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Seven-year dynamics of testate amoeba communities driven more by stochastic than deterministic processes in two subtropical reservoirs

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ABSTRACT

Testate amoebae are widely distributed in natural ecosystems and play an important role in the material cycle and energy flow. However, community assembly of testate amoebae is not well understood, especially with regard to the relative importance of the stochastic and deterministic processes over time. In this study, we used Illumina high-throughput sequencing to explore the community assembly of testate amoebae from surface waters in two reservoirs of subtropical China over a seven-year period. Majority of testate amoebae belonged to the rare taxa because their relative abundances were typically lower than 0.01% of the total eukaryotic plankton community. The testate amoeba community dynamics exhibited a stronger interannual than seasonal variation in both reservoirs. Further, species richness, rather than species turnover, accounted for the majority of community variation. Environmental variables explained less than 20% of the variation in community composition of testate amoebae, and the community assembly appeared to be strongly driven by stochastic processes. Based on the Sloan neutral community model, it was found that neutral processes explained more than 65% of community variation. More importantly, the Stegen null model analysis showed that the stochastic processes (e.g., ecological drift) explained a significantly higher percentage of community assembly than deterministic processes over seven years, although deterministic processes were more influential in certain years. Our results provide new perspectives for understanding the ecological patterns, processes and mechanisms of testate amoeba communities in freshwater ecosystems at temporal scale, and have important implications for monitoring plankton diversity and protecting drinking-water resources.

1. Introduction

Testate amoebae are a polyphyletic group of single-celled amoeboid protists in which the cytoplasm is enclosed within an external shell (Meisterfeld, 2002; Mitchell et al., 2008; Adl et al., 2019). They are widely distributed in freshwater, brackish water and soil from tropical to polar regions (Yang et al., 2010; Regalado et al., 2018), and they play an important role in the biogeochemical cycles and energy flow of aquatic and terrestrial ecosystems (Wilkinson, 2008). Studies of testate amoebae are highly relevant for our understanding of evolutionary and ecological relationships among protists (Shen et al., 1990; Yang et al., 2006; Mitchell et al., 2008). Globally, there are about 2000 described morphospecies of testate amoebae (Yang et al., 2006; Mitchell et al., 2008). The shells of testate amoebae are relatively resistant to decay and are generally well preserved in lake sediments and peatlands (Mitchell et al., 2008). Therefore, testate amoebae can be used to record past changes in environment (Mitchell et al., 2008) and they are increasingly used as a biotic proxy of climate and environmental changes (Swindles and Roe, 2007; Wall et al., 2010; Qin et al., 2013b; Ju et al., 2014; Ndayishimiye et al., 2019) and as biological indicators of water quality and pollution (Jeelani et al., 2018; Steele et al., 2018; Cockburn et al., 2020; Nasser et al., 2020).

In previous limnological studies, testate amoebae have been used to reveal changes in different environmental factors such as temperature, pH, and salinity (Asioli et al., 1996; Patterson and Kumar, 2000; Mattheeussen et al., 2005; Roe and Patterson, 2014; Ndayishimiye et al., 2019). However, most of these studies have focused on wetlands and/or lake sediments and are based on subfossil records (Swindles et al., 2015; Ndayishimiye et al., 2019). To date, research on the community structure dynamics of tes-

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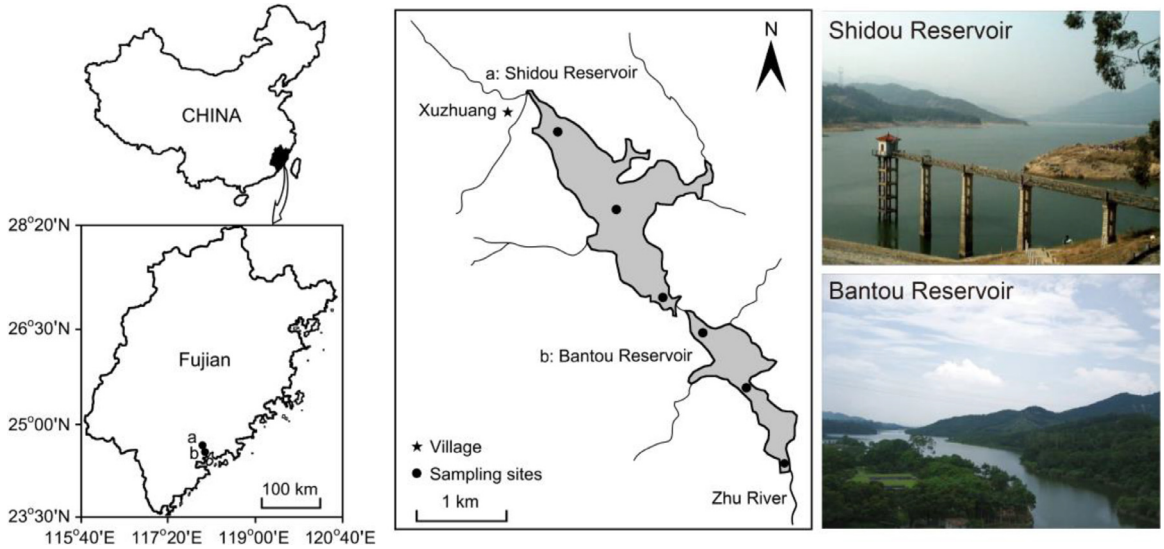

Fig. 1. Location of the two studied reservoirs and six sampling sites in Fujian province, southeast China (revised from Yang et al., 2017).

tate amoebae in the planktonic phase of inland water bodies across long time series (more than five years) has been lacking.

Recently, understanding the fundamental ecological processes underlying the assembly of microbial community has become a core topic in microbial ecology (Nemergut et al., 2013; Zhou and Ning 2017; Tripathi et al., 2018) and the major ecological process shaping microbial eukaryotic communities is hotly debated (Chase, 2010 ; Powell et al., 2015; Chen et al., 2019). Ecological processes include deterministic and stochastic processes that can simultaneously influence the assembly of local communities (Stegen et al., 2012; Zhou et al., 2014; Chen et al., 2019). In the traditional theoretical understanding of niche-based processes, the deterministic factors, including local environmental conditions, species traits, and interspecies interactions (e.g., competition, predation, mutualisms and tradeoffs), control the composition of local communities (Chesson, 2000; Fargione et al., 2003; Powell et al., 2015). However, stochastic processes such as random birth, death and dispersal events (colonization, extinction and speciation) also play an important role for the species composition in a given locality (Chave, 2004; Fukami et al., 2005). It is generally accepted that the assembly of microbial communities is regulated by deterministic and stochastic processes simultaneously (Chase, 2010; Ofiteru et al., 2010; Stegen et al., 2013; Zhou and Ning, 2017; Chen et al., 2019). The relative importance of deterministic and stochastic processes is influenced by the rate of species dispersal and strength of ecological selection along spatial and temporal scales (Caruso et al., 2011; Chisholm and Pacala, 2011; Stegen et al., 2012; Dini-Andreote et al., 2015; Evans et al., 2017). Fortunately, advent of high-throughput sequencing techniques has revolutionized microbial ecology and these techniques now enable us to obtain detailed and comprehensive information about plankton communities by analyzing environmental samples (Liu et al., 2017; Ren et al., 2018; Xue et al., 2018; Chen et al., 2019).

