that the canonical relationship was significant ($p = 0.007$ after 1000 permutations) and the first two canonical axes accounted together for ~65% of the variation in the OTUs data ($p<0.05$ after 1000 permutations). Then, OTUs scores on the first two canonical axes were used as synthetic descriptors of OTU environmental optimum and between-OTU optimum differences were calculated as Euclidean distances of the scores on these two canonical axes in order to produce a typology of taxa on the assemblages (Legendre & Legendre, 1998) (for a

similar approach see Stegen *et al*. 2012). On the other hand, we calculated cophenetic distances to convert the tree into a phylogenetic distance matrix using the package Picante for R (Kembel *et al*, 2010).

Measuring and testing community phylogenetic structure

We estimated, using Picante, the unweighted mean pairwise distance (MPD) and the unweighted mean nearest taxon distance (MNTD) to analyze the phylogenetic relatedness of OTU in each lake (Webb *et al*, 2002; Kembel *et al*, 2010.). To compensate for random processes in the observed phylogenetic community structure, we calculated a standardized effect size ($_{SES}$MPD and $_{SES}$MNTD) following Kembel (2009). The null model algorithm used was 'independentswap' with 999 randomized null communities and 1000 iterations (Gotelli 2000). The obtained standardized effect size measure (i.e. $_{SES}$MPD and $_{SES}$MNTD) were used to test for phylogenetic clustering or overdispersion (Webb *et al*, 2002). Negative SES values and low quantiles ($p< 0.05$) indicate that community members are clustered, i.e., that phylogenetic distances across a phylogenetic tree in a given community, relative to the regional pool of taxa, are more closely related than expected by chance. On the other hand, positive SES values and high quantiles ($p>0.95$) indicate phylogenetic overdispersion, i.e. greater phylogenetic distances among co-occurring taxa than expected.

Spearman rank correlation coefficient for correlations between environmental factors and phylogenetic indices were calculated.

RESULTS

Across all 20 samples we obtained a total of 73432 quality sequences, which ranged from 1299 to 7763 per sample, with an average read length of 240 bp after primer and barcode removal. Based on the OTU identification, a data matrix that contained 1817 unique OTUs was constructed. Of the total of sequences, > 99% could be classified within Bacteria, while <0.01% of the sequences were classified as Archaea. Across all the lakes, 45 bacterial phyla and one phylotype which could not be classified beyond Archaea were assigned. Rarefaction curves showed that the sequencing effort was variable among samples and, in general terms, they did not reach an asymptote suggesting insufficient sequencing to capture the full diversity of the communities (**Supplementary Figure S2 a-c**).

Verrucomicrobia is the dominant Phylum in the three studied lakes

On the whole, free living bacterial communities in these lakes were dominated by sequences related to Verrucomicrobia (47.3%), followed by Planctomycetes (11.1%) and unclassified Bacteria (9.1%). Among the 1,817 phylotypes assigned, 28% were exclusively found in turbid CH, 18.5% in alternating LAC and 28% in clear TRI lake; while only 9.4% were shared among the three systems (**Figure 1)**. Similarity percentage analysis (SIMPER) based on OTU's relative reads indicated an overall dissimilarity among systems of ~87%. The OTUs that contributed most strongly to the differences belonged to phyla Verrucomicrobia (27% of cumulative dissimilarity), Planctomycetes (12.5%), and also the unclassified Bacteria (11.4%) resulted important in the distinction among lakes.

Common and abundant OTU's were detected; albeit most of the taxa found were rare (57.7%). According to our definition, a total of 38 OTUs comprised the "core microbiome" in these systems (2.1% of total OTUs accounting for 57% of total reads) (**Supplementary Figure S3**). Among these 38 abundant OTUs, IndVal analysis evidenced 16 OTUs that tended to be present in only one system and in most samples of that habitat type and thus, could be considered as habitat specialists (**Suplementary Table S2**). For turbid CH lake 7 OTUs were identified as specialists and comprised 22% of total reads for that system. Among them, only two OTUs related to the orders Verrucomicrobiales (OTU ID 1418) and Phycisphaerales (OTU ID 23) accounted for 16% out of 22% of total reads. In the case of alternating LAC lake, 5 specialists were identified which represented 40% of total reads obtained for that lake. Only one OTU related to order Spartobacteriales (OTU ID 1475) accounted for 27% of total reads obtained for LAC. Finally, for clear TRI, 4 habitat specialists were

detected representing 40% of total reads obtained for this system with only one OTU related to order Puniceicoccales (OTU ID 875) comprising 36% out of 40% of these total reads.

Deterministic processes are the most important in shaping bacterial community composition

The NMDS plot obtained based on Raup Crick metric showed that bacterial communities clustered according to the alternative state of the lake (PERMANOVA F= 88.636, p < 0.001, **Figure 2**). Overall, the observed mean Raup-Crick dissimilarities significantly differ from the null expectation (p< 0.001) suggesting that deterministic processes are the most important in explaining bacterial community assembly patterns in these systems (**Figure 3)**. According to β_{RC} estimations and the permutational analysis of multivariate dispersion test (**Table 1**), lowest values were recorded for the turbid system, which also evidenced a high level of compositional consistency indicating the strongest habitat filtering condition. On the contrary, permutation dispersion showed that the clear and the alternating bacterial communities were considerable more variable in their intra-OTU composition.

Differential distribution of OTU's among lakes underlie phylogenetic clustering

The Mantel correlogram showed significant positive correlations across short phylogenetic distances (p<0.01) (**Figure 4**) indicating similar habitat associations among closely related bacterial taxa.

The phylogenetic approach evidenced that within each system, bacteria tend to co-occur with other bacteria that were more closely related than expected by chance. In agreement with the Mantel correlogram, this phylogenetic clustering was more evident at terminal levels in the phylogeny as sesMNTD values tended to increase in significance relative to sesMPD (**Figure 5 a-b, Supplementary Table S3**). These clustered distributions, together with the taxonomical results, reinforce the idea of habitat filtering selecting for traits conserved, particularly, at terminal levels in the phylogeny. However, when analyzing the progressive succession of sesMNTD through time, we found that the strength of this phylogenetic clustering varied temporally within each site, even resulting in some statistically random communities.

