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Decade-scale change in testate amoebae community primarily driven by anthropogenic disturbance than natural change in a large subtropical reservoir

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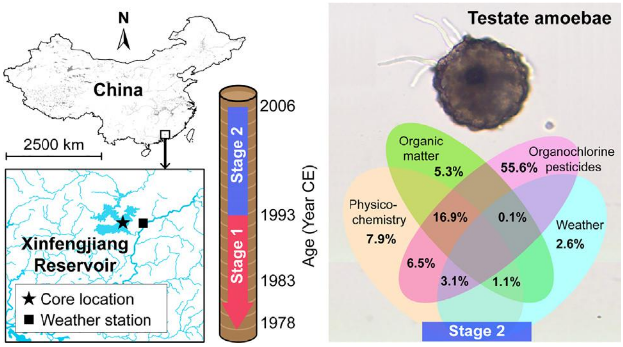
HIGHLIGHTS

- Two development stages were defined for a testate amoebae community in a large subtropical reservoir.
- In the first stage, the community was influenced by both anthropogenic and natural changes.
- In the second stage, the community was impacted more by anthropogenic disturbance.
- The testate amoeba community turnover rate was greater during the second than the first stages.
- The testate amoebae community change was closely associated with the organochlorine pesticides.

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding the extent of human activities leading to an influx of chemical pollutants that cause substantial environmental transformations is the focus of much ongoing research. In this study, we present a multi-proxy record based on a sediment core from a large subtropical reservoir (Xinfengjiang Reservoir) in south China with an emphasis on the changes in testate amoebae community, in combination with sedimentological (radioactivity, physicochemistry, nutrient and organochlorine pesticides) and climatological (air temperature and precipitation) data over the last three decades. Twenty-seven testate amoebae species belonging to seven genera (*Arcella*, *Centropyxis*, *Cyclopyxis*, *Difflugia*, *Netzelia*, *Euglypha* and *Pseudodifflugia*) were observed. Species richness, abundance and biomass of testate amoebae were in ranges of 18–26 species, 616–825 ind. ml^{-1} and 9.0–19.4 μg C ml^{-1}, respectively. Two development stages of the reservoir, dated to 1978–1993 (stage 1) and 1993–2006 (stage 2), were distinguished based on testate amoebae communities. Stage 1 was characterized by elevated dry bulk density, carbon-to-nitrogen ratio and p,p'-DDE in the sediment core and an impact of nitrogen and sulfur

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Sediment multi-proxy
Testate amoebae community
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deficiency on testate amoebae. Stage 2 was marked by a decrease of dry bulk density, elevated concentrations of aluminum, iron and carbon, low carbon-to-nitrogen ratio and organochlorine pesticides, fluctuations in rainfall on shorter and yearly timescales, and a stronger influence of the organochlorine pesticides on testate amoebae. Testate amoebae community change and the identified two-stage development were consistent with atmospheric deposition of organochlorine pesticides from anthropogenic sources inside and outside the reservoir watershed, nutrient influx and sediment physicochemistry. The testate amoebae community dynamics and a strong community-environment relationship in stage 2 were linked with non-random patterns in the biotic neighborhoods of species (deterministic processes). The results suggest a stronger impact of anthropogenic disturbance than natural environmental change on testate amoebae community variation of Xinfengjiang Reservoir over time.

1. Introduction

Reconstructing past conditions based on sediment provides a foundation for understanding long-term environmental changes (Charman, 2001; Berke, 2018; Birks et al., 2012). The sediment records from reservoirs of Guangdong Province in the southeast China have so far been underutilized for understanding the impact of global warming or change on the habitats and microbial communities, or human activities, of the ecosystems (Han and Dumont, 2011; Lin et al., 2010). More than 6000 reservoirs were constructed since the 1950s in Guangdong Province. However, there is a shortage of paleolimnological work to understand the environmental change in reservoirs over decadal time scale (Beyens and Meisterfeld, 2001; Hayes et al., 2017).