Here, we used Illumina high-throughput sequencing to investigate the dynamics and community assembly of testate amoebae. We collected 150 samples from surface waters of two subtropical reservoirs in southeast China over a period of seven years and combined community analysis with measurement of environmental variables. The two reservoirs are connected with water from the higher reservoir flowing directly into the lower one (the two reservoirs being separated by a dam). Such connectivity condition allows the comparison of results from two adjacent water bodies. In this study, we aim at answering the following questions: 1) How does the testate amoeba community change in surface waters of subtropical reservoirs over seven years; 2) What is the relative importance of deterministic and stochastic processes in shaping the community assembly of testate amoebae.

2. Materials and methods

2.1. Study sites and sampling

Field samplings were conducted every season from 2010 to 2016 in both Shidou and Bantou reservoirs, which are part of the Xiamen Reservoir Time-series (XRT) program in Xiamen city of Fujian province, southeast China (Fig. 1). Xiamen has a subtropical monsoon climate with annual mean temperature of 20.7 °C and annual mean precipitation of 1335.8 mm (Yang et al., 2017). Water temperature in the two reservoirs ranged from 14.6 to 33.4 °C during the study period. The Shidou Reservoir with a total storage capacity of 61.4 million m^3 is located immediately upstream of Bantou Reservoir (total storage capacity 4.4 million m^3). The two reservoirs are separated by a dam, and the water flows into Bantou Reservoir when the water level is high in Shidou Reservoir. Normally, the water flow is very low or almost zero, leading to a long water residence time. The two reservoirs are important drinking-water sources for Xiamen city (Yang et al., 2016, Yang et al., 2017).

In total, 150 samples were collected from the surface waters (0.5 m depth) in Shidou and Bantou reservoirs. There were 25 sampling visits from July 2010 to July 2016 for Shidou and Bantou reservoirs, respectively. Water samples were taken in the months of January, April, July and October in each year (Fig. 1, Fig. 2, Table S1). Replicate samples were collected from upstream, midstream, and downstream zones in each reservoir (Yang et al., 2017). Upstream, midstream and downstream zones are riverine, transitional, and lacustrine zones in each reservoir, respectively (Yang et al., 2017). For each sample, about 500 mL water was prefiltered through a 200 μm mesh to remove large particles and metazoans, and then filtered using 0.22 μm pore-size polycarbonate filters (47 mm diameter, Millipore, Billerica, MA, USA) by using vacuum pump. To reduce the likelihood of contamination, the filter bowls were rinsed successively by sterile water and sample water before each sample filtering. The filters were stored at −80 °C until DNA extraction (Liu et al., 2017).

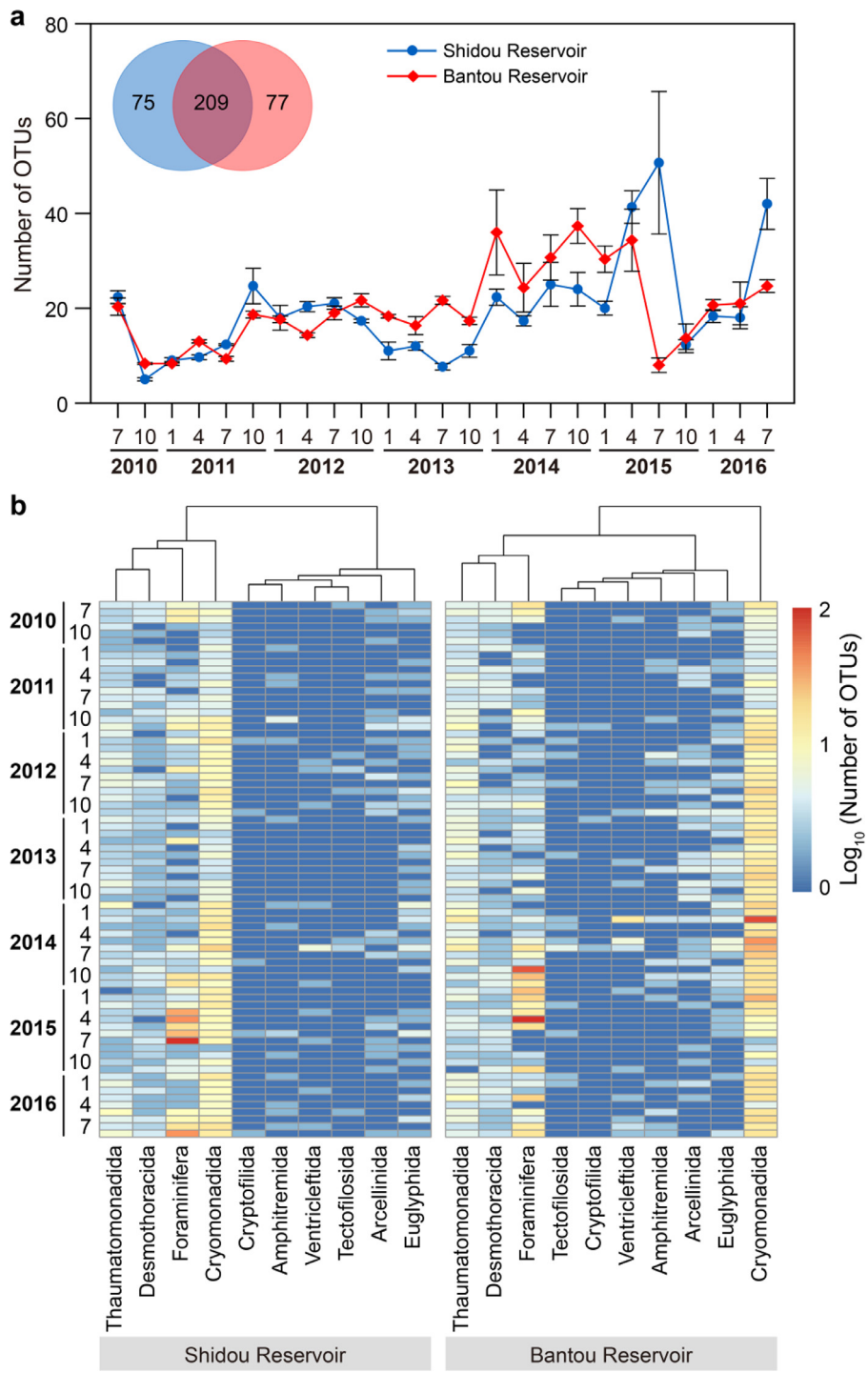

Fig. 2. Dynamics of testate amoeba communities in Shidou and Bantou reservoirs from 2010 to 2016. (**a**) The OTU richness of testate amoebae. All data are mean ± SE (n = 3). (**b**) Community composition and hierarchical cluster of testate amoebae over seven years. The numbers 1, 4, 7, and 10 indicate January, April, July and October, respectively.