We also observed that phylogenetic indices exhibit significant trends with environmental parameters that were identified in a prior study as important in structuring bacterial communities in these lakes. We found that sesMPD increased significantly with chlorophyll-*a*, CDOM absorption coefficient at 440 nm and turbidity ($r^2_{chl\text{-}a}$= -0.62, p= 0.02; r^2_{CDOM}= -0.56, p= 0.03; $r^2_{turbidity}$= -0.53, p= 0.02). On the other hand, sesMNTD decreased significantly with alkalinity (r^2_{alk}= 0.53, p= 0.02).

Habitat preference is phylum-dependent

We observed that, with very few exceptions, OTUs from dominant phyla were systematically recorded under the same alternative regime. That is to say, an OTU retrieved from the turbid lake was also found in the alternating system during the "turbid phase" and the same holds true for those OTUs retrieved from the clear lake when compared with the "clear phase" of the alternating regime (**Figure 6**). At the phylum level, dominant Verrucomicrobia as well as Alphaproteobacteria and Bacteroidetes comprise phylotypes occupying unique niches in turbid as well as in clear systems, suggesting a wide range of physiological adaptation that characterize these phyla. On the contrary, Betaproteobacteria and, to a lesser extent, candidate division ZB2 were consistently more often found in "clear" conditions while Planctomycetes representatives were mostly associated to the turbid regime.

DISCUSSION

As a whole, our combined null model analyses indicate that the taxonomic as well as the phylogenetic structure significantly deviated from the stochastic expectation, indicating that bacterioplankton communities' local assembly from these hypertrophic shallow lakes is ruled primary deterministically, via a strong selection imposed by the abiotic conditions that characterize turbid and clear regimes.

Nevertheless, as not only the spatial but also the temporal dimension was considered, our combined taxonomic/ phylogenetic approach let us evidence a temporal dependency in the stochastic/deterministic balance indicating an important but secondary role of death and random recruitment processes structuring these alternative communities.

These results agree with the notion that bacterial species sorting is a widespread process (Tamames *et al*, 2010; Lindsrtöm & Langenheder, 2012; Pontarp *et al*, 2012; Schmidt *et al*, 2016) and that this process is particularly prevalent in aquatic systems (Heino *et al*, 2015). Taxonomic as well as phylogenetic indices showed that this abiotic control was maximized under turbid conditions, which is in line with the notion that habitat filtering increases with environmental extremes (Horner-Devine & Bohannan, 2006; Wang *et al*, 2012).

We further observed that the prevailing process of habitat filtering resulted in a similar community composition under the same alternative state. The temporal succession observed in alternating LAC from a "clear type" assemblage to a "turbid type" one indicates high connectivity by dispersal among sites (Chase 2003) and demonstrates that spatial factors structuring these communities are not important at the scale considered here. Hence, the non-random segregation patterns detected reflect a mutual exclusion between OTUs, rather than spatially structured assembly composition (Soinien *et al* 2013, Soinien 2014).

This predominant deterministic scenario would suggests that, as these shallow lakes switch towards turbid states, there is the possibility that a single regional equilibrium would eventually be achieved as superior competitors begin to dominate, thus eroding β-diversity and, eventually, affecting community functional attributes (McCann 2000; Chase 2003, Curtis & Sloan *op. cit.*). Nevertheless, we observed that the strength of the phylogenetic clustering varied temporally within each site, even resulting in some statistically random communities.

These empirical results fit well with recent theoretical frameworks that postulate that both, deterministic and stochastic processes, are important in shaping community's assembly and succession, and that their relative importance varies temporally (*e.g.* Ferrenberg *et al*, 2013; Zhou *et al*, 2014; Dini-Andreote *et al*, 2015). The ecological implications of these shifts between stochastic and deterministic control during succession result evident. Particularly, neutral processes have a profound effect on transitions between alternative regimes, especially when considering features such as limited dispersal or spatial heterogeneity (Martín *et al*. 2015).

From the "metacommunity" perspective this "stochastic background" indicates that there exist many bacterial taxa that frequently appear by chance in the different systems and this is possible because they are represented in the metacommunity. The ~10% of shared OTU's among the three systems together with temporal succession observed in alternating LAC, suggest that efficiently dispersing taxa are temporarily present at sites that are not environmentally favorable for them due to the strong influx of colonists from other sites.

On the other hand, from the "alternative states" modeling perspective, our results ratify the relevant role of demographic stochasticity in generating alternative states communities. That is to say, these neutral processes directly affect populations dynamics in such a way that, ultimately, can cause communities to move from one locally stable configuration to another without a permanent change in environmental parameters ("communities perspective" of alternative states *sensu* Beisner *et al*, 2003).

It follows that shifts in the alternative state of the systems would not affect dramatically the reservoir of diversity in this metacommunity pool as there exists a chance that at least some bacterial taxa will respond differentially to variable conditions and perturbations assuring the regional coexistence of bacterial communities in multiple organizational states. Then, the greater the variance in species responses' contained in the community, the more these systems are protected against environmental variability (Yachi & Loreau, 1999, Loreau *et al*, 2003, Shanafelt *et al*, 2015).

Still, the overriding strong local niche selection denotes that OTUs ' abilities to establish in these lakes are determined by their traits, and suggests a strong selective pressure for specific physiological features required to persist when these systems undergo a shift from a clear- to a turbid- regime (Webb *et al*, 2002; Chase & Leibold, 2003). Although in reality we cannot determine the actual

distribution of traits within these complex bacterial assemblages, the taxonomic resolution at which bacterioplankton composition varies among sites together with the phylogenetic analysis performed provide clues about the traits under selection.

Recent studies reveal that microbial traits are differentially conserved across the tree of life and appear to be conserved in a hierarchical fashion, possibly linked to their biochemical complexity (Martiny *et al*. 2015 and references therein). According to Martiny *et al*. (2015), environmental selection on traits deeply conserved in the phylogeny leads to broad taxonomic scale shifts among samples, whereas selection on shallower conserved traits leads to finer-scale shifts.

In this analysis we found a prevailing co-ocurrence of Verrucomicrobia and Planctomycetes phyla dominating these lakes, with local abiotic conditions promoting shifts at finer scale taxonomic resolution. This taxonomic pattern suggest a hierarchy in the environmental sorting wherein dominant phyla are regionally selected from the metacommunity pool, and variations within each alternative state further select taxa from these main players. Noteworthy, this strong local environmental control selecting for specific OTUs translated for some groups in a consistent within-phylum habitat preference.