Testate amoebae are a polyphyletic group of unicellular shelled amoeboid protists in which the cytoplasm is enclosed within an external shell (Charman, 2001; Mitchell et al., 2008; Prentice et al., 2018). They are widely distributed in freshwater and soil from tropical to polar regions, and they are responsible for the biogeochemical cycles and energy flow of aquatic and terrestrial ecosystems (Ju et al., 2014; Ogden and Hedley, 1980; Patterson et al., 1985; Roe and Patterson, 2006; Todorov and Bankov, 2019). The shells of testate amoebae are resistant to decay and are largely well preserved in peatlands and sediments (Beyens and Meisterfeld, 2001; Mitchell et al., 2008). The testate amoebae from sediments can be identified to genus and species levels and utilized in the biostratigraphic description of Quaternary sediments (Charman, 2001). They have been employed to record past changes in the environment and they are increasingly utilized as a biotic proxy of climate and environmental changes and as bioindicators of water quality and pollution (Patterson et al., 1996; Qin et al., 2009; Roe et al., 2009; Wanner et al., 2020). Testate amoebae proxies have been successfully used to detect changes in environmental variables such as water table, pH, nutrients in wetlands and water temperature in lakes (Beyens and Meisterfeld, 2001; Ndayishimiye et al., 2019; Patterson et al., 2012; Tsyganov et al., 2019). Previous limnological work reveals that the populations of testate amoebae are impacted by both natural and anthropogenic changes (Ndayishimiye et al., 2019, 2020a).

To date, investigation of human-induced influence on the testate amoebae community over decadal-timescale has been lacking. Additionally, understanding the important ecological processes underlying the community assembly has become an important topic in the ecology of testate amoebae (Ren et al., 2018; Wang et al., 2020). It is generally accepted that ecological processes such as deterministic and stochastic processes can concurrently influence the assembly of local communities (Chen et al., 2019; Nyirabuhoro et al., 2020; Ren et al., 2018; Wang et al., 2020). The deterministic variables, including local environmental conditions, species traits, and interspecies interactions (e.g., competition, predation, mutualism and tradeoff), control the composition of local communities. The stochastic processes such as birth, death and dispersal events (e.g., colonization, extinction and speciation) also play a considerable role for the species composition in certain locality (Dini-Andreote et al., 2015; Zhou and Ning, 2017). The relative importance of deterministic and stochastic processes is impacted by the rate of species dispersal and strength of ecological selection along spatial and temporal scales (Dini-Andreote et al., 2015). Fortunately, fossil testate amoebae are a remarkable gift that has modernized microbial ecology and these records today permit us to gain comprehensive information about the testate amoebae community change at different time scales (Ndayishimiye et al., 2020a).

There is a lack of work on developing paleolimnological tools to separate the impacts of natural and anthropogenic changes on the testate amoebae community over decadal time-scale (Beyens and Meisterfeld, 2001; Mitchell et al., 2008). Questions still remain on how we can distinguish the effects of natural changes on testate amoebae community from those caused by human-driven pollution at fine temporal scale specifically in Anthropocene Epoch (Qin et al., 2009; Wang et al., 2020). In this work, the record of testate amoebae together with sedimentological (radioactivity, physicochemistry, nutrient and organochlorine pesticides) and climatological data (air temperature and precipitation) of the largest reservoir in Guangdong Province in south China are presented. We measured more than 40 environmental factors particularly those related to human activities. The research hypotheses are: 1) Increasing human-driven pollution in Xinfengjiang Reservoir may result in distinct development stages of testate amoebae community at decadal scale. 2) Deterministic processes have a greater influence on the community assembly of testate amoebae than stochastic processes in Xinfengjiang Reservoir over three decades. In this study, we aim at answering the following questions: 1) How does the testate amoebae community change in a large subtropical reservoir impacted by human activities over the past three decades; 2) What is the relative importance of deterministic and stochastic processes in shaping the community assembly of testate amoebae in a large subtropical reservoir over the past three decades.