2.2. Environmental and phytoplankton information

Water depths of sampling sites were measured with a Speedtech SM-5 Depthmate portable sounder (Speedtech Instruments, Great Falls, VA, USA), and water transparency was measured with a Secchi disk. We used a water quality analyzer (Hydrolab DS5, Hach Company, Loveland, CO, USA) to measure water temperature (WT), pH, dissolved oxygen (DO), chlorophyll-*a* (Chl-*a*), turbidity, electrical conductivity (EC) and oxidation-reduction potential (ORP) *in situ*. Total carbon (TC), total organic carbon (TOC), total nitrogen (TN), ammonium nitrogen (NH_4-N), nitrate and nitrite nitrogen (NO_x-N), total phosphorus (TP), and phosphate phosphorus (PO_4-P) were measured as described in our previous study (Liu et al., 2013). Phytoplankton was identified on an inverted microscope (Motic AE31, China) using Hu and Wei (2006) as taxonomic reference.

2.3. DNA extraction and sequence processing

The FastDNA spin Kit (MP Biomedicals, Santa Ana, CA, USA) was used to extract the total DNA from filters following the manufacturer's instruction. Before DNA extraction, the filter that collected the 0.2–200 μm eukaryotic plankton was cut into small pieces using flame-disinfected scissors. The hypervariable V9 region of 18S rRNA gene was amplified using eukaryotic primers 1380F and 1510R (Amaral-Zettler et al., 2009; Liu et al., 2017). The PCR amplification and library construction was performed according to Liu et al. (2017). The 150 samples were sequenced on the Illumina HiSeq2500 platform (Illumina, Inc., San Diego, CA, USA) using a paired-end approach. Illumina high-throughput sequencing provides a quick and effective way to obtain information of DNA in environmental samples, allowing the identification of testate amoebae based on the sequence similarity. The original sequence data have been deposited in the NCBI Sequence Read Archive (SRA) database under the BioProject number PRJNA415265 and the accession number SRP121028.

2.4. Bioinformatics

Low-quality reads were removed with default parameters using "make.contigs" in Mothur software and merged paired-end reads of the 18S rRNA gene sequences were processed using VSEARCH 1.9.1 (Rognes et al., 2016). Quality filtered reads were assigned to operational taxonomic units (OTUs) at 97% sequence similarity threshold. Representative sequences from each OTU were matched against the Protist Ribosomal Reference (PR2) database using the RDP classifier (Guillou et al., 2013; de Vargas et al., 2015). All singletons, doubletons, mitochondrial sequences, chloroplast sequences, and unknown organisms (sequences with less than 80% similarity to the protist reference sequence) were removed before the downstream analyses. In order to standardize the sequencing effort, a randomly selected subset of 29,809 sequences from each sample was used to standardize sequencing effort and the final dataset retained 4471,350 reads at 97% similarity level. Here, we defined the rare taxa as the OTUs with a relative abundance $<$ 0.01% in a sample and which never showed $>$ 1% abundance in any sample (Chen et al., 2019; Liu et al., 2019).

The sequences were assigned to different taxonomic groups using the PR2 database (Guillou et al., 2013). Based on the revised classification of eukaryotes (Adl et al., 2019), we selected the taxonomic groups containing amoebae from the members of Amoebozoa, Rhizaria and Excavata, and then removed the sequences of naked amoebae. The final data set included 10 main categories, namely, Amphitremida, Arcellinida, Cryomonadida, Cryptofilida, Desmothoracida, Euglyphida, Foraminifera, Tectofilosida, Thaumatomonadida, and Ventricleftida. In total, we retained 361 OTUs and 7808 sequences of testate amoebae from the 150 samples. The OTUs were defined at 97% sequence similarity level. At this level we can roughly treat them as species.

2.5. Community composition

Non-metric multidimensional scaling (NMDS) of testate amoeba communities was performed based on the Bray-Curtis similarity with relative abundance of OTUs. Analysis of similarity (ANOSIM) was used to investigate differences in the testate amoeba communities between groups. The statistic global R represents the degree of separation between groups.

The beta-diversity (Bray-Curtis dissimilarity) of the testate amoeba community between different samples was partitioned into two components, the abundance gradient (richness) and the balanced variation (turnover), using the "bray.part" function of the "betapart" R package (Jiao et al., 2017; Xue et al., 2018). We used time-lag analysis to quantify the contributions of richness and turnover dissimilarity components to the Bray-Curtis dissimilarity between each pair of samples to reveal temporal patterns in the testate amoeba community, and the time-lag was then plotted against the dissimilarity matrix using the "ggplot2" R package (Collins et al., 2000).

2.6. Relationships between testate amoebae and environment variables

The Euclidean distances of environmental variables were calculated. The pairwise Spearman's rank correlations (r) between environmental factors were calculated. Preliminary detrended correspondence analysis (DCA) on the testate amoeba data revealed that the longest gradient length in Shidou and Bantou reservoirs was 4.42 and 7.24 ($>$ 3 standard deviations), respectively. Therefore, canonical correspondence analysis (CCA) was used to explore relationships between the testate amoeba community and environmental factors. To improve normality and homoscedasticity, all environmental factors, except pH, were log $(x + 1)$ transformed before analysis. In order to reduce multicollinearity among environmental factors, all variance inflation factors (VIFs) were kept below 10 (Nakazawa and Nakazawa, 2017). The environmental factors influencing the composition of the testate amoeba community were selected by 999 Monte Carlo permutation tests at the significant level ($P < 0.01$). In addition, the relative importance of water temperature, physico-chemical factors and nutrients were assessed using the variation partitioning analysis (VPA). Mantel test was also used to investigate the correlations between Euclidean distance of environmental factors and Bray-Curtis dissimilarity of communities. These analyses were performed using the software packages CANOCO for Windows 4.5 (ter Braak and Smilauer, 2002) and the "vegan" package in R software (version 3.4.3) (Oksanen et al., 2018).