Also, this taxonomic configuration collectively with the significant positive correlations across short phylogenetic distances shown by our correlogram analysis, suggest that variations within each alternative state would select for traits shallower conserved in the phylogeny. In this regard, as dissolved organic carbon (DOC) quality plays a role in shaping bacterial production in these shallow lakes (Torremorell *et al*, 2015) and as it has already been proved a shallow phylogenetic conservatism of traits related to the ability to grow on a diverse array of carbon substrates (Martiny *et al* 2013) we hypothesize that these alternative communities specifically diverge in effect traits regarding carbon use.

Yet globally distributed, the unusual permanent co-ocurrence of Verrucomicrobia and Planctomycetes dominating these communities is striking.

The ubiquity of Verrucomicrobia in these systems coincides with previous reports on the presence of this phylum in a variety of contrasting environments (*e.g.* Scheuermayer *et al*, 2006; Yoon *et al*, 2011; Freitas *et al*, 2012; Parveen *et al*, 2013); while the differential distribution of phylotypes agrees with the notion of a phylum composed of several ecologically distinct linages characterized by a high metabolic versatility (Freitas *et al op.cit.*; Chiang, 2015). Despite little is reported on the abundance and ecology of aquatic Verrucomicrobia, its dominance in these shallow lakes might be result of the extremely high nutrient concentration that characterize these systems as it has been found that the prevalence of this phylum in lakes is positively correlated with nutrient-richness and phosphorus availability (Arnds *et al*, 2010 and references therein). In addition, it has been suggested that phytoplankton diversity is an important contributing factor structuring verrucomicrobial communities (Parveen *et al*, 2012). In line with this, we suggest that the already established differences in phytoplankton composition in these shallow lakes (Allende *et al*, 2009; Izaguirre *et al*, 2012) constitute one of the main habitat filters that locally select for specific verrucomicrobial phylotypes as the prevailing algal assemblage dominating these systems represents, indeed, different micro-patches of concentrated substrates that are differentially used by these bacteria (Azam, 1998).

On the other hand, the subdominant phylum Planctomycetes also represents a widely distributed but relatively understudied bacterial group which have been detected in environments as diverse as soils, marine and freshwater systems, humans, termite guts, permafrost and wastewater treatment plant (Steven *et al*, 2011 and references therein). As regards our observations, we found, with a few exceptions, that members of this phylum were preferentially retrieved under turbid conditions, which is in line with previous reported associations of this phylum with eutrophic conditions and algal blooms (Steven *et al*, 2011). Nevertheless, this consistent phylum-level habitat preference is challenging as only a few genera have been clearly described and diversity surveys covering all known genera of Planctomycetes in natural environments are scarce (*e.g.*Wang *et al*, 2013 and references therein).

AKNOWLEDGEMENTS

We gratefully thank Roberto Escaray and José F. Bustingorry for the technical assistance in lake sampling and chemical analyses and Horacio Zagarese for his helpful discussions related to this work

FUNDINGS

This research was supported by the 'Argentine network for the assessment and monitoring of Pampean shallow lakes ($PAMPA^2$-CONICET)', and 'Agencia Nacional de Promoción Científica y Tecnológica' (PICT 2011-1029, PICT 2014-1290 and PICT 2014-2898).

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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DATA ACCESSIBILITY

Raw sequences have been archived to the NCBI's Sequence Read

Archive (SRA) database under the BioProject ID PRJNA324643, accession SRP076211

Author Contributions

P.H. and S.M. contributed with the raw sequence data analysis pipeline and the construction of the phylogenetic trees. M.E.LL has performed the statistical analyses and M.E.LL and F.U. have written the paper.

FIGURE LEGENDS

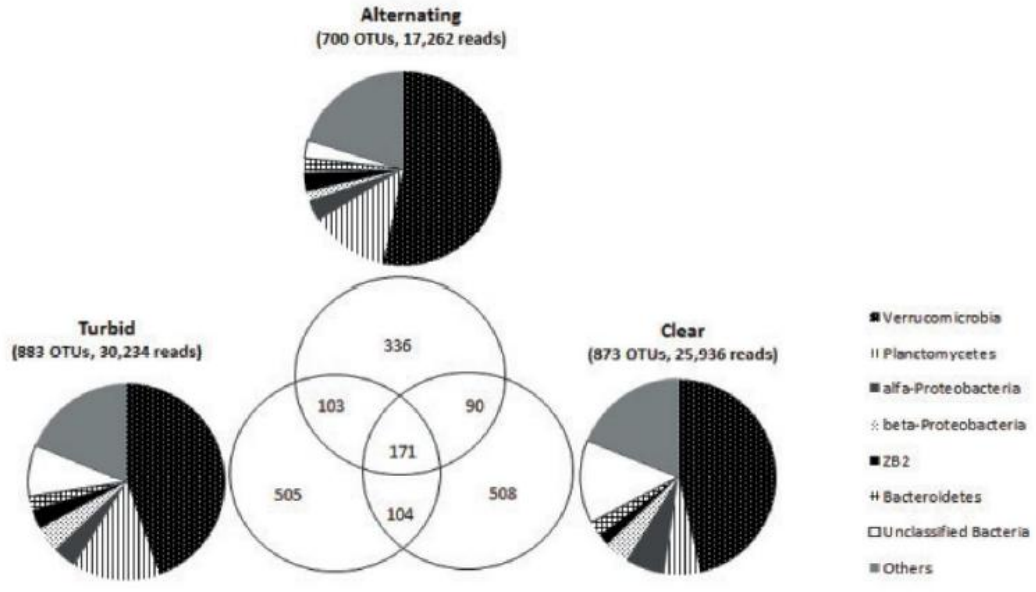

Figure 1: Comparison of bacterial community composition among lakes. Figures in brackets indicate the total number of OTUs assigned for each system as well as total reads. Pie charts indicate the relative contribution of different phyla to total reads, averaged through the different sampling dates. Venn diagram summarizes unique and shared OTUs among systems.

