



Planktonic ciliate community driven by environmental variables and cyanobacterial blooms: A 9-year study in two subtropical reservoirs

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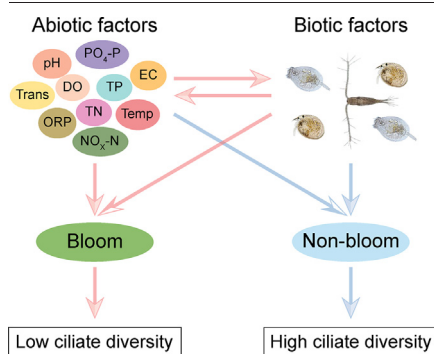
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HIGHLIGHTS

- Inter-annual variability was stronger than seasonality in ciliate communities.
- Ciliate community structure was impacted by cyanobacterial blooms.
- Ciliate community assembly was mainly controlled by stochastic processes.
- Cyanobacterial bloom increased selection pressure on ciliate community.
- Environment affected directly, but cyanobacteria indirectly shaped the ciliate community.

GRAPHICAL ABSTRACT



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ABSTRACT

It is well-established that environmental variability and cyanobacterial blooms have major effects on the assembly and functioning of bacterial communities in both marine and freshwater habitats. It remains unclear, however, how the ciliate community responds to such changes over the long-term, particularly in subtropical lake and reservoir ecosystems. We analysed 9-year planktonic ciliate data series from the surface water of two subtropical reservoirs to elucidate the role of cyanobacterial bloom and environmental variabilities on the ciliate temporal dynamics. We identified five distinct periods of cyanobacterial succession in both reservoirs. Using multiple time-scale analyses, we found that the interannual variability of ciliate communities was more strongly related to cyanobacterial blooms than to other environmental variables or to seasonality. Moreover, the percentage of species turnover across cyanobacterial bloom and non-bloom periods increased significantly with time over the 9-year period. Phylogenetic analyses further indicated that 84 %–86 % of ciliate community turnover was governed by stochastic dispersal limitation or undominated processes, suggesting that the ciliate communities in subtropical reservoirs were mainly controlled by neutral processes. However, short-term blooms increased the selection pressure and drove 30 %–53 % of the ciliate community turnover.

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We found that the ciliate community composition was influenced by environmental conditions with nutrients, cyanobacterial biomass and microzooplankton having direct and/or indirect significant effects on the ciliate taxonomic or functional community dynamics. Our results provide new insights into the long-term temporal dynamics of planktonic ciliate communities under cyanobacterial bloom disturbance.

1. Introduction

Cyanobacterial blooms are a recurring phenomenon in many aquatic environments worldwide, reflecting high human-induced nutrient loading (Plass and Paerl, 2021; Wang et al., 2020a; Huisman et al., 2018). Some cyanobacterial species produce cyanotoxins, which can negatively impact a wide range of co-occurring species and reduce water quality (Tan et al., 2021; Liu et al., 2019; Xue et al., 2018; Rabalais et al., 2010). They also have indirect negative impacts on aquatic organisms, for example, by decreasing light penetration during blooms and by causing oxygen depletion (Plass and Paerl, 2021; Huisman et al., 2018; Rabalais et al., 2010). Thus, cyanobacterial blooms can significantly decrease aquatic ecosystem functions and services (Tan et al., 2021; Wang et al., 2020b). Different planktonic organisms may respond differently to cyanobacterial bloom-induced environmental and ecological changes (Kosiba and Krzton, 2022; Tirjakova et al., 2016). For example, zooplankton may become food limited (Napiorkowska-Krzebietke et al., 2021; Kosiba et al., 2018; Moustaka-Gouni et al., 2006), while some heterotrophic bacteria can benefit from cyanobacterial blooms (Shao et al., 2014; Ploug et al., 2011). Further, some microeukaryotes (protists and zooplankton) may play key functional roles in the processing of cyanobacterial blooms (Gao et al., 2022; Liu et al., 2019; Xue et al., 2018) and specific communities are strongly correlated with cyanobacterial blooms (Napiorkowska-Krzebietke et al., 2021; Liu et al., 2019; Kosiba et al., 2018). Of these microeukaryotes, ciliates perhaps exhibit the widest variety of ecological strategies, from free-living to symbionts, and they have a wide range of optimal prey types (Canals et al., 2020; Simek et al., 2019; Adl et al., 2019; Agatha, 2011). They are, therefore, a relevant group to focus on in relation to cyanobacterial blooms.

Despite comprehensive evidence that ciliates are inherently integrated and may control bottom-up interactions in the aquatic food webs (Scheuerl and Kaitala, 2021; Ger et al., 2016, 2019; Boyer et al., 2011) and predate on bacteria, cyanobacteria, microalgae and nano-flagellates (Meira et al., 2021; Weisse, 2017; Wang et al., 2017), their community compositional and functional responses to cyanobacterial bloom-induced environmental and ecological changes have only recently been addressed (Weisse and Montagnes, 2022; Qu et al., 2021; Haraguchi et al., 2018; Andrushchyshyn et al., 2006). It is known that a short-term cyanobacterial bloom increases the environmental selection and decreases the stochasticity of bacterioplankton (Wang et al., 2020a; Woodhouse et al., 2016). However, we do not know to what extent interannual variability in environmental conditions and recurring short to long-term cyanobacterial blooms alters the relative importance of stochastic and deterministic processes for ciliate community composition. Recent work has shown that taxonomic and functional turnover in ecological communities have great significance to elucidate community changes in heterogeneous environments (Graco-Roza et al., 2022), such as pollution and biological disturbances (i.e., algal bloom and invasion).

Time-series data are valuable for disentangling how stochastic and deterministic processes drive the compositional changes of ciliate communities. For example, short-term studies have provided evidence of seasonal recurrence and succession of ciliates (Pitsch et al., 2019; Sommer et al., 2012), while only weak patterns of community changes appeared in reaction to disturbance events (i.e., algal bloom) (Martin-Platero et al., 2018; Simon et al., 2015; Mueller et al., 1991). Time series-based sampling has shown that, although the abundance of given species may fluctuate substantially, the plankton communities change according to environmental fluctuations (Martin-Platero et al., 2018). The community composition of plankton and ecological factors determining their community structure are

known (Lima-Mendez et al., 2015), while there is no clear evidence for how interannual ciliate community changes in relation to cyanobacterial blooms.

In this study, we analysed a 9-year time series of data (2010–2018) collected from subtropical Shidou and Bantou reservoirs in Xiamen, southeast China (Fig. S1) to elucidate the planktonic ciliate community dynamics and its responses to short- to long-term dominance of cyanobacteria along with reservoir multi-annual environmental variability. We aimed to investigate: (1) the variability of planktonic ciliate communities in subtropical reservoirs over the 9-year period; (2) how the ciliate taxonomic and functional communities change with time-related changes in cyanobacterial blooms, and (3) how the intensity of cyanobacterial blooms changes the relative importance of deterministic and stochastic processes in shaping ciliate community dynamics.