We further explored the relationships between physico-chemical factors, nutrients, phytoplankton and testate amoeba richness using a partial least squares path modeling (PLS-PM) in the R "plspm" package (Wang et al., 2016; Gao et al., 2019). This model is well known to estimate the complex cause-effect relationships between multiple variables. Four groups of variables were used: physico-chemical factors (water depth, transparency, water temperature, pH, dissolved oxygen, turbidity, electrical conductivity, oxidation-reduction potential and water level fluctuations), nutrients (total carbon, total organic carbon, total nitrogen, ammonium nitrogen, nitrate and nitrite nitrogen, total phosphorus, and phosphate phosphorus), phytoplankton (abundance (ind/L) of Bacillariophyta, Chlorophyta, Chrysophyta, Cryptophyta, Cyanobacteria, Euglenophyta, Pyrrophyta, and Xanthophyta) and richness of the testate amoeba communities (the OTUs number of testate amoebae). We ran PLS-PM using 999 bootstraps to test the direct and indirect effects of predictor variables on the testate amoebae. Path coefficients reflect the direction and strength of the linear relationships between variables, and indirect effects include the multiplied path coefficients between a predictor and a response variable. The goodness-of-fit index (GOF) was used to evaluate the performance of the model (Sanchez, 2013).

2.7. Community assembly

The contributions of neutral or stochastic processes to testate amoeba community assembly were assessed using the Sloan neutral community model (NCM), which can generate the best fit distribution curve with least-squares method (Sloan et al., 2006). The random immigrations, births and deaths are assumed to determine the relative abundance of taxa in a metacommunity. This model

predicts the relationship between occurrence frequency of individuals (OTUs) and mean relative abundance in the metacommunity (Sloan et al., 2006). The N describes the metacommunity size and m estimates immigration rate (Chen et al., 2019). The estimated immigration rate can be interpreted as a measure of dispersal limitation. All analyses were performed in R (version 3.4.3) (R Core Team, 2017).

In order to better understand the mechanisms underlying the community assembly of testate amoebae, we evaluated the contributions of deterministic and stochastic processes to community assembly using the Stegen null model (Stegen et al., 2013; Tripathi et al., 2018). This model uses the phylogenetic trees to estimate the process of each pairwise turnover for testate amoebae OTUs (Stegen et al., 2013). The null model has recently received much attention in microbial ecology (Zhou et al., 2014; Tripathi et al., 2018). In this study, approximately-maximum-likelihood phylogenetic tree constructed with the sequences of representative OTUs was used. The phylogenetic signal occurs when more closely related species are more ecologically similar, and it was used to quantitatively estimate the percentage of compositional turnover linked to deterministic and stochastic processes (Webb et al., 2002; Kembel et al., 2010; Evans et al., 2017). We calculated the pairwise phylogenetic turnover between communities using the β-mean nearest taxon distance (βMNTD) based on the Stegen null model of picante package in R 3.4.3 (Webb et al., 2002; Stegen et al., 2013). The β-nearest taxon index (βNTI) is the difference between observed βMNTD and mean of the null distribution of βMNTD normalized with its standard deviation. The relative contribution of variable selection and homogeneous selection was estimated from the percentage of pairwise comparisons whose βNTIs were > 2 and < -2, respectively. We further calculated the Bray-Curtis based Raup-Crick index (RC_{bray}) to investigate pairwise comparisons that deviated from selection (Stegen et al., 2013). The relative contribution of dispersal limitation was estimated as the percentage of pairwise comparisons with $|\beta NTI| < 2$ and $RC_{bray} > 0.95$. The relative contribution of homogenizing dispersal was estimated as the percentage of pairwise comparisons with $|\beta NTI| < 2$ and $RC_{bray} < -0.95$. The ecological drift was therefore estimated as the percentage of pairwise comparisons with $|\beta NTI| < 2$ and $|RC_{bray}| < 0.95$ (Dini-Andreote et al., 2015; Tripathi et al., 2018).

The generalized additive model (GAM) was used to evaluate the effects of seven years on βMNTD and βNTI across all datasets in Shidou and Bantou reservoirs, respectively. The GAM was fitted using the "mgcv" R package (Wood, 2016). We used integrated smoothness estimation for each explanatory variable and study site as a random factor (Tripathi et al., 2018).

3. Results

3.1. Composition and diversity of testate amoeba communities

From the 150 samples of Shidou (75 samples) and Bantou (75 samples) reservoirs, we obtained 4471,350 high-quality sequences of the 18S rRNA gene of eukaryotic plankton. These sequences clustered into 13,311 OTUs at 97% similarity level and were distributed among nine super-groups of eukaryotes (Fig. S1). The percentage of testate amoebae in the total eukaryotic community was 2.61% of OTUs and 0.16% of sequences for the Shidou Reservoir, and 2.43% of OTUs and 0.19% of sequences for the Bantou Reservoir, respectively (Fig. S2). The sequences were mainly belonged to the lineages Amoebozoa (Arcellinida) and Rhizaria (Cryomonadida, Cryptofilida, Desmothoracida, Euglyphida, Foraminifera, Tectofilosida, Thaumatomonadida, and Ventricleftida) with lower numbers from Stramenopiles (Amphitremida) (Fig. 2). The NMDS results showed that the difference in the testate amoeba community was non-significant between Shidou and Bantou reservoirs ($P = 0.20$) (Fig. S3). However, the interannual variations of the testate amoeba communities were significantly greater than seasonal variations in both Shidou and Bantou reservoirs (Mann-Whitney U test, $P < 0.01$). The results of ANOSIM test further confirmed the significant interannual changes of the testate amoeba community from 2010 to 2016 (Table 1).

Table 1
Analysis of similarity (ANOSIM) statistics testing the temporal difference in testate amoeba communities from Shidou and Bantou reservoirs.

Grouping by	Shidou		Bantou	
	R	*P*	R	*P*
Year (7 years)	**0.304**	**0.001**	**0.294**	**0.001**
2010 vs. 2011	**0.311**	0.020	**0.293**	0.028
2010 vs. 2012	**0.279**	0.022	0.056	0.273
2010 vs. 2013	**0.507**	**0.001**	**0.300**	**0.007**
2010 vs. 2014	0.212	0.040	0.030	0.352
2010 vs. 2015	0.226	0.021	**0.263**	0.023
2010 vs. 2016	**0.342**	**0.008**	**0.557**	**0.001**
2011 vs. 2012	0.065	0.078	0.241	**0.001**
2011 vs. 2013	**0.337**	**0.001**	**0.373**	**0.001**
2011 vs. 2014	**0.254**	**0.001**	**0.346**	**0.001**
2011 vs. 2015	**0.448**	**0.001**	**0.518**	**0.001**
2011 vs. 2016	**0.422**	**0.001**	**0.506**	**0.001**
2012 vs. 2013	**0.369**	**0.001**	0.249	**0.003**
2012 vs. 2014	0.189	**0.001**	0.190	**0.004**
2012 vs. 2015	**0.497**	**0.001**	**0.478**	**0.001**
2012 vs. 2016	**0.378**	**0.001**	**0.291**	**0.006**
2013 vs. 2014	0.165	**0.002**	0.142	**0.008**
2013 vs. 2015	**0.486**	**0.001**	**0.476**	**0.001**
2013 vs. 2016	**0.650**	**0.001**	**0.490**	**0.001**
2014 vs. 2015	**0.303**	**0.001**	0.218	**0.006**
2014 vs. 2016	**0.318**	**0.002**	0.187	0.019
2015 vs. 2016	0.122	0.052	0.029	0.337
Season (4 seasons)	0.118	**0.001**	0.127	**0.001**
Jan. vs. Apr.	0.028	0.158	0.107	**0.006**
Jan. vs. Jul.	0.221	**0.001**	0.246	**0.001**
Jan. vs. Oct.	0.104	**0.006**	0.231	**0.001**
Apr. vs. Jul.	0.102	0.011	0.054	0.077
Apr. vs. Oct.	0.073	0.039	0.017	0.257
Jul. vs. Oct.	0.167	**0.001**	0.133	**0.004**