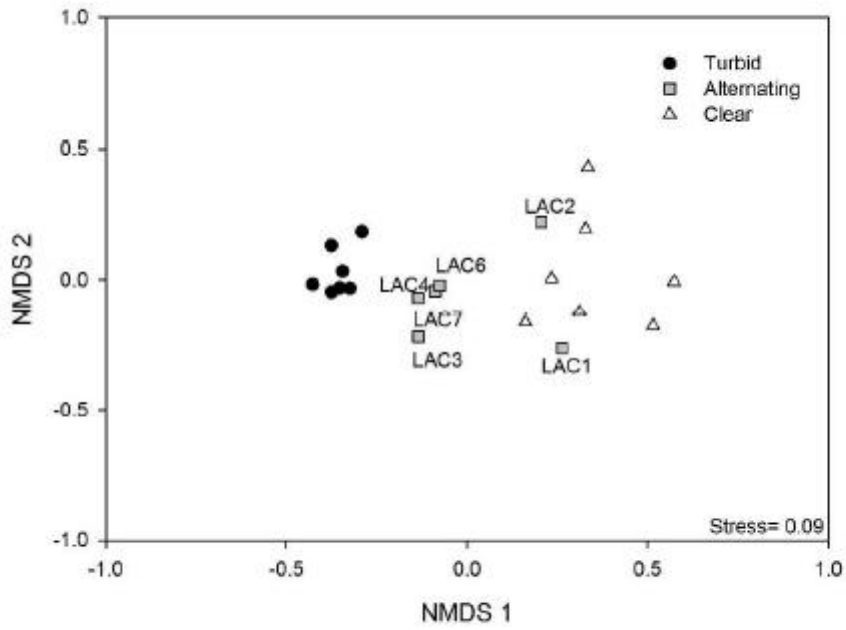

Figure 2: Non-metric multidimensional scaling ordination based on modified Raup-Crick dissimilarities. Communities that are closer together, using modified are more deviant from the null expectation, whereas communities that are farther apart are less deviant from the null expectation.

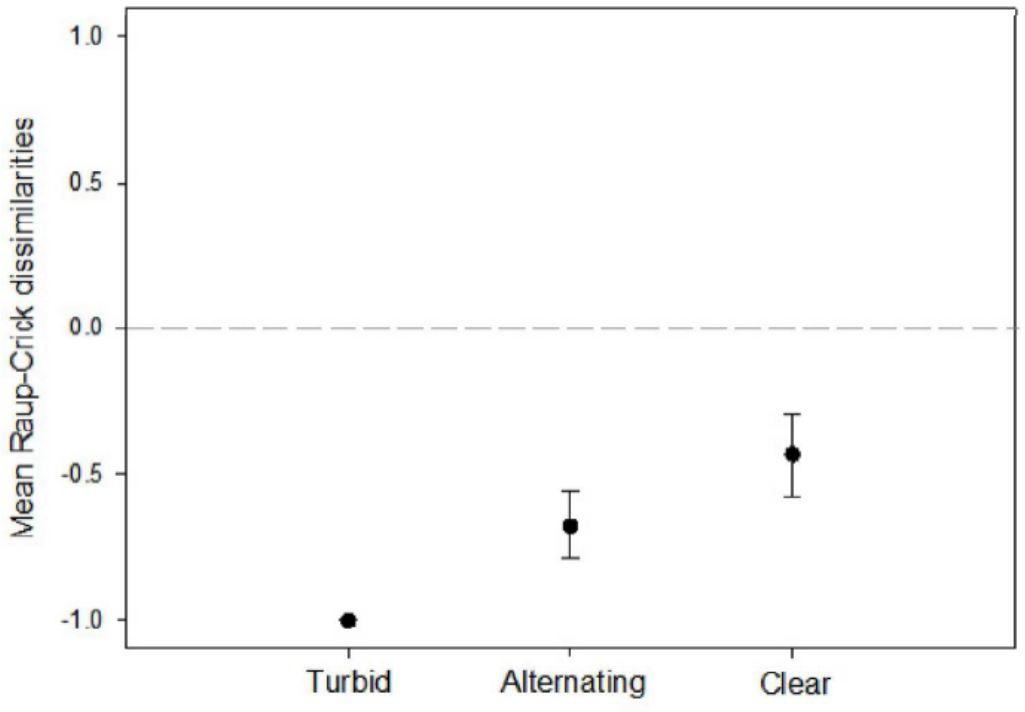

Figure 3: Null deviations of the bacterial communities from each lake. A null deviation close to zero suggests that neutral processes are more important in structuring the community, whereas larger negative null deviations suggest that niche-based processes are more important.

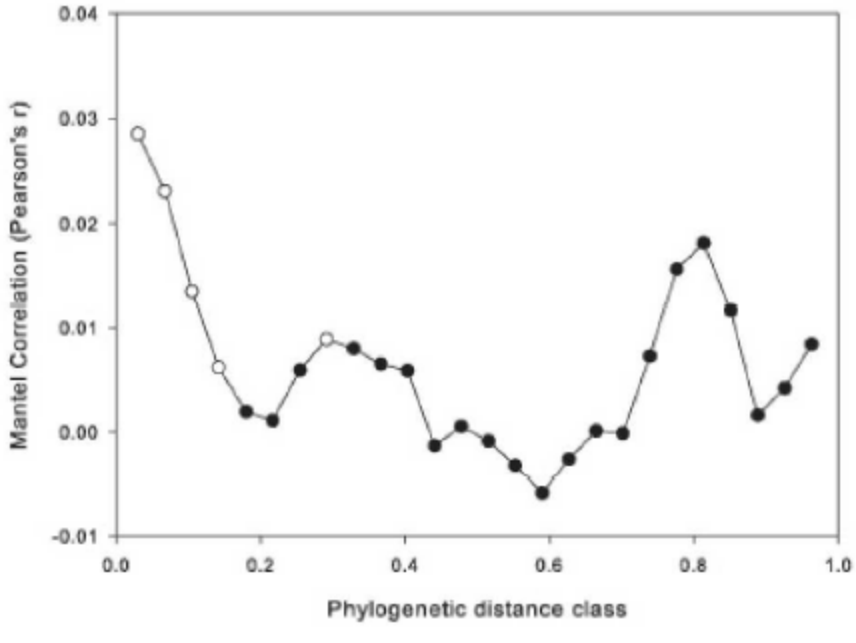

Figure 4: Phylogenetic Mantel correlogram showing significant phylogenetic signal across short phylogenetic distances. Open symbols denote significant correlations at $p < 0.01$. Significantly positive correlations indicates that ecological niches distance between OTUs increase with their phylogenetic distance, but only across the phylogenetic distance class being evaluated, that is, there is phylogenetic signal in OTU environmental niches.