2. Materials and methods

2.1. Study area

The sampling sites were located in two reservoirs, Shidou Reservoir and Bantou Reservoir in Xiamen, subtropical China (Liu et al., 2019; Yang et al., 2017). Xiamen has a subtropical monsoon climate, characterized by long, humid and hot summers and short, dry, and mild winters (Fig. S1). The annual mean temperature is 20.7 °C, and the annual mean precipitation is 1335.8 mm (Xiamen meteorological department). Shidou Reservoir is a large drinking water reservoir, having a total water storage capacity of 61.4 million m³ with a mean water depth of 13.9 m (25.8 m maximum depth), while Bantou Reservoir is a smaller and shallower reservoir, having a total water storage capacity of 4.4 million m³ with a mean water depth of 7.2 m (12.4 m maximum depth) (Yang et al., 2017). The Bantou Reservoir is located downstream of the Shidou Reservoir, and water flows into Bantou Reservoir through an openable switch gate when the water level is high enough in the Shidou Reservoir (generally in the rainy season). The hydrological conditions in the reservoirs are tightly linked to the surrounding forested landscape and periodic rainfall events (Liu et al., 2019; Yang et al., 2017). Rainfall changes the water levels, temperature, pH and dissolved oxygen concentrations, and the dissolved nutrient levels may rise several times higher than the normal state (Shabarova et al., 2021; Yang et al., 2017).

2.2. Water sample collection

Water samples (20 L) were collected from the surface waters (0.5 m depth, near the inflow, near the outflow, and the middle of reservoir) in each reservoir using water sampler (5 L polycarbonate bottle). Only surface water was sampled because we focus on the effects of cyanobacteria blooms that typically occur here. In total, 252 samples (126 samples from each reservoir with three replicates) were collected monthly from May 2010 to April 2011 (36 samples from each reservoir, a total of 72 samples) and in each season (spring, summer, autumn and winter from 2011 to 2018, 90 samples from each reservoir, a total of 180 samples) over the 9-year period. The water samples were pre-filtered through a 200 µm sieve to remove large particles/debris and macro- to mesoplankton. After that, plankton were filtered using 0.2 µm pore-size polycarbonate membrane (47 mm diameter, Millipore, Billerica, MA, USA) from 500 to 1000 mL of water following our previous study (Liu et al., 2019). The filtered membranes were immediately stored at −80 °C.

2.3. DNA extraction, PCR amplification and bioinformatics

The planktonic DNA was extracted directly from the membrane using FastDNA SPIN Kit and the Instrument (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The hypervariable V9 region of the microeukaryotic 18S rRNA gene was amplified using the universal primer pair 1380F and 1510R (Amaral-Zettler et al., 2009) with attached Illumina (San Diego, CA, USA) adapters. Amplification of each sample was performed in triplicate reactions using 15 µL of Phusion HighFidelity PCR Master Mix (New England Biolabs, Beverly, MA, USA) with 0.4 µM of primers (0.2 µM of each primer) and 10 ng of target DNA. The amplification conditions included an initial denaturation at 98 °C for 1 min, followed by 30 cycles of 10 s at 98 °C, 30 s at 50 °C and 60 s at 72 °C. At the end of the amplification, the amplicons were subjected to final 10 min extension at 72 °C. PCR products from triplicate reactions per sample were pooled and gel purified. The purified PCR products were pooled in equal quantity and then paired-end sequenced (2 × 150 bp) on an Illumina HiSeq platform (Illumina Inc., San Diego, CA, USA) (Liu et al., 2017).

The raw sequence data were assembled using Mothur v.1.39.5 (Schloss et al., 2009) and further processed with Usearch v.10 (Edgar, 2010). Singletons and chimeras were discarded using default settings in the Usearch (with cluster algorithm). Quality-filtered reads were assigned to zero-radius OTUs at a 97 % sequence similarity threshold, and representative sequences from each OTU were identified by the Protist Ribosomal Reference (PR2) database using RDP Naive Bayesian Classifier (Guillou et al., 2013). Unassigned (sequence similarity to a reference sequence was <80 %) OTUs were removed before the downstream analyses. We found 12,428 microeukaryotic OTUs (8.2 % OTUs and 5.6 % sequences of ciliate in Shidou, whereas 7.7 % OTUs and 6.1 % sequences in Bantou) after normalization (29,062 reads/sample). We normalized the ciliate sequence number to 999 for each of 252 samples. Of these, 685 and 674 OTUs were retrieved in Shidou and Bantou reservoirs, respectively (Fig. S2).

2.4. Physicochemical and nutrient variables

Water transparency (Trans) was measured with a Secchi disk. Water temperature (WT), electrical conductivity (EC), pH and dissolved oxygen (DO) were measured *in situ* using a Hydrolab DS5 multi-parameter water quality analyzer (Hach, Loveland, CO, USA). Total nitrogen (TN), ammonium nitrogen (NH₄-N), nitrite and nitrate nitrogen (NO_x-N), phosphate phosphorus (PO₄-P) and total phosphorus (TP) were analysed according to standard methods (Greenberg et al., 1992).

2.5. Phytoplankton analysis

A total of 2.5 L of water was obtained from each sample and fixed *in situ* with 1.5 % Lugol's iodine solution (Yang et al., 2017). To enumerate phytoplankton, 50 mL subsamples of the Lugol's fixed samples were concentrated according to Zhang and Huang (1991). For each sample at least 500 phytoplankton individuals were identified and counted using an inverted microscope (Motic A-31, Xiamen, China) following taxonomic references (Hu and Wei, 2006; Zhang and Huang, 1991; Shen et al., 1990). The abundance of each phytoplankton species was first transformed to biovolume and then to biomass following Hillebrand et al. (1999).

2.6. Cyanobacterial blooms

A cyanobacterial bloom was considered when the biovolume exceeded a threshold value of 10 mm³/L according to the Water Quality Research Australia framework (Woodhouse et al., 2016).

In this study, *Raphidiopsis raciborskii* showed massive cyanobacterial blooms in Shidou and Bantou reservoirs (Tan et al., 2021). A particularly long-term continuous bloom occurred from May 2010 to April 2011 (BP1: bloom period 1) in both reservoirs. The intensity of other cyanobacterial blooms differed between the two reservoirs. The reservoir-specific bloom periods were winter 2014, from spring to winter 2015 (BP2: bloom period

2) and from spring to autumn 2018 (BP3: bloom period 3) in Shidou Reservoir. Frequent cyanobacterial blooms were observed in Bantou Reservoir, particularly in autumn 2014, summer 2015 and autumn 2015 (BP2: bloom period 2) and autumn 2018 (BP3: bloom period 3).

2.7. Functional attributes of ciliates

The feeding categories (proxies for ecological role) of ciliates were assigned to the OTUs (or species) (Majaneva et al., 2022; Adl et al., 2019; Shen et al., 1990; Lynn, 2008; Foissner and Beger, 1996). The categories were based on Adl et al. (2019) and included algivorous (A), bacterivorous (B), commensal-bacterivorous (B*), parasitic-histophagous-bacterivorous (BP*), cytotoxic (C), fungi, cyanobacteria and filamentous-algae feeding (F), omnivorous (O), osmotrophic (OC), predatory/raptorial (R), saprotrophic (S) and parasitic (X) species as well as species with unknown functions.

2.8. Data analyses

Canonical analysis of principal coordinate followed by discriminant analysis based on Bray-Curtis dissimilarity of the ciliate community composition was conducted with 9999 permutations in PRIMER v.7.0.21 (Clarke and Gorley, 2015; Anderson et al., 2008). In the analysis, the program determined the appropriate number of dimensions (m) with the lowest misclassification error (%) to be included in the principal coordinate and discriminant analysis. The significant differences between samples were tested by analysis of similarity (ANOSIM) with 999 permutations using the PRIMER. To investigate the percentage of variation in ciliate community composition between and within periods, a permutational multivariate analysis of variance (PERMANOVA) with Monte Carlo simulation (9999 permutations) was performed using Bray-Curtis dissimilarity of the relative abundance of OTUs in PRIMER 7.0.21 + PERMANOVA (Clarke and Gorley, 2015; Anderson et al., 2008).