The ANOSIM statistic R is calculated by the difference of the between-group mean rank similarities, thus it displays the degree of separation between groups. Higher R value indicates higher difference between groups. Bold font means the significance at $P < 0.01$ level or R value > 0.25, respectively.

Clearly, the number of OTUs in both reservoirs varied greatly over time (Fig. 2). The shared number of OTUs (209) from Shidou and Bantou reservoirs accounted for 73.6% and 73.1% of total richness, respectively (Fig. 2a). The time-lag regression showed that the community dissimilarity increased at the beginning and then declined, indicating that the testate amoeba community first underwent a directional change followed by a convergent change later in the sampling period (Fig. 3). Partitioning of beta-diversity further revealed that species richness (richness), rather than species replacement (turnover), accounted for the majority of the changes in community dissimilarity over time (Fig. 3). The community of testate amoebae showed recoverability in both reservoirs, especially with regard to temporal community changes contributed by the species richness.

3.2. Relationship between the testate amoeba community and environmental variables

The environmental stability of the two reservoirs was evaluated by the Euclidean distance. The Euclidean distance was slightly larger in Shidou than in Bantou reservoirs indicating that the environments were more stable in Bantou Reservoir (Fig. S4a). Spearman correlations among environmental factors revealed that there

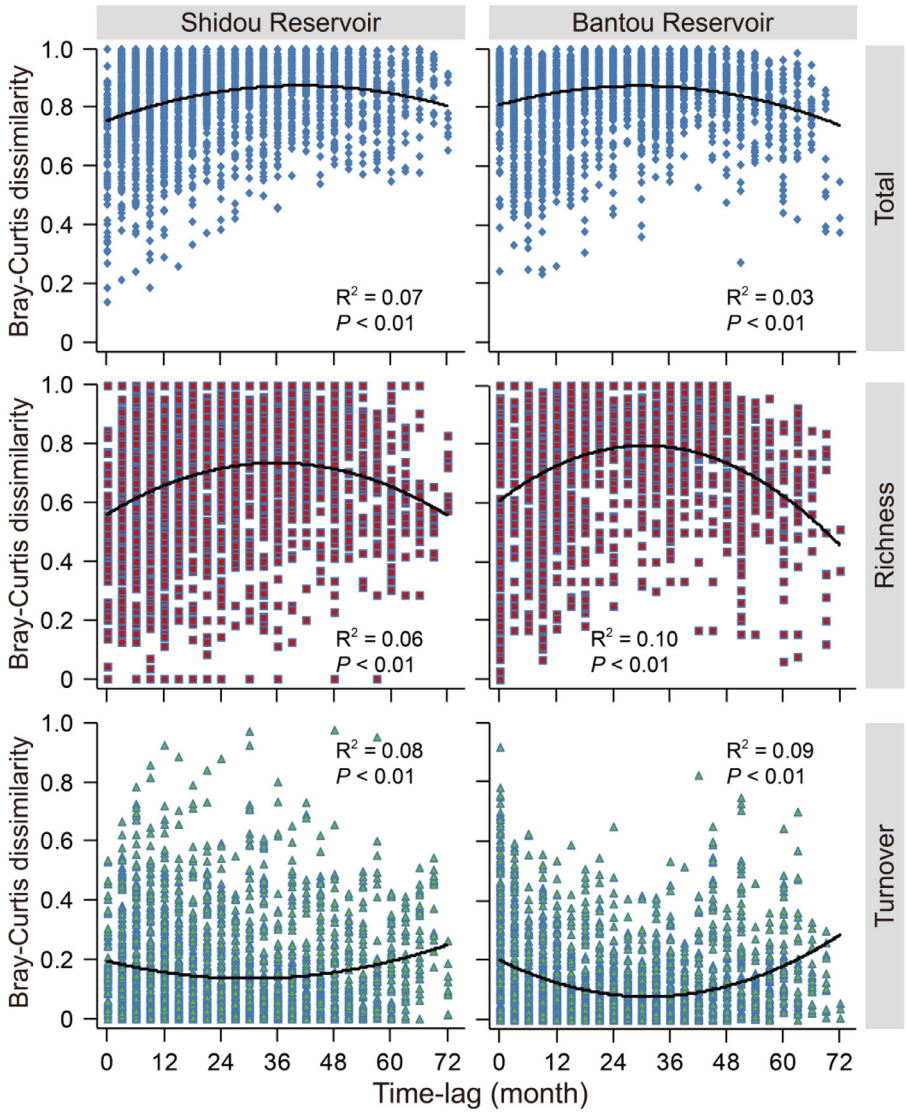

Fig. 3. The contributions of richness (**middle**) and turnover (**bottom**) components to the beta-diversity (**top**) of testate amoeba communities in Shidou and Bantou reservoirs, respectively.

were 42 significant correlations in Shidou Reservoir (22 positive and 20 negative) and 37 significant correlations in Bantou Reservoir (16 positive and 21 negative) (Fig. S4b). Based on the ANOSIM test, difference of the environmental factors between two reservoirs (Global R = 0.059, $P < 0.01$) was very minor but statistically significant. However, there was no any significant difference among the environmental factors of upstream, midstream, and downstream within each of reservoir (Table S2). More importantly, we found that the transparency, water temperature, pH, dissolved oxygen, electrical conductivity, and total nitrogen had significant effects on the testate amoeba community in Shidou Reservoir ($P < 0.01$) (Fig. 4a). In Bantou Reservoir, the significant environmental factors included water depth, water temperature, chlorophyll-*a*, electrical conductivity, total nitrogen (TN) and, phosphate phosphorus (Fig. 4b). The variation partition analysis (VPA) indicated that all environmental factors explained 12–15% of testate amoeba community variability in Shidou and Bantou reservoirs (Fig. 4c, d). Nutrients had the highest effects on the testate amoeba communities among the environmental factors in both reservoirs (Fig. 4c, d). Combined with a Mantel test, we found that water temperature had stronger effects on testate amoeba communities in Bantou than Shidou reservoirs (Fig. 4c, d, Table S3). In the Partial least squares path model, the physico-chemical factors, nutrients, and phytoplankton communities had positive direct effects on the testate amoeba richness in Shidou Reservoir, however, those variables had negative direct effects on the testate amoeba richness in Bantou Reservoir (Fig. S5).