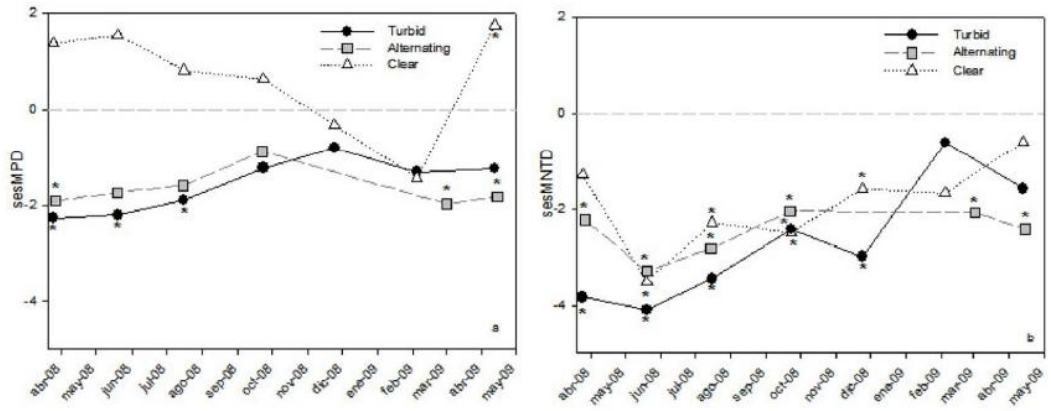

Figure 5: Variation of the Standardized effect size of the unweighted mean pairwise distance (sesMPD) and the unweighted mean nearest taxon distance (sesMNTD) for the 16S rDNA sequences during the studied period for the three lakes. (*) Communities that are significantly structured at the p= 0.05 level.

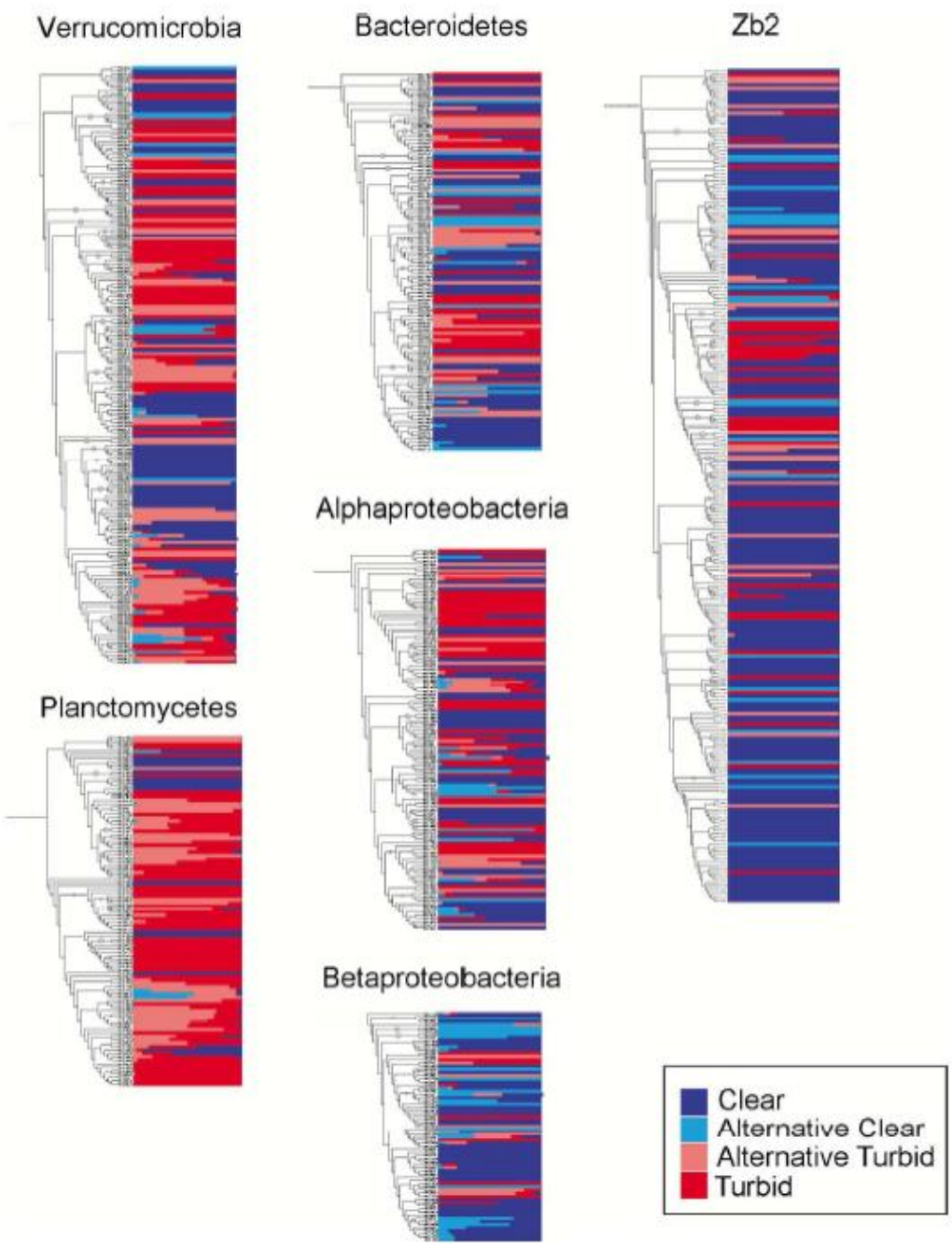

Figure 6: Phylogenetic tree of dominant bacterial phyla inferred from partial 16S rRNA sequences. Colored bars represent the proportional distribution of each OTU reads between turbid and clear regimes.

Table 1: Raup-Crick dissimilarity estimations for each system (β_{RC}) and results for the permutational analysis of multivariate dispersion test. Communities differ significantly at $p < 0.05$.