Beta-diversity (Bray-Curtis dissimilarity) was partitioned into two components, the abundance gradient (richness) and the balanced variation (turnover) using the "bray.part" function of the "betapart" R package (Baselga et al., 2022) to reveal temporal patterns of the ciliate taxonomic and functional communities, respectively. Further, a time-lag regression analysis (Collins et al., 2000) was used to quantify the contributions of turnover dissimilarity components, functional composition and richness variation over time.

To understand the mechanisms underlying the ciliate community assembly and the contributions of deterministic and stochastic processes, the phylogenetic bins-based null-model (NM) (Stegen et al., 2012) and Sloan neutral community model (NCM) were used (Sloan et al., 2006). These analyses were conducted in R packages "iCAMP", "Hmisc", "minpack.lm" and "stats4" (R Core Team, 2020). Applying the NCM approach, the contributions of neutral processes can generate the best fit distribution curve (with least-squares method), which predicts the relationship between the occurrence frequency of the individual OTUs and their mean relative abundance in the metacommunity (Sloan et al., 2006). Random immigration, births and deaths are assumed to determine the relative abundance of OTUs in a metacommunity. The *N* in the NCM model describes the metacommunity size, and *m* estimates the immigration rate that can be interpreted as a measure of dispersal limitation (Zhou and Ning, 2017; Sloan et al., 2006).

The null model approach uses phylogenetic trees to estimate the process of each pairwise turnover for randomized OTUs (Stegen et al., 2012). The phylogenetic signal occurs when more closely related OTUs are more ecologically similar and quantitatively estimates the percentage of compositional turnover linked to deterministic and stochastic processes (Zhou and Ning, 2017; Stegen et al., 2012). A phylogenetic tree of ciliates was constructed in Qiime2 (Bolyen et al., 2019). The pairwise phylogenetic turnover between communities was calculated as the mean nearest taxon distance metric (βMNTD). The β-nearest taxon index (βNTI) is the difference between observed βMNTD and the mean of the null distribution of βMNTD normalized with its standard deviation. Four major ecological

processes were quantified: (1) β NTI value >2 and <-2 indicated that communities were driven by heterogeneous selection and homogeneous selection, respectively; (2) the relative contribution of dispersal limitation was calculated as the percentage of pairwise comparisons with $|\beta\text{NTI}| < 2$ and $\text{RC}_{\text{bray}} > 0.95$; (3) the relative contribution of homogenizing dispersal was assessed as the percentage of pairwise comparisons with $|\beta\text{NTI}| < 2$ and $\text{RC}_{\text{bray}} < -0.95$; and (4) the ecological drift (undominated fraction) was calculated as the percentage of pairwise comparisons with $|\beta\text{NTI}| < 2$ and $|\text{RC}_{\text{bray}}| < 0.95$ (Isabwe et al., 2022).

Random Forest (RF) analysis was used to explore significant predictors for long-term ciliate community dynamics and across cyanobacterial bloom and non-bloom periods, respectively. This analysis was conducted in the “randomForest” and “rfPermute” packages in R (Archer, 2022; Liaw and Wiener, 2002). The number of trees was 5000 within 9999 permutations. A high percentage of mean sum square (%IncMES) of a given predictor indicates high contribution of that predictor to the community dynamics. Further, to investigate the grazing impacts on ciliate community dynamics, the relative abundances (sequences) of four microzooplankton groups (Branchiopoda, Gastrotricha, Maxillopoda and Rotifera) were included in the RF analysis. Their abundances were incorporated from normalized total 18S rRNA gene sequence data. Moreover, the relative importance of physicochemical variables, cyanobacterial biomass, nutrients and microzooplankton were partitioned by variation partitioning analysis (VPA) using the “vegan” and “fmsb” packages in R (Nakazawa, 2022; Oksanen et al., 2020). Finally, the direct and indirect effects of biotic (cyanobacteria and microzooplankton) and abiotic environmental conditions (physicochemical and nutrients) on ciliate community dynamics were assessed by partial least square path model (PLS-PM) using the “plsmpm” package in R (Sanchez, 2013). Environmental data were $\log(x + 1)$ transformed, with the exception of pH, and normalized before analysis (Clarke and Gorley, 2015; Anderson et al., 2008).

3. Results

3.1. Temporal dynamics of cyanobacterial blooms

Time series analysis revealed five distinct cyanobacterial periods and each period was distinct in term of cyanobacterial biomass ($P < 0.05$). The mean cyanobacterial biomass of these five periods was recorded as 1739.60 mg/L, 0.66 mg/L, 42.30 mg/L, 0.88 mg/L and 26.56 mg/L during BP1, NBP1, BP2, NBP2 and BP3, respectively, in Shidou Reservoir, while it was 58.45 mg/L, 1.22 mg/L, 32.70 mg/L, 1.44 mg/L and 22.40 mg/L, respectively, in Bantou Reservoir (Fig. 1a). The contribution of cyanobacterial biomass to total phytoplankton biomass was 99 %, 6.4 %, 86.7 %, 13.8 % and 87.5 % during BP1, NBP1, BP2, NBP2 and BP3 in Shidou Reservoir, and 85.1 %, 14.1 %, 77.3 %, 24.2 % and 86.6 % in Bantou Reservoir, respectively. In the smaller reservoir (Bantou), the same periods were observed with the exception that the second bloom period was not continuous but interrupted for a short time by a cyanobacterial biovolume given a threshold value of 10 mm³/L. In addition, a short-term bloom was observed in Bantou Reservoir in autumn 2012 within the first non-bloom period (Fig. 1a).

3.2. Temporal dynamics of ciliate community

The planktonic ciliate community composition exhibited a stronger response to cyanobacterial blooms than seasonality in both reservoirs revealed by ANOSIM (Table 1) and CAP analyses (Fig. S3a, b). The permutational multivariate analysis of variance (PERMANOVA) and following pairwise comparisons among the periods revealed that the greater differences occurred between BP1 (bloom period 1) and BP3, BP1 and NBP2 (non-bloom period 2), BP2 and BP3, NBP1 and BP3 as well as between NBP1 and NBP2, showing 64.9 %, 61.6 %, 62.0 %, 62.7 % and 57.7 % community compositional variation in Shidou Reservoir, and 59.1 %, 57.9 %, 57.0 %, 57.8 % and 55.7 % variation in Bantou Reservoir, respectively (Table 2). Among the ciliate communities, Colpodea (47 OTUs),

Heterotrichea (22 OTUs), Litostomatea (184 OTUs), Oligohymenophorea (143 OTUs), Plagiopylea (13 OTUs), Prostomatea (52 OTUs) and Spirotrichea (211 OTUs) were dominant over the 9-year study period and exhibited marked interannual variability in terms of relative abundance and function (Fig. 1b, c). Therefore, our 9-year time series analysis revealed that the community composition and richness of planktonic ciliates underwent pronounced changes following interannual pattern corresponding to cyanobacterial succession and that this pattern was overall stronger than the seasonal pattern.

The changes in OTU richness and Shannon-Wiener diversity were strongly related to the cyanobacterial succession than the seasonality in Shidou Reservoir, while seasonality was found to be stronger for OTU richness in Bantou Reservoir (Fig. 2). Further, time-related changes in taxonomic beta-diversity accounted for 83.8 % and 87.8 % of the total variability in ciliate community composition in Shidou and Bantou reservoirs, respectively, and 82.4 % and 67.3 % of the functional variability, respectively (Fig. 3a, c). Time accounted for 23.9 % of the variation in OTU richness in Shidou Reservoir and for 14.6 % in Bantou Reservoir (Fig. 3a, c), indicating that large-scale species turnover/replacements occurred over the 9-year period. The time related changes of environmental factors, cyanobacterial biomass and microzooplankton abundance were 4.5 %, 2.7 % and 0.3 % in Shidou Reservoir, and 5.4 %, 0.4 % and 6.6 % in Bantou Reservoir, respectively (Fig. 3b, d), suggesting that water ecological conditions have significant influence on directional change of the ciliate community composition and richness in both reservoirs.