3.3. The community assembly of testate amoebae

The testate amoeba community in both Shidou and Bantou reservoirs showed a good fit to the Sloan neutral community model (Fig. S6). Neutral processes explained 65–66% of the community variation (Fig. S6a, b) and more than 90% of the OTUs could be predicted as neutrally distributed OTUs with 95% confidence (Fig. S6c). The GAM analysis based on the null model showed that the βMNTD was significantly related to the time-lag values ($P < 0.01$) (Fig. S7a, b). The general patterns of changes in the testate amoeba community were similar between Shidou and Bantou reservoirs, however, there were some minor differences. In Shidou Reservoir, the βMNTD values increased from the time-lag 0 to 44 months and exhibited a sharp decline when time-lag > 44 months. In Bantou Reservoir, the βMNTD values increased from the time-lag 0 to 26 months and exhibited a gradual decline during time-

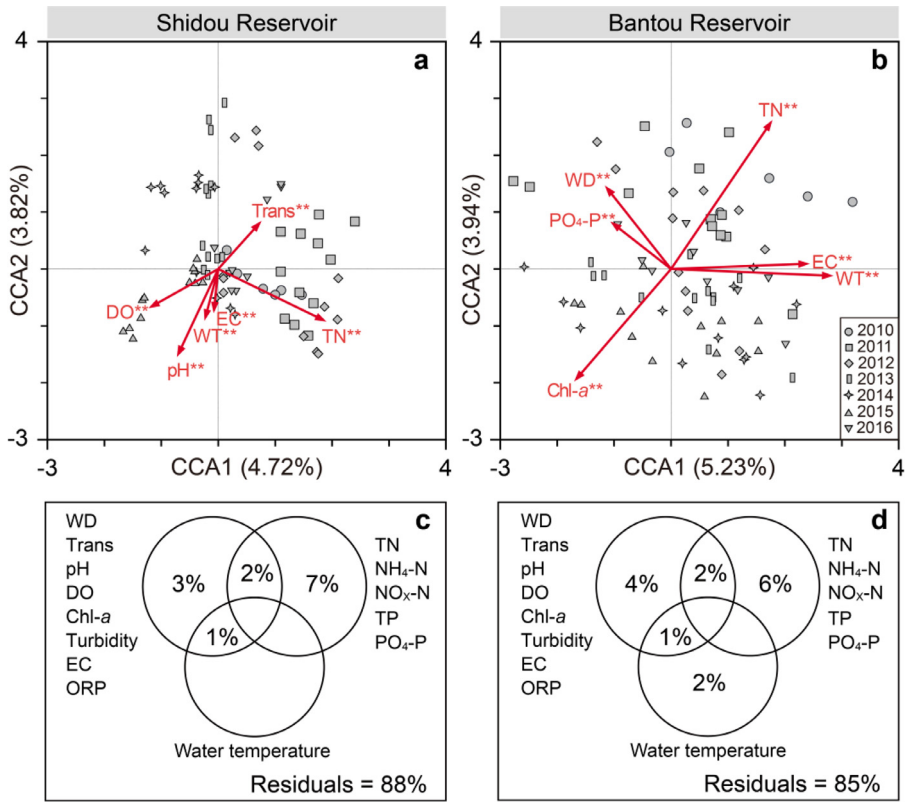

Fig. 4. The effect of environmental variables on testate amoeba communities. Canonical correlation analysis plots and variance partitioning analysis in Shidou (**a, c**) and Bantou (**b, d**) reservoirs, respectively. WD: water depth; Trans: transparency; DO: dissolved oxygen; Chl-*a*: chlorophyll-*a*; EC: electrical conductivity; ORP: oxidation-reduction potential; TN: total nitrogen; NH_4-N: ammonium nitrogen; NO_x-N: nitrate and nitrite nitrogen; TP: total phosphorus; PO_4-P: phosphate phosphorus. ** $P < 0.01$. For simplicity, the explanations < 1% are not shown in the Venn diagrams (**c, d**).

lag 26–44 months and abrupt decrease after the 44th month (Fig. S7). We further explored the relationship between βNTI and successional times, and obtained similar trends to βMNTD. In Shidou and Bantou reservoirs, the βNTI distributions gradually shifted over times-lag most in stochastic community assembly ($|\beta NTI| < 2$) (Fig. S8). Then we quantified the contributions of the deterministic (e.g., homogeneous selection and variable selection) and stochastic (e.g., dispersal limitation, homogenizing dispersal, and ecological drift) processes to the assembly of the testate amoeba community using βNTI and RC_{bray} values across different years. The results showed that most βNTI and RC_{bray} values ranged from −2 to 2 and −0.95 to 0.95, respectively (Fig. S9). This suggested that the community assembly of testate amoebae was primarily controlled by stochastic rather than deterministic processes in all seven years.

Overall, the stochastic processes (dispersal limitation, homogenizing dispersal, and ecological drift) accounted for 83% and 85% of the community assembly processes in Shidou and Bantou reservoirs over seven years, respectively (Fig. 5). There were some differences between different years and the deterministic processes were more influential in certain years (e.g., 2013 and 2016 in Shidou Reservoir, and 2016 in Bantou Reservoir) (Fig. 5). In Shidou Reservoir, the stochastic processes (mainly ecological drift) had a relatively higher contribution in 2011, 2012, 2014, and 2015. However, in 2010, 2013 and 2016, the deterministic processes, mainly homogeneous selection (33%, 58% and 61% for each of the three years, respectively), contributed relatively more (Fig. 5 top). In Bantou Reservoir, the stochastic processes (mainly ecological drift) had a relatively higher contribution in 2011, 2013, 2014, and 2015. However, in 2010, 2012 and 2016, the deterministic processes, mainly homogeneous selection (33%, 35% and 47%), showed a high contribution to the community assembly (Fig. 5 bottom). Moreover, in order to assess if our results were biased by the inclusion of samples from different periods, we chose different sampling periods to explore the variation of community assembly of testate amoebae. The analyses further confirmed that the community assembly of testate amoebae was primarily controlled by stochastic rather than deterministic processes over two years (2013, 2014) or four years (2013, 2014, 2015, 2016) (Fig. S10), as seen for the analysis over seven-year period (Fig. 5).