	Turbid	**Alternating**	**Clear**
Min βRC	-1.00	-1.00	-1.00
Max βRC	-0.99	0.39	0.74
Mean βRC (± SE)	-1.00 ± 0.00	-0.68 ± 0.12	-0.43 ± 0.14
Permuted analysis of betadispersion			
Average distance to mean compositional centroid	0.00003	0.21182	0.27388
Multiple comparisons	***p values***		
Turbid-Alternating	0.016		
Turbid-Clear	0.002		
Clear- Alternating	0.626		

SUPPLEMENTARY MATERIAL

STUDY SITE

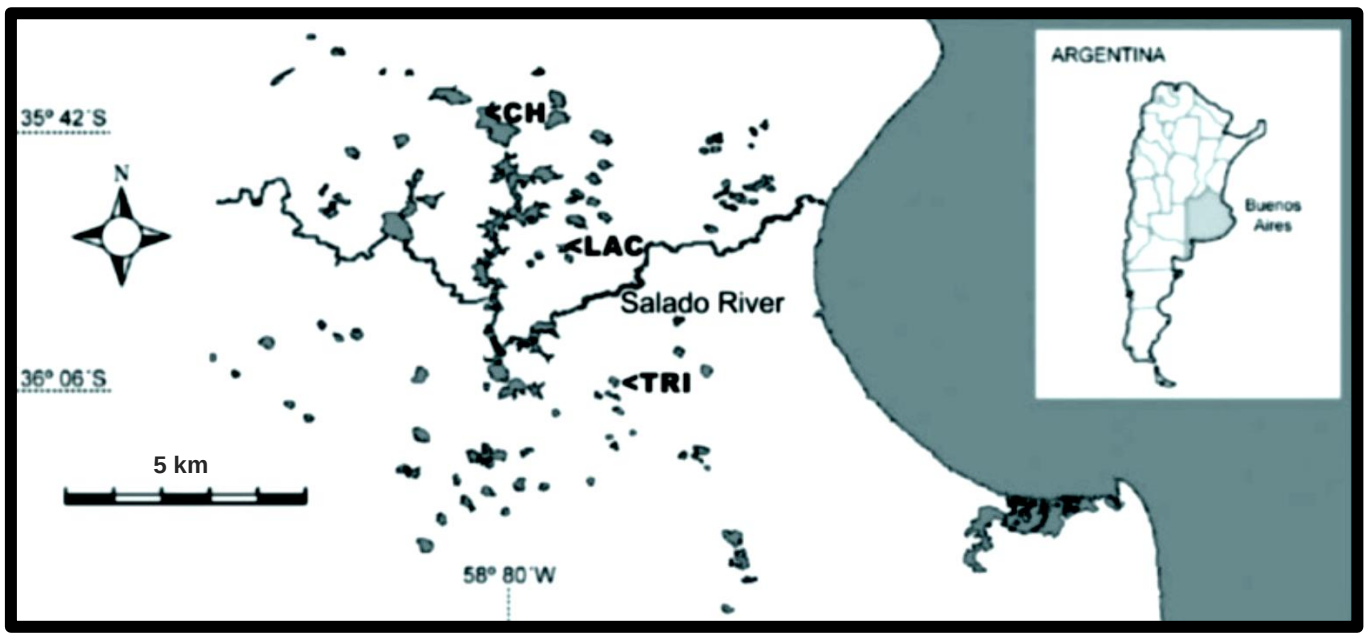

Figure S1: Location of the three lakes seleted. CH: Turbid Chascomús; LAC: alternating Lacombe and TRI: clear-vegetated El Triunfo

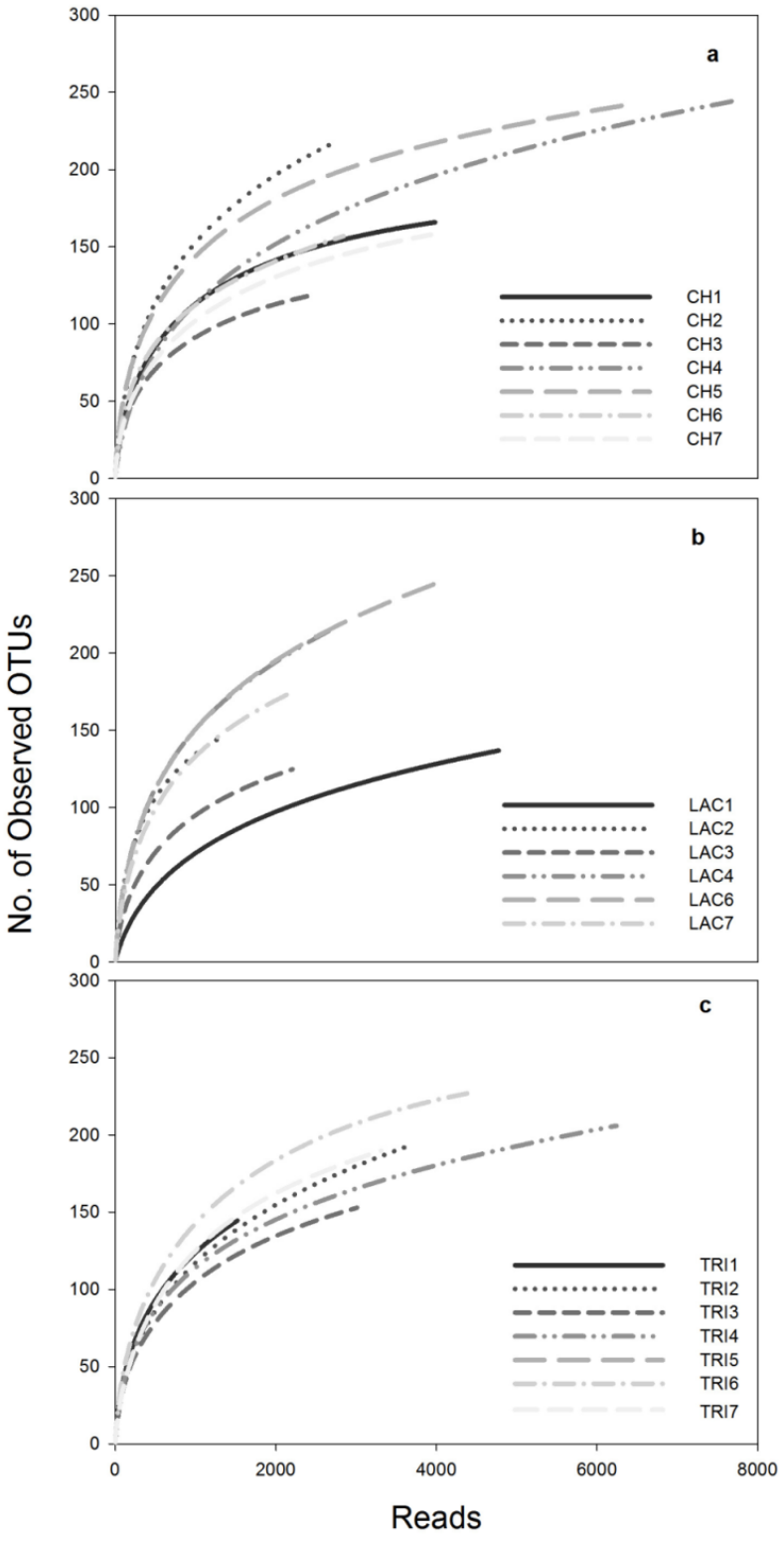

Figure S2: Rarefaction curves for the different samples comprising each system.