3.3. The ciliate community assembly

The relationship between distribution and the relative abundance of planktonic ciliate OTUs was well fitted to the Sloan neutral community model (Fig. 4a, b), indicating that ciliate community assembly was mainly driven by neutral processes. The NCM model explained 81.6 % and 80.3 % of the overall community variation of planktonic ciliates in Shidou and Bantou reservoirs, respectively. Further, the NCM model roughly explained 76.4 % and 78.5 % of ciliate community variations for bloom samples from Shidou and Bantou reservoirs, respectively, and 80.6 % and 78.9 % for non-bloom samples, respectively (Fig. 4a). The metacommunity size (N_m) determined by dispersal/or drift was found to be higher in non-bloom than in bloom periods in Shidou Reservoir ($N_m = 110$ for bloom and 127 for non-bloom), but higher for the bloom periods than non-bloom periods in Bantou Reservoir ($N_m = 138$ for bloom and 108 for non-bloom) (Fig. 4a).

The percentage of turnover in the ciliate community assembly was governed primarily by stochastic processes (dispersal limitation: 63.8 %, undominated: 19.1 %, homogenizing dispersal: 1.2 %, total stochastic contribution: 84.1 %), while a small portion was explained by deterministic processes (homogeneous selection: 10.8 % and heterogeneous selection: 5.1 %, total deterministic contribution: 15.9 %) in Shidou Reservoir (Fig. 4c). Accordingly, dispersal limitation (65.8 %), undominated processes (19.3 %), homogenizing dispersal (1.1 %), homogeneous selection (8.8 %) and heterogeneous selection (5 %) (total stochastic contribution was 86.2 % and total deterministic processes contribution was only 13.8 %) drove ciliate community assembly in Bantou Reservoir (Fig. 4c). When considering bloom and non-bloom periods, 81.3 % and 85.6 %, respectively, of turnover in ciliate community assembly was explained by stochastic processes (i.e., dispersal limitation, homogenizing dispersal and undominated processes), while 18.7 % and 14.4 % of variation explained by deterministic processes (i.e., homogeneous selection and heterogeneous selection processes) in Shidou Reservoir, respectively (Fig. 4c). In Bantou, the stochastic processes explained 84.0 % and 85.9 %, whereas deterministic processes explained 16.0 % and 14.1 % of ciliate community turnover in bloom and non-bloom periods, respectively (Fig. 4c).

The influence of the stochastic and deterministic processes on ciliate community assembly showed different patterns with respect to the strong and weak cyanobacterial bloom periods (Fig. 4b). The NCM model accounted for 79.0 %, 89.3 %, 81.8 %, 75.1 % and 61.6 % of community

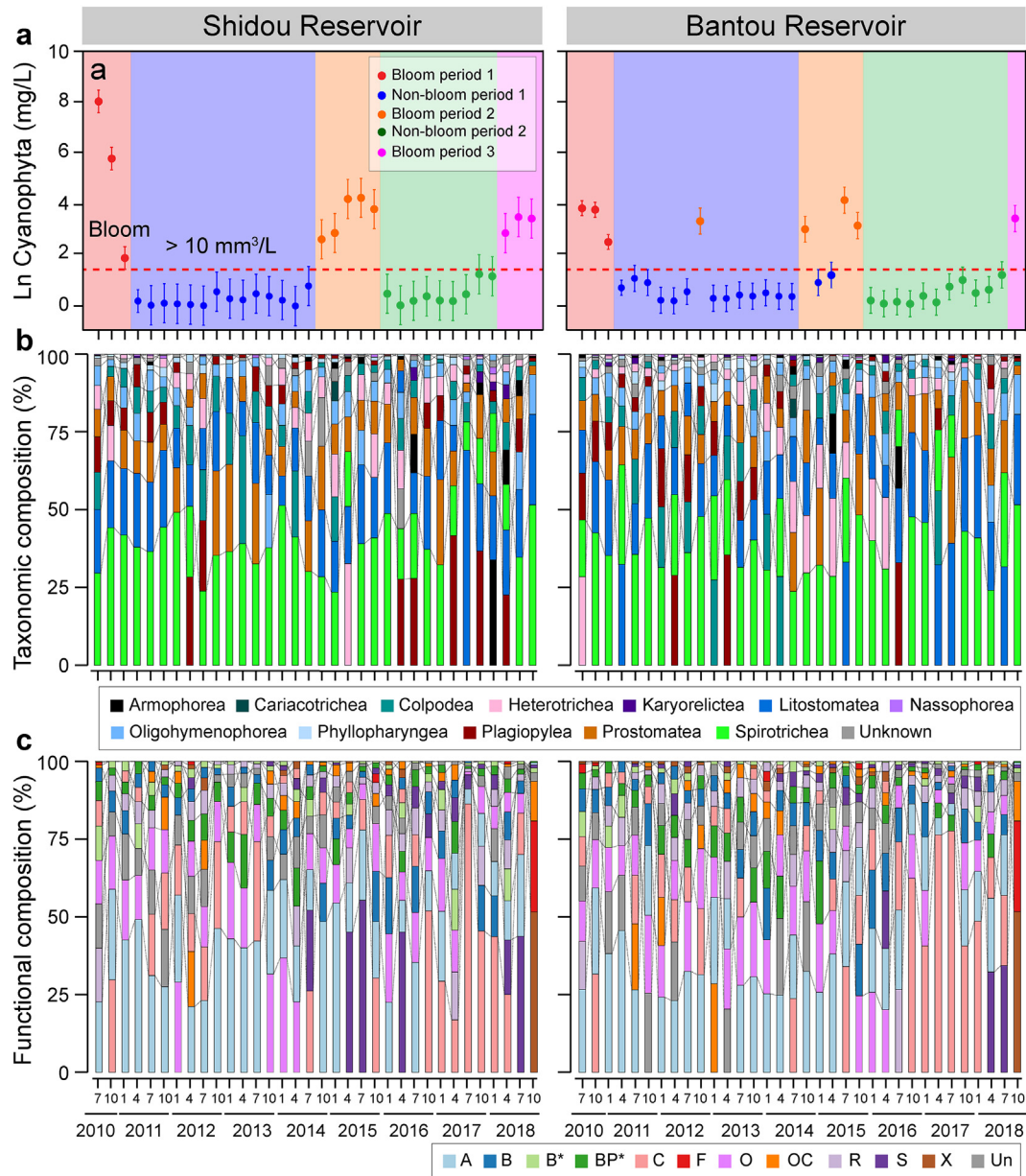


Fig. 1. Nine-year dynamics of cyanobacterial biomass (a), ciliate taxonomic composition (b) and functional composition (c) in two subtropical reservoirs.

Ciliate functional groups: A, algivorous; B, bacterivorous; B*, commensals-bacterivorous; BP*, histophagous-bacterivorous; C, cytotrophic; F, fungi, cyanobacteria and filamentous-algae feeders; O, Omnivores; OC, osmotrophic; R, predators/raptors; S, saprotrophs; Un, unknown function; and X, parasitic. Note that Cyanophyta biomass was $\ln(x + 1)$ transformed in Fig. 1a.

variations for BP1, NBP1, BP2, NBP2 and BP3, respectively, in Shidou Reservoir, whereas the model accounted for 76.8 %, 88.9 %, 47.6 %, 78.2 % and 61.1 % community variations, respectively, in Bantou Reservoir (Fig. 4b). Our results thus revealed stronger stochastic than deterministic

processes in shaping the ciliate community assembly in both reservoirs, but a higher degree of deterministic influence was found during weak bloom period (i.e., BP3) in both reservoirs (Fig. 4c). The contribution of deterministic processes was 5.7 %, 4.0 %, 10.5 %, 25.7 % and 52.8 % for BP1, NBP1, BP2, NBP2 and BP3, respectively, in Shidou Reservoir, whereas it was 18.6 %, 6.7 %, 13.6 %, 23.0 % and 30.0 %, respectively, in Bantou Reservoir (Fig. 4c), indicating an increased selection pressure during weak bloom period in 2018.