4. Discussion

The temporal dynamic of testate amoeba communities in inland waters is not well-described, especially in freshwater reservoirs. The use of deep high-throughput sequencing now allows us to quickly and effectively obtain more biological information - also of the relatively rare taxa in the plankton communities (Logares et al., 2013; Xue et al., 2018; Chen et al., 2019). Here, we used this approach to explore the community changes of testate amoebae, their driving factors, and mechanisms of community assembly in two subtropical reservoirs over seven years.

4.1. Interannual and seasonal variability in testate amoeba communities in reservoirs

In contrast to previous studies, focusing only on testate amoebae based on the microscopic approach (Yang et al., 2010; Qin et al., 2013a; Ju et al., 2014; Nasser et al., 2019), here, we first quantified the percentages of testate amoebae in the whole eukaryotic plankton, and found that the majority of the testate amoebae belonged to rare (low-abundance) taxa of the plankton communities. The difference in community composition between the

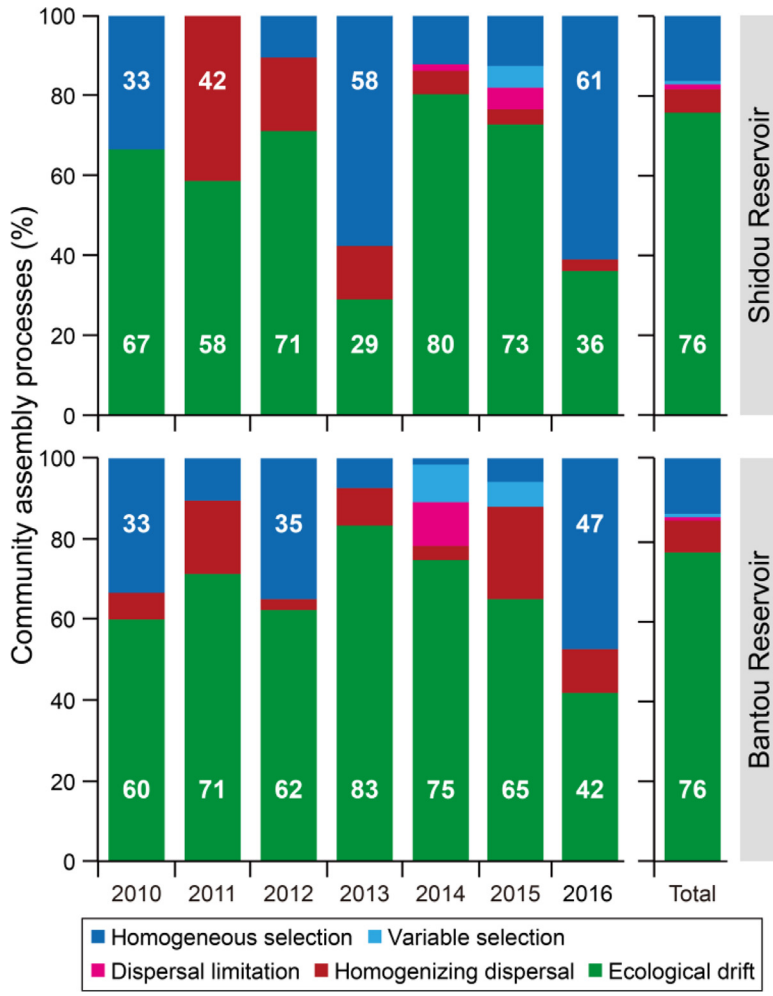

Fig. 5. The percentage of turnover in testate amoeba community assembly governed primarily by various deterministic (homogeneous selection and variable selection) and stochastic (dispersal limitation, homogenizing dispersal, and ecological drift) processes in Shidou and Bantou reservoirs. Note that the contributions > 25% are indicated by numbers.

two reservoirs was not significant (Fig. 2a, Fig. S3), which is probably due to the high connectivity between the reservoirs.

Previous long-term studies were not able to detect repeated seasonal trends in the dynamics of phytoplankton or zooplankton communities from freshwater lakes (Anneville et al., 2002; Rettig et al., 2006). Similar results were obtained in our study since the interannual differences in the composition of testate amoeba communities were stronger than the seasonal repeated patterns (Table 1). This suggested that the influence of the unpredictable interannual variation in climate change and human activities on testate amoeba community might exceed their ability to recover from the seasonal variations. In this study, the variations of climate factors mainly included rainfall, warming and cooling events, and they varied among different years (Yang et al., 2017). We further partitioned the beta-diversity into species richness and species replacement components to quantify the key process driving the community dynamics of testate amoebae (Fig. 3). Interestingly, the species richness contributed more variation than species turnover in driving the dynamics of testate amoeba community composition. In contrast, Xue et al. (2018) found that the species turnover explained a higher percentage of the eukaryotic plankton communities following a reservoir cyanobacterial bloom. Therefore, variability in taxonomic group or level, environmental gradients, and temporal scale must be considered when investigating the key mechanisms of eukaryotic community dynamics. Moreover, the recoverability can also be described as the resilience to an alternative stable state following an environmental change (Moustaka-Gouni et al., 2006). Resilience is often defined as the degree to which a system returns to pre-disturbance conditions, in state or function, after a disturbance or perturbation (Konopka et al., 2015). Here we found that the testate amoeba community exhibited some recoverability over seven years, which is similar to the findings of other studies of eukaryotic microbial communities in freshwater or marine ecosystems (Kim et al., 2014; Brannock et al., 2016; Liu et al., 2019).

4.2. Minor influence of environmental variables on the testate amoeba community over seven years

The water environmental conditions were relatively more stable in Bantou than in Shidou reservoirs (Fig. S4). This may be due to the fact that Shidou Reservoir is located upstream of Bantou Reservoir, and the former was subjected to more external disturbance (Yang et al., 2017). The external disturbance perhaps refers to the water source of the reservoirs. In Shidou Reservoir, the water originates from several tributaries, whereas the water in Bantou Reservoir mainly comes from Shidou Reservoir. The difference of environmental factors between two reservoirs was minor but significant (Global R = 0.059, $P < 0.01$). Generally, the local testate amoeba community can respond differently to different en-

vironmental conditions (Qin et al., 2013a; Regalado et al., 2018; Ndayishimiye et al., 2019).