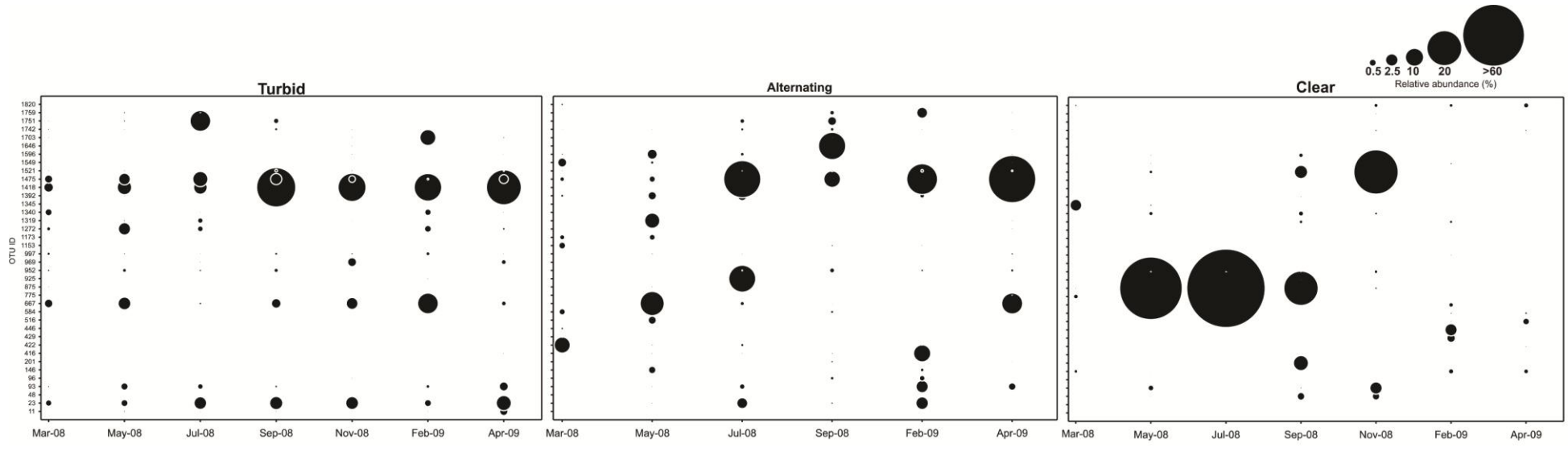

Figure S3: Temporal variation of core OTUs in each lake. Bubble areas are proportional to total relative abundance of the indicated OTU during each sampling date, in each lake.

Table S1: Geographical coordinates and mean (± S.D.) values for physical and chemical characteristics of the three lakes.

Alternative state	**Turbid**	**Alternating**	**Clear**
	Chascomús (CH)	**Lacombe (LAC)**	**El Triunfo (TRI)**
Location	35° 36'S 58° 02'W	35° 49'S 57° 49'W	35° 51'S 57° 52'W
Surface area (km^2)	28.7	1.6	1.5
Temperature (°C)	18.4 (± 4.1)	20.8 (± 4.4)	18.1 (± 3.6)
Conductivity (µS cm^{-1})	2600 (± 400)	3300 (± 1100)	1590 (± 260)
pH	9.1 (± 0.1)	9.0 (± 0.4)	9.3 (± 0.3)
Dissolved Oxigen (mg l^{-1})	8.8 (± 0.8)	8.0 (± 1.7)	9.5 (± 1.8)
Alkalinity (µeq l^{-1})	10665 (± 3305)	19940 (± 9499)	9218 (± 3106)
Secchi (cm)	8.7 (± 2.4)	31.3 (± 12.3)	> 100
Turbidity (NTU)	162.0 (± 79.7)	19.3 (± 13.7)	3.2 (± 2.2)
K_d PAR (m^{-1})	20.2 (± 6.5)	7.1 (± 2.3)	4.6 (± 1.7)
Seston (mg l^{-1})	223.8 (± 107.0)	45.5 (± 24.0)	5.2 (± 3.7)
% Organic Matter in seston	40.7 (± 5.6)	66.1 (± 25.9)	89.0 (± 11.8)
Total Nitrogen (µg l^{-1})	3928 (± 1980)	3791 (± 2210)	3126 (± 1690)
Total Phosphorous (µg l^{-1})	714 (± 209)	289 (± 80)	95 (± 23)
CDOM absorption at 440 nm (m^1)	1.22 (± 0.27)	2.24 (± 0.47)	1.53 (± 0.33)
Ratio 250:365 nm	10.64 (± 0.51)	7.93 (± 2.72)	9.94 (± 0.49)
Chlorophyll-a (µg l^{-1})	322.0 (± 198.0)	91.8 (± 49.3)	19.5 (± 18.9)

METHODS

DNA extraction and pyrosequencing analysis

Samples for DNA were pre-filtered sequentially through a 50 µm and then through a 20 µm- nylon mesh to remove larger protists, large particles and particle-associated bacteria. On average, between 100 ml (turbid system) and 1000 ml (clear system) of sample were filtered (*i.e.* until filter colmatation) with a vacuum pump, first through a 3 µm pore-size polycarbonate filter and then, through a 0.2 µm pore-size polycarbonate filter (Millipore). During the sampling period, mean bacterial concentration ranged between 10^5 cells ml^{-1} in the clear lake (TRI) to 10^7 -10^8 cells ml^{-1} in the turbid lake (CH) (data not shown). Filters were placed into separate sterile eppendorffs, and stored at -80 °C until nucleic acid extraction was performed.

Only DNA from material retained in the 0.2 µm pore-size filters was extracted and analyzed. Filters were extracted using a CTAB protocol (Fernández Zenoff *et al* 2006). Briefly, warm CTAB lysis buffer (2% CTAB; 1.4M NaCl, 100 mM Tris-Cl pH 8, 20 mM EDTA pH 8) was added to the filters and were incubated at 60 °C for 30 min. After incubation, two purification steps were performed adding 0.7 ml of chloroform-isoamilic alcohol (24:1) and centrifugations at 14000 rpm for 10 min. After that, DNA was precipitated in cold isopropanol and then centrifuged at 14000 rpm for 30 min. Finally, a washing step of the pellet using cold ethanol (80%) was performed and air-dried extracted DNA was resuspended in 40 µl of TE buffer. Nucleic acid extracts were stored at -80°C until analysis.