Table 1

Summary of the ANOSIM and PERMANOVA (Adonis) results of ciliate taxonomic composition in Shidou and Bantou reservoirs, respectively.

Group	ANOSIM global R		PERMANOVA pseudo-F	
	Shidou	Bantou	Shidou	Bantou
Season (4 seasons)	0.191**	0.164**	5.102**	3.415**
Year (9 years)	0.527**	0.557**	7.853**	7.441**
Inter-annual (5 periods)	0.534**	0.472**	9.052**	7.822**
Bloom vs non-bloom	0.190**	0.124**	6.389**	5.995**

Global R represents degree of separation between groups (** $P < 0.01$).

3.4. The factors responsible for ciliate community dynamics

The interannual variability in environmental variables was more pronounced than seasonal variation in both reservoirs (ANOSIM; interannual variability: $R = 0.306$, $P < 0.01$ and $R = 0.331$, $P < 0.01$ in Shidou and Bantou, respectively, and seasonal variability: $R = 0.232$, $P < 0.01$ and $R = 0.226$, $P < 0.01$ in Shidou and Bantou, respectively). For both reservoirs,

Table 2

The pairwise comparison of the ciliate taxonomic composition in Shidou and Bantou reservoirs, respectively.

Group	ANOSIM global R		PERMANOVA dissimilarity (%)	
	Shidou	Bantou	Shidou	Bantou
BP1 vs NBP1	0.480**	0.279**	53.404**	49.105**
BP1 vs BP2	0.323**	0.191*	52.900**	49.015**
BP1 vs NBP2	0.564**	0.476**	61.552**	57.868**
BP1 vs BP3	0.841**	0.773**	64.870**	59.107**
NBP1 vs BP2	0.431**	0.525**	52.014**	51.203**
NBP1 vs NBP2	0.607**	0.593**	57.722**	55.696**
NBP1 vs BP3	0.866**	0.896**	62.681**	57.768**
BP2 vs NBP2	0.258**	0.345**	56.935**	56.483**
BP2 vs BP3	0.723**	0.934**	62.044**	56.960**
NBP2 vs BP3	0.062	−0.048	55.079**	50.545

Global R represents degree of separation between groups (* $P < 0.05$ and ** $P < 0.01$). Five periods were identified: BP1 indicates the first bloom period (21 samples in Shidou and 21 samples in Bantou during 2010–2011); NBP1 indicates the first non-bloom period (54 samples in Shidou and 51 samples in Bantou during 2011 to 2014); BP2 indicates the second bloom period (15 samples in Shidou and 12 samples in Bantou during 2014–2015); NBP2 indicates the second non-bloom period (27 samples in Shidou and 37 samples in Bantou during 2016–2018); and BP3 indicates the third bloom period (9 samples in Shidou and 5 samples in Bantou during 2018).

random forest (RF) prediction analyses revealed that the most important predictive variables for planktonic ciliate community dynamics were water temperature, transparency, pH, dissolved oxygen, ORP, total nitrogen, $\text{NO}_x\text{-N}$, total phosphorus, $\text{PO}_4\text{-P}$ and cyanobacterial biomass (Table S1). Electrical conductivity, Branchiopoda (including cladocerans) and Maxillopoda (including planktonic copepods) were important predictors in Shidou Reservoir, while $\text{NH}_4\text{-N}$, Gastrotricha and Rotifera were important predictors in Bantou Reservoir (Table S1).

We found the physicochemical variables, nutrients, cyanobacterial biomass and microzooplankton abundance had a significant relationship with the taxonomical and functional beta-diversity of planktonic ciliate communities (Fig. S5). Further, the variance partitioning analysis (VPA) showed

that 38 % and 34 % of the community variation could be explained by these predictive variables in Shidou and Bantou reservoirs, respectively (Fig. 5a). The individual effect of the physicochemical variables, cyanobacterial biomass, nutrients and microzooplankton abundance accounted for 13 %, 12 %, 5 % and 1 % in Shidou Reservoir, and 7 %, 10 %, 5 % and 2 % in Bantou Reservoir, respectively (Fig. 5a). Furthermore, PLS-PM analysis showed that physicochemical variables and nutrients had significant direct effects, while cyanobacterial biomass and microzooplankton had indirect effects on the ciliate communities in Shidou Reservoir (Fig. 5b). In Bantou Reservoir, physicochemical variables, nutrients and microzooplankton had significant direct effects, while cyanobacterial biomass had indirect effects on ciliate community dynamics (Fig. 5b). Placing all our results in a conceptual framework, changes in water ecological conditions promoting cyanobacterial blooms in the reservoirs led to enhanced dispersal and environmental selection of planktonic ciliate communities, showing increased cyanobacterial bloom-dependency of ciliate communities and lower diversity of ciliate during bloom periods than non-bloom periods in these subtropical reservoirs.

4. Discussion

4.1. Ciliate community changes from the bloom to non-bloom periods

We found that the relative abundance of the taxa Colpodea, Heterotricha, Oligohymenophorea and Spriotricha peaked during the bloom period 1 when cyanobacterial biomass was remarkably high (1740 mg/L and 58 mg/L in Shidou and Bantou, respectively), while Litostomatea peaked during the bloom period 3 when cyanobacterial biomass was relatively lower than bloom period 1 (26.6 mg/L and 22.4 mg/L in Shidou and Bantou, respectively), and Prostomatea dominated during bloom period 2 when cyanobacterial biomass was 42.3 mg/L and 32.7 mg/L in Shidou and Bantou, respectively (Figs. 1b & S4a). In comparison a study in shallow hypertrophic reservoir in Slovakia, Central Europe (Tirjakova et al., 2016), and eutrophic lakes in China (Li et al., 2016), they have shown that only few common taxa flourished and dominated during the bloom periods. Furthermore, our results showed that three bloom periods were dominated by different functional groups, such as algivorous, bacterivores (including commensals and