Although the difference of testate amoeba community composition was not significant between Shidou and Bantou reservoirs, environmental factors influenced testate amoebae in different or complex ways in this study. First, the transparency, water temperature, pH, dissolved oxygen, electrical conductivity, and total nitrogen were significant variables in shaping the testate amoeba community in Shidou Reservoir, however, water depth, water temperature, chlorophyll-*a*, electrical conductivity, total nitrogen, and phosphate phosphorus were significant variables in Bantou Reservoir (Fig. 4a, b). In addition, the water temperature had slightly higher importance for the testate amoeba community in Bantou than Shidou reservoirs (Fig. 4c, d). Second, many studies have shown that the physico-chemical factors and nutrients have both direct and indirect effects on microeukaryotic and bacterial communities in aquatic ecosystems (Powell et al., 2015; Wang et al., 2016; Ren et al., 2018; Xue et al., 2018). In our two reservoirs, the testate amoeba communities were also impacted by both direct and indirect effects from the physico-chemical factors and nutrients, as indicated by the partial least squares model (Fig. S5). Nevertheless, only up to 15% of the community variation could be explained by measured environmental variables in our VPA analysis. This indicated that these environmental factors, or deterministic processes, played a minor role in community assembly of the testate amoebae over seven years. Recently, stochastic processes shaped microeukaryotic community assembly was reported across wet and dry seasons in a subtropical river by Chen et al. (2019). In addition, the large unexplained community variation in this study could also be due to the absence of some important environmental factors that were not taken into account in the VPA.

4.3. The community assembly of testate amoebae driven by stochastic processes

In general, both deterministic and stochastic processes occur simultaneously in the assembly of local communities (Chase, 2010; Ofiteru et al., 2010; Xue et al., 2018; Chen et al., 2019). It is a central goal in ecology to quantify the relative importance of deterministic and stochastic processes for the assembly of microbial communities (Morrison-Whittle and Goddard, 2015; Zhou and Ning, 2017). Our results clearly indicated that the stochastic processes are more important than deterministic processes for the assembly of the testate amoeba communities in the two subtropical reservoirs over seven years (Fig. 5). Hence, in our study the environmental variables could only explain a minor part of the variation in the testate amoeba communities.

In this study, the testate amoeba community showed a good fit to the neutral community model which explained over 65% of the frequency distribution of the testate amoeba taxa in the two reservoirs (Fig. S6). This means that community assembly could be mainly shaped by stochastic processes. This conclusion is supported by the results of Xue et al. (2018), which indicated that the stochastic processes are particularly pronounced for the rare eukaryotic plankton. Further, in the Stegen null model results, the stochastic processes (mainly ecological drift) explained over 80% of the community variation in the seven-year succession, whereas the deterministic processes explained less than 20% community variation in the two reservoirs (Fig. 5). Combined with the assessment of the neutral model, this result further corroborated the importance of stochastic processes for community assembly of testate amoebae. The importance of stochastic processes was previously illustrated for other eukaryotic microbial communities from subtropical rivers and reservoirs (Ren et al., 2018; Chen et al., 2019).

In addition, we found that the contributions of deterministic and stochastic processes to the community assembly fluctuated across different years. For example, the community assembly of testate amoebae was almost completely explained by stochastic processes for Shidou Reservoir in 2011. This may be due to the relocation of local residents in the upper reaches and the water quality improvement in 2010 (Yang et al., 2017). Total nitrogen (TN), total phosphorus (TP), and comprehensive trophic state index (TSIc) showed a significant decrease after human population emigration in late 2010 (Yang et al., 2017). The people emigration could have reduced the impact or stress of environmental factors on the testate amoeba community, which in turn led to an increased contribution of the stochastic processes to the community assembly. Anthropogenic influence can cause disturbance of the water environment, for example by increasing the nutrients and promoting cyanobacterial bloom; such changes can strongly affect the community assembly of plankton communities (Liu et al., 2019). In 2016, however, the contribution of the deterministic processes (mainly from the homogeneous selection of environmental factors) to community assembly of testate amoebae increased significantly, suggesting that the response of the community to environmental homogeneous selection was delayed, perhaps due to the cyanobacterial bloom in 2015 (Fig. 5) (Yang et al., 2017; Liu et al., 2019). Reservoir ecosystems are typically subject to disturbances of different frequencies and intensities. Natural communities are usually not in equilibrium, and temporal snapshots can be heavily influenced by short-term variations, for example storm and flooding events (Lin and Patel, 2008; Perdomo et al., 2012; Luo et al., 2015). In order to assess if our results were biased by the inclusion of samples from different periods, we performed a second null-model analysis only including samples from two- or four-year periods (Fig. S10). The results were almost identical to those of the full data set over all seven years and confirmed that the community assembly of testate amoebae was primarily controlled by stochastic rather than deterministic processes.

Overall, the stochastic processes were the main driving force or mechanism for community assembly of testate amoebae in the two subtropical reservoirs, but relative contributions of deterministic and stochastic processes exhibited a high degree of fluctuation between individual years.

In addition, our results may potentially challenge the accuracy of the traditional model or transfer functions employing testate amoeba communities as indicators for reconstruction of the past environment or climate based on a single sediment core sample. To improve the accuracy of the model or transfer functions, more community assembly processes and ecological factors should be taken into account. Therefore, the increased understanding of the processes governing the community assembly of testate amoebae may have important implications for monitoring plankton diversity and protecting the water resources, especially drinking-water conservation management in the Anthropocene.

5. Conclusions

We highlighted the importance of stochastic processes in shaping the testate amoeba community from inland waters for the first time. Our study provides a better understanding of long-term dynamic patterns, processes, and mechanisms underlying the testate amoeba community in two subtropical reservoirs. We found that the majority of testate amoebae belonged to the rare eukaryotic plankton, because they had a relative abundance $< 0.01\%$ in the plankton community. In both reservoirs, the testate amoeba communities exhibited a stronger interannual variation than seasonal change, and they first showed a directional change, then a convergent change from 2010 to 2016. Further, species richness, rather than species turnover, accounted for the majority of community variation. More than 85% of community variation could not be explained by our measured environmental variables over the seven-

year period. Stochastic processes appeared to play a more important role in the structuring of testate amoeba community. However, the relative importance of deterministic and stochastic processes varied greatly among the seven successional years. Altogether, our results indicate that both stochastic and deterministic processes can influence the community assembly of testate amoebae, but the stochastic processes (e.g., ecological drift) appear to be the dominant mechanism in this study. Therefore, we should consider the importance of stochastic processes, as well as the disturbance caused by climate change and human activities, when designing studies to investigate the community assembly of plankton and to reconstruct the environment changes based on testate amoebae in future.

Author contributions

JY conceived the idea and designed the experiments. WW and KR performed the DNA sequencing and bioinformatics. WW, HC, XG and JY collected the samples and determined the environmental parameters. WW, JY and XG analyzed data. WW and JY wrote the first draft of the manuscript, and all authors contributed to and have approved the final manuscript.

Supplementary data

Supplementary data related to this article can be found online.

Declaration of Competing Interest

The authors declare no competing financial interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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