A total of 20 DNA samples were submitted to INDEAR (Rosario, Santa Fe, Argentina) for tag-pyrosequencing. The amplicons were sequenced using 454 GS FLX (Roche-454 Life Sciences, 454 Life Sciences, Branford, CT, USA) with Titanium chemistry. Samples were amplified with modified primers for region V4 of 16S rRNA (*i.e.* F515-R806) (Caporasso *et al*. 2011) to achieve the typical sequence length range for the 454 FLX chemistry used in this study.

Data analysis

Pyrosequencing data generated from the 454-sequencing runs were processed using QIIME (Caporaso et al., 2010). Briefly, quality sequences were binned into phylotypes (≥ 97% similarity) using UCLUST (Edgar, 2010) and grouped by samples according to their unique barcode. A representative sequence of each phylotype was chosen and the phylogenetic relationships of the 16S sequences obtained were determined by a BLAST search within the Ribosomal Database Project (RDP) database (http://rdp.cme.msu.edu/). Representative sequences were then aligned using PyNAST (Caporaso et al., *op. cit.*) with a phylogenetic tree constructed using the maximum likelihood algorithm implemented in FastTree (Price et al., 2009).

Singletons were removed from the OTU table obtained and a randomly subsample OTU table was generated by subsampling down to the lowest number of reads in order to correct for possible biases introduced by unequal sequencing efforts (normalized OTU table). This normalization was performed in the *R* environment (www.r-project.org) using the package Phyloseq (Mac Murdie & Holmes 2013) and the resulting normalized matrix was used for further analyses.

OTU ID	Domain	Phylum	Class	Order	Family	Confidence (%)	Indicator Values			Regime
							Specificity (A)	Fidelity (B)	*p* value	
11	Bacteria	Verrucomicrobia	Methylacidiphilae	Methylacidiphilales	LD19	100	1.000	1.000	0.001	**T**
23	Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales		92	0.703	1.000	0.005	**T**
48	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	100	1.000	0.571	0.016	**C**
93	Bacteria	Verrucomicrobia	Spartobacteria	Spartobacteriales	Spartobacteriaceae	98				
96	Bacteria	Proteobacteria				100				
146	Bacteria	Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	100				
201	Bacteria	Verrucomicrobia	Spartobacteria	Spartobacteriales	Spartobacteriaceae	99				
416	Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales		99	0.969	0.833	0.019	**A**
422	Bacteria					97	0.975	0.667	0.008	**A**
429	Bacteria	OP3	PBS-25			100				
446	Bacteria	Proteobacteria	Betaproteobacteria			100				
516	Bacteria					100				
584	Bacteria					100				
667	Bacteria	Verrucomicrobia	Spartobacteria	Spartobacteriales	Spartobacteriaceae	97				
775	Bacteria					85	1.000	0.714	0.002	**C**
875	Bacteria	Verrucomicrobia	Opitutae	Puniceicoccales	Puniceicoccaceae	100	0.990	0.714	0.024	**C**
925	Bacteria	Proteobacteria				100				
952	Bacteria	Planctomycetes	Planctomycea	Pirellulales	Pirellulaceae	100				
969	Bacteria	ZB2				99	1.000	0.857	0.001	**T**
997	Bacteria	Verrucomicrobia	Spartobacteria	Spartobacteriales	Spartobacteriaceae	97	0.706	1.000	0.002	**T**
1153	Bacteria					99	1.000	0.833	0.001	**A**
1173	Bacteria	Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	100				
1272	Bacteria	Acidobacteria	Chloracidobacteria			93	0.973	0.857	0.001	**T**
1319	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	99				
1340	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	100				
1345	Bacteria	Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	94				
1392	Bacteria	Verrucomicrobia	Opitutae	Puniceicoccales		100	0.880	0.833	0.008	**A**
1418	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales		98	0.998	1.000	0.001	**T**
1475	Bacteria	Verrucomicrobia	Spartobacteria	Spartobacteriales	Spartobacteriaceae	96	0.699	1.000	0.006	**A**
1521	Bacteria	Verrucomicrobia	Spartobacteria	Spartobacteriales		100				
1549	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	99				
1596	Bacteria	Spirochaetes	Leptospirae	Leptospirales		100				
1646	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales		100				
1703	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales		98	0.929	0.571	0.042	**T**
1742	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	97				
1751	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales		100				
1759	Bacteria	Planctomycetes	Planctomycea	Pirellulales	Pirellulaceae	94				
1820	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales		100	0.843	1.000	0.002	**C**

Table S2: Phylogenetic assignment and sequence distribution of 'core' OTUs. Taxonomic assignments are the finest level that passed the Ribosomal Database Project classifier's (80% confidence threshold). Grey lines corresponds to indicator OTUs of each type of regime (*i.e.* turbid (T), alternating (A) and clear (C) system) based on OTU fidelity and relative abundance.

Table S3: Values estimated for the standardized effect size of the unweighted mean pairwise distance (sesMPD) and the unweighted mean nearest taxon distance (sesMNTD) for the 16S rDNA sequences during the studied period for the three lakes.

	Turbid (CH)				Alternating (LAC)				Clear (TRI)			
Sampling date	**sesMPD**	***p* value**	**sesMNTD**	***p* value**	**sesMPD**	***p* value**	**sesMNTD**	***p* value**	**sesMPD**	***p* value**	**sesMNTD**	***p* value**
March-08	-2.3	0.016	-3.8	0.001	-1.9	0.028	-2.2	0.014	1.4	0.913	-1.3	0.107
May-08	-2.2	0.016	-4.1	0.001	-1.7	0.053	-3.3	0.001	1.5	0.932	-3.5	0.001
July-08	-1.9	0.035	-3.4	0.001	-1.6	0.061	-2.8	0.004	0.8	0.781	-2.3	0.012
September-08	-1.2	0.114	-2.4	0.009	-0.9	0.195	-2.0	0.013	0.6	0.722	-2.5	0.005
November-08	-0.8	0.214	-3.0	0.003	N.A.	N.A.	N.A.	N.A.	-0.3	0.371	-1.6	0.05
February-09	-1.3	0.100	-0.6	0.272	-2.0	0.022	-2.1	0.018	-1.4	0.088	-1.7	0.049
April-09	-1.2	0.119	-1.6	0.057	-1.8	0.033	-2.4	0.005	1.7	0.964	-0.6	0.279

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