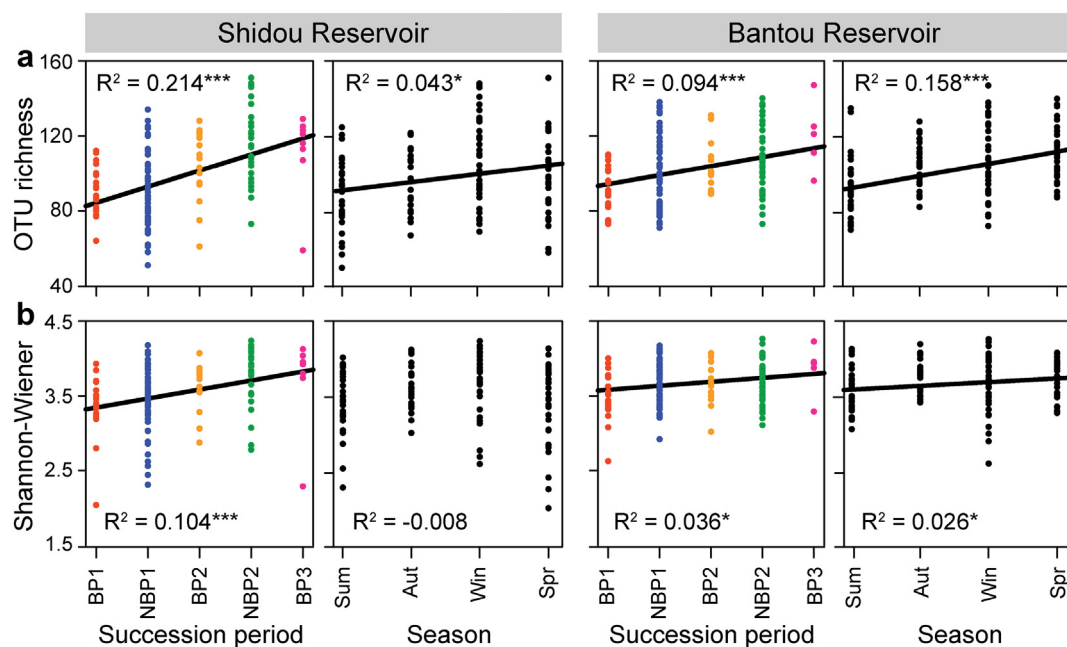


Fig. 2. Temporal dynamics of OTU richness (a) and Shannon-Wiener diversity (b) of ciliate communities corresponding to the cyanobacterial bloom succession and among the four seasons in Shidou and Bantou reservoirs, respectively.

Sum, summer; Aut, autumn; Win, winter; Spr, spring. R^2 indicates coefficient of determination, and star marks indicate significant level at 0.05* and 0.001***. BP1, bloom period 1; NBP1, non-bloom period 1; BP2, bloom period 2; NBP2, non-bloom period 2; BP3, bloom period 3.

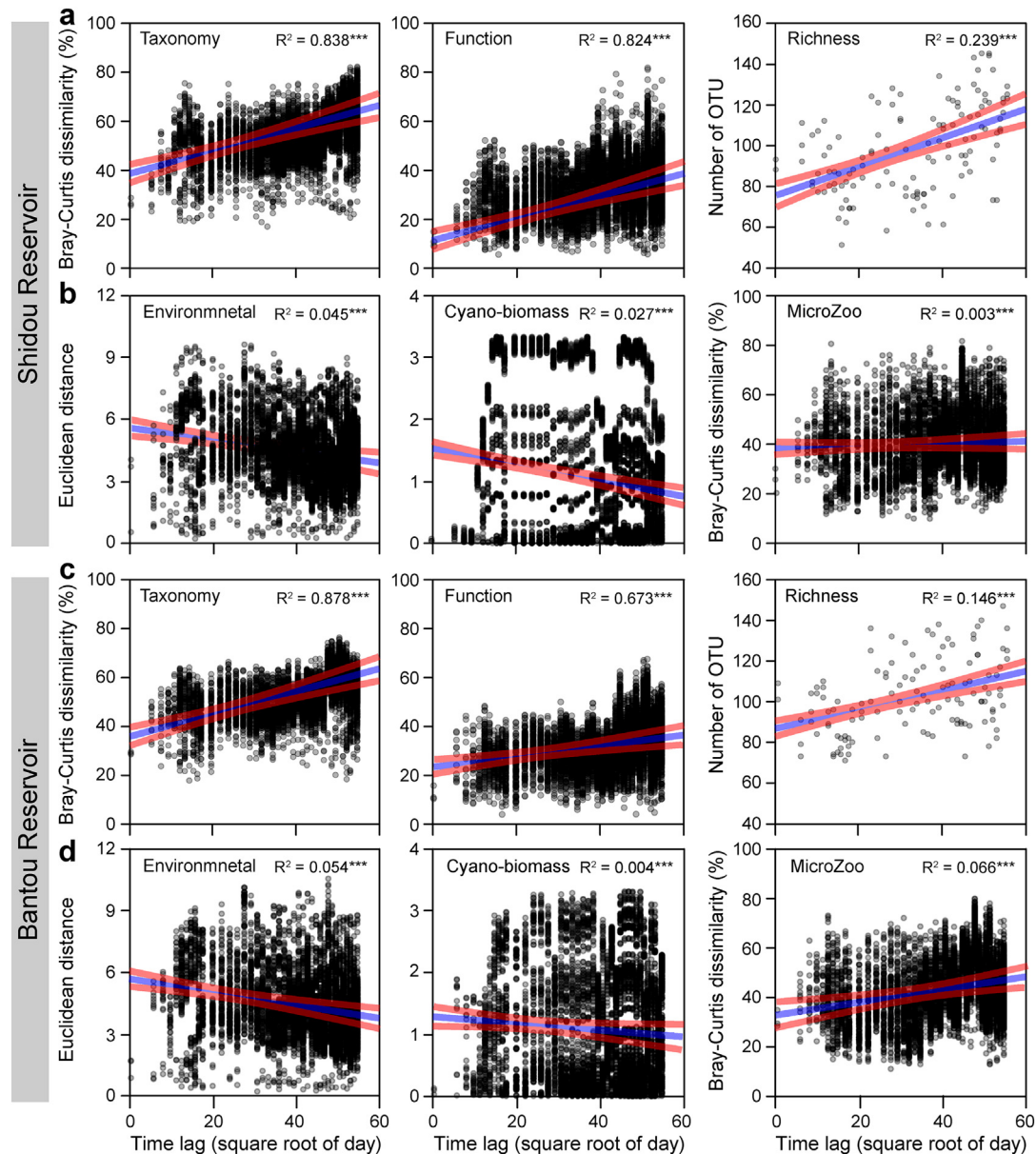


Fig. 3. Time-lag regression analysis of ciliate taxonomic composition, functional composition, richness, environmental factors (physicochemical and nutrients), cyanobacterial biomass and microzooplankton abundance over the nine-year period in Shidou (a, b) and Bantou reservoirs (c, d), respectively.

Blue lines indicate the fitted model and red lines the 95 % confident intervals of the fitted model. Adjusted R square (R^2) indicates the strength of the model fit. Taxonomy, taxonomic composition; Function, functional composition; Richness, ciliate OTU richness; Environmental, environmental factors (physicochemical and nutrients); Cyano-biomass, cyanobacterial biomass; MicroZoo, relative abundance of microzooplankton.

parasitic-histophagous), omnivores and predators being higher in first bloom period (BP1), while both algivorous and saprotrophs in the second bloom period (BP2), and the third bloom period (BP3) was dominated by algivorous, cytotoxic, and saprotroph ciliates (Figs. 1c & S4b). The different responses of ciliate functional feeding groups might reflect variations in resources (*i.e.*, quality and quantity of food, shape and size of the prey) (Meira et al., 2021; Weisse, 2017; Li et al., 2016), as we found relationship between planktonic ciliate feeding groups and cyanobacterial biomass, suggesting that food items contributed to structuring ciliate functional groups.

4.2. Marked interannual variability of ciliate community

Previous studies of ciliate diversity patterns have mainly covered short-term periods (*i.e.*, 1 or 2 years) across specific environmental gradients (Canals et al., 2020; Sommer et al., 2012; Agatha, 2011; Tirok and

Gaedke, 2006), while long-term (*i.e.*, decadal scale) studies are very rare. Our 9-year time series data analyses did not reveal a clear repeatable seasonal pattern in the ciliate community composition in the two studied reservoirs, but we found a strong cyanobacterial bloom dependent interannual dynamic pattern (Tables 1 & 2). This finding is different from previously studied ciliate and protistan communities (Pitsch et al., 2019; Simon et al., 2015), and likely reflect a strong influence of the cyanobacterial bloom on ciliate community and diversity (Fig. 2). The species turnover showed strong directional changes and the community dissimilarity between samples increased significantly with time (Fig. 3), implying an action of internal or external forces driving the community succession from its original position over time. Others have found that species turnover plays an important role in the community dynamics of eukaryotic plankton, particularly during bloom-induced disturbances (Xue et al., 2018; Kampichler and van der Jeugd, 2013; Soininen, 2010). Another possible

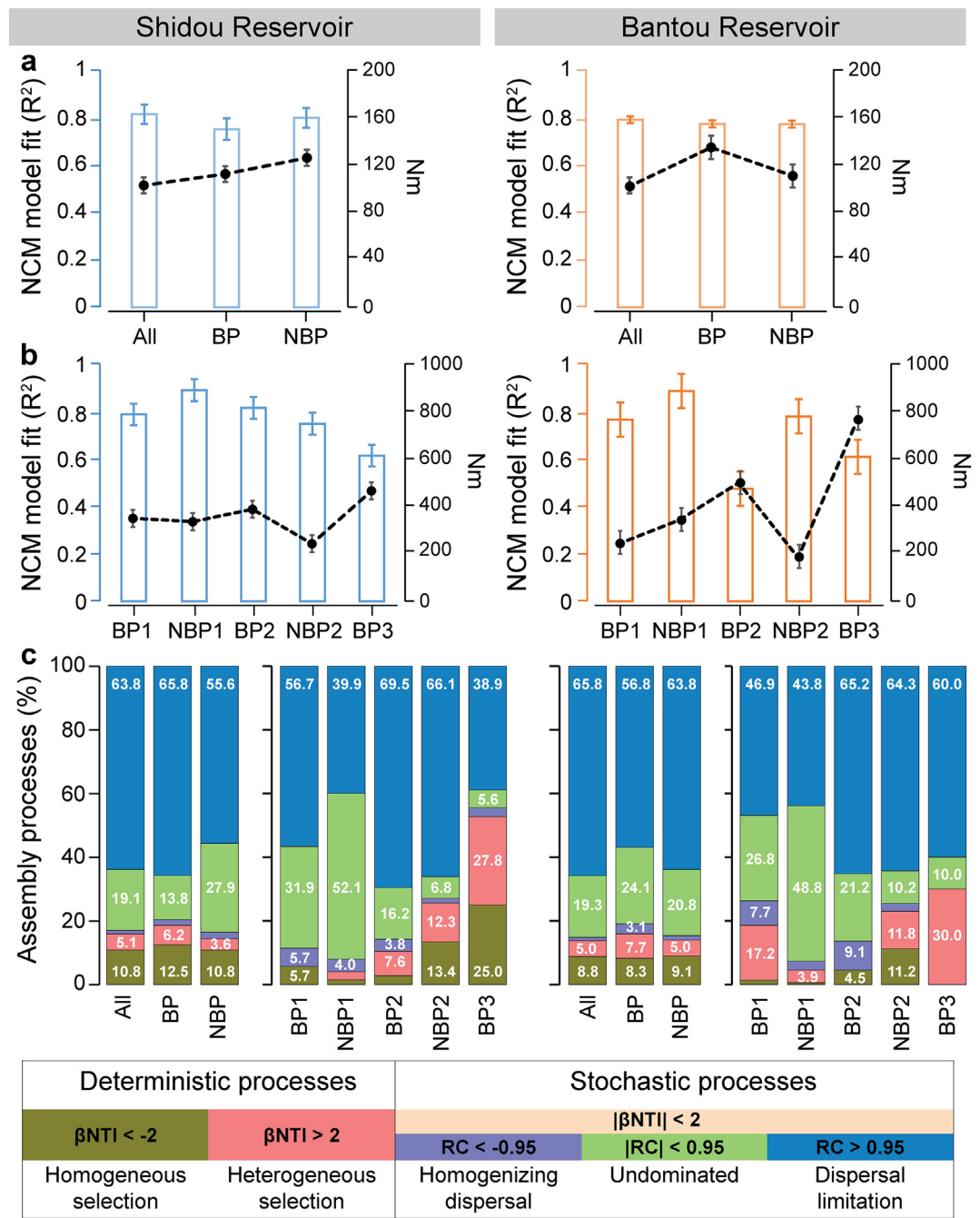


Fig. 4. The contribution of stochastic and deterministic processes on community assembly of ciliate communities. Fit of the neutral model of ciliate community assembly (a, b), and the relative importance of different processes on community assembly across cyanobacterial bloom successions in Shidou and Bantou reservoirs (c), respectively. The bars with standard deviation represent results from the fitted neutral model and the dotted lines represent the metacommunity size (a, b). The numbers in the bar plots represent the percentage of different portions of stochastic and deterministic processes on community assembly and number is shown >3 % (i.e., >3 %) to avoid complexity (c).

reason for the strong interannual variability, supported by phylogenetic turnover, is higher dispersal ability and drift (Novotny et al., 2021; Canals et al., 2020). In general, higher dispersal ability and drift allow the microbial communities to have a higher probability of colonizing suitable habitats (Canals et al., 2020; Stegen et al., 2012; Sloan et al., 2006), reducing the variation of community composition. Consistently, the community compositions in the three cyanobacterial bloom periods were rather similar and distinct from those of the two non-bloom periods (Fig. S3c), suggesting that the intensity of environmental selection being stronger in bloom periods than in two non-bloom periods. These results concur with a recent study of a drinking water reservoir in Ohio, USA, which showed stronger

effects of environmental factors on freshwater bacterioplankton communities during cyanobacterial bloom period than during non-bloom period (Wang et al., 2020a). Such an increase environmental effects might result in niche diversification for the microeukaryotic plankton (Novotny et al., 2021; Xue et al., 2018; Tirok and Gaedke, 2006).

4.3. Ecological processes governing the assembly of ciliate communities

Our results suggest that the planktonic ciliate community of both reservoirs was mainly determined by stochastic processes (Fig. 4a), which was in accordance with previous studies of freshwater microeukaryotes (Chen

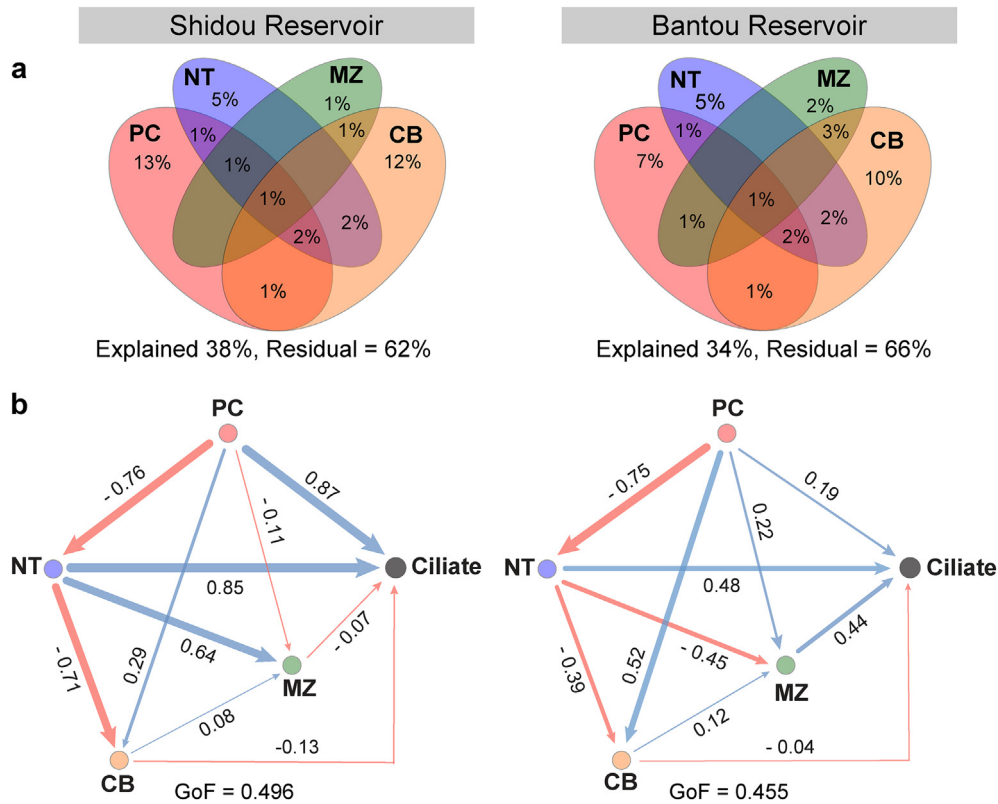


Fig. 5. Variation partition analysis of the random forest predictive variables responsible for ciliate community dynamics (a), and partial least square path model (PLS-PM) showing the direct and indirect effects of biotic and abiotic factors on ciliate community dynamics in Shidou and Bantou reservoirs (b), respectively. PC, water physicochemical variables; NT, water nutrients; MZ, microzooplankton; CB, cyanobacterial biomass. The significant predictive variables (significant level at $P < 0.05$) for ciliate community dynamics are given in Table S1 (a). Red lines represent indirect effects and blue lines represent direct effects. GoF, goodness of fit of the model (b).

et al., 2019; Xue et al., 2018), amoebae (Wang et al., 2020b; Ren et al., 2018) and bacterioplankton (Nyirabuhoro et al., 2021; Wang et al., 2020a). Our results revealed that dispersal limitation and undominated processes explained more of the variation in community assembly during non-bloom periods than during cyanobacterial bloom periods in both reservoirs (Fig. 4b), indicating that under the cyanobacterial bloom condition the ciliate community composition changes were more dissimilar than during non-bloom periods. This may reflect that the most abundant taxa show rapid growth under suitable environmental conditions (Liu et al., 2019; Xue et al., 2018) and some ciliate taxa are functionally redundant (Meira et al., 2021; Weisse, 2017), suggesting that low dispersal rate during strong bloom period was the primary cause of dissimilar community structure. Our results also showed that homogeneous and heterogeneous selections were largely responsible for the community assembly of ciliates during the weak bloom period (in year 2018) in both reservoirs (30 %–53 %, Fig. 4c), suggesting similar community composition during the weak bloom periods. This is because of ciliate communities are shaped by environmental selection owing to different habitat preferences and fitness of the species (Wang et al., 2020b; Chen et al., 2019; Stegen et al., 2012; Aberle et al., 2007), and the different species respond differently to environmental factors (Simon et al., 2015; Stegen et al., 2012).

4.4. Reservoirs environmental variability related to ciliate community dynamics

We found significant changes of environmental condition, cyanobacterial biomass and microzooplankton abundance with time (Fig. 3), even though the changes were modest and explained a minor fraction of the planktonic ciliate community variability along the 9 years. Our results suggest that even small changes in the ecological conditions (transparency, water temperature, pH, dissolved oxygen, electrical conductivity, oxidation reduction

potential and nutrients as well as cyanobacterial biomass) in reservoirs may result in major disturbance of ciliate communities (Fig. 5a). Similar results have been found for stream biofilm-associated ciliates, showing major changes in ciliate community structure owing to variation in environmental conditions, especially in the level of dissolved oxygen, temperature, turbidity, pH and availability of nutrients (Dopheide et al., 2009). Previous studies have shown that temperature, nitrogen and microzooplankton grazing can play an important role during cyanobacterial blooms since blooms enhance the positive relationship between metazoans and ciliates (Kosiba et al., 2018; Ger et al., 2016; Boyer et al., 2011; Aberle et al., 2007). Since eutrophication tends to strengthen the relationship between ciliate functional groups and microzooplankton (Kosiba and Krzton, 2022; Tirjakova et al., 2016; Boyer et al., 2011; Aberle et al., 2007), we found both positive and negative significant correlations between ciliate feeding groups and microzooplankton (Fig. S5), indicating an impact of microzooplankton likely through predation and mutual competition (Shabarova et al., 2021; Ger et al., 2019; Kosiba et al., 2018; Moustaka-Gouni et al., 2006).

A recent global scale study of taxonomic and functional turnover in ecological communities has shown that freshwater ecosystems had faster rate of decay along environmental changes than those of marine ecosystems (Graco-Roza et al., 2022), concurrently, our 9-year study expounded that the taxonomic and functional structure of planktonic ciliate communities in freshwater reservoirs in subtropical region affected by both environmental changes and cyanobacterial blooms induced biological disturbances. Therefore, our results imply that the direct and indirect effects of environmental factors (physicochemical and nutrient variables) and biological disturbances (cyanobacterial biomass and microzooplankton grazing) are likely collectively determining the ciliate community assembly. Altogether, our findings shed light on the mechanisms undelaying long-term ciliate community dynamics in subtropical reservoirs, and our 9-year time series

analyses provided novel evidence for non-repeatable season succession of ciliate communities related to variation in the intensity of cyanobacterial blooms.

5. Conclusion

Our study provided detailed insight into the nine-year dynamics of the planktonic ciliate communities in two subtropical reservoirs within three cyanobacterial bloom and two non-bloom periods. We found five distinct periods determined by cyanobacterial biomass, and the ciliate community composition was closely related to these periods. The interannual variability of ciliates was significant and twofold higher than seasonal variability. Moreover, there was a strong directional change in the ciliate taxonomical and functional structure over the nine years. The ciliate community composition was primarily determined by stochastic processes but also by environmental variables and microzooplankton. However, disturbances caused by cyanobacterial blooms (happening because of sudden changes in environmental conditions) affected ciliate communities and caused changes in the taxonomical and functional composition. Thus, cyanobacterial blooms led to an increase in the deterministic community assemblages of the ciliate community. Generalizing these results, we can say that every time a bloom occurs, the ciliate changes to a community that is more similar to a previous bloom, but different communities occurring during non-bloom periods. Whereas during non-bloom periods, the ciliate community is more driven by neutral processes and becomes different with time, and if interrupted with a bloom, the community after the bloom is different from the community before the bloom. By investigating the ecological characteristics of ciliate communities and long-term variability of environmental variables, cyanobacterial biomass and microzooplankton relative abundance in subtropical reservoirs, this study has expanded our understanding of ciliate community dynamics in freshwater ecosystems under cyanobacterial bloom disturbance.

CRedit authorship contribution statement

J. Yang: Conceived the idea, methodology and designed the experiment. **J. Yang** and **M. Abdullah Al:** Conducted formal data analyses and wrote first draft. **M. Abdullah Al** and **WP. Wang:** Data validation and interpretation. **HH. Chen** and **WP. Wang:** Collected and processed samples and measured environmental variables. **L. Jin:** Performed phytoplankton microscopy. **YY. Xue:** Conducted DNA extraction and bioinformatics. **E. Jeppesen, M. Majaneva** and **H. Xu:** Research collaboration and revised manuscript. **J. Yang** and **M. Abdullah Al:** Revised final version. Finally, all authors have agreed in submission to the journal.

Data availability

All raw sequences data of 18S rRNA gene from this study have been submitted to the NCBI with accession BioProject number PRJNA415265. These raw sequences were also stored in the National Omics Data Encyclopedia database under the Project ID: OEP002057.

Declaration of competing interest

The authors declare no competing financial interests. The authors alone are responsible for the content and writing of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.159866>.

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