2. Material and methods

2.1. Site description and sampling approach

Xinfengjiang Reservoir, also called Evergreen Lake (Fig. 1a), is located in Guangdong Province, South China, about six km far from Heyuan City. It was created by the damming of the Xinfeng River, an affluent of East River. It is the largest reservoir in Guangdong Province covering an area of about 370 km^2 and with a storage capacity of about 13.9 km^3. The reservoir has a drainage area of about 5734 km^2, which covers about 25% of the East River watershed area (Cheng et al., 2015). The reservoir area is located within the boundaries of subtropical monsoon climate, and the wet season is from April to September. The mean annual rainfall and annual inflow at the dam site are 1974.7 mm and 192 m^3 s^{-1}, respectively (Table 1). Xinfengjiang Reservoir was planned and constructed to serve multiple purposes, and its operational requirements are largely connected with reservoir-operation water levels (Wang et al., 2019). Since coming into operation in October 1960, it has provided a considerable number of benefits including potable water supply, flood-prevention, hydropower generation, shipping and irrigation (Fig. 1b). The decline of water quality and eutrophication

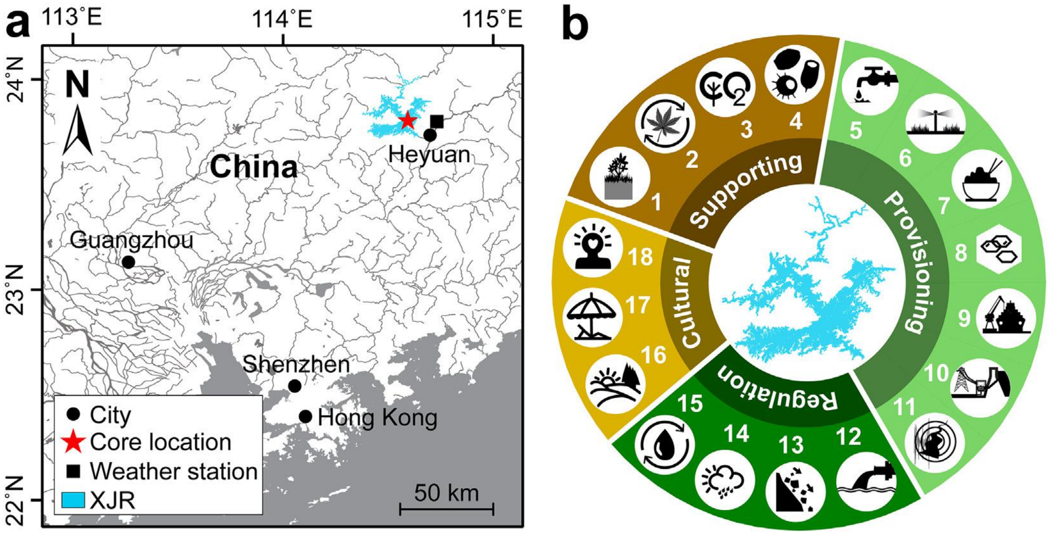

Fig. 1. Xinfengjiang Reservoir (XJR). (a) Map showing the coring location (star) and weather station (square). (b) A flowchart of supporting, provisioning, regulation and cultural ecosystem services of Xinfengjiang Reservoir. Arabic number from 1 to 18 show water storage, nutrient cycling, carbon cycling; aquatic life forms and fossilized organic remains; drinking water, irrigation water, aquafarming, raw materials (construction), transport, electricity generation, research (seismology); water purification, erosion regulation, climate regulation, water regulation; aesthetic values, recreation, and ecotourism. The map was created by QGIS version 3.12.2 (QGIS Development Team, 2020).

of Xinfengjiang Reservoir are largely due to the nutrient-rich domestic and industrial wastewater and fertilizers discharged from agricultures and fisheries (Han and Dumont, 2011).

In November 2006, sediment cores were collected from one of the deepest zones (water depth = 65 m) in Xinfengjiang Reservoir using a Kajak corer (80 mm diameter). In this study, one sediment core (length = 20 cm) was used for testate amoebae analysis because the sediment deposition rate in the reservoir is small and relatively constant over time (about 1.42 cm yr^{-1}) (Lin et al., 2010). The sampled sediment core was kept at −4 °C and shipped to laboratory. The sample was sliced into twenty-nine sub-samples. Twenty sub-samples were collected at 0.5 cm interval along the core for depths 0 to 10 cm and the other nine ones were gathered at 1 cm interval of the core depths 10 to 20 cm.

Table 1
Description of Xinfengjiang Reservoir.

Parameter	Measurement
Dam and spillway	
Construction began	1958
Opening year	1962
Dam height (m)	124
Dam length (m)	440
Dam width (m)	5
Service spillway capacity (10^3 m^3 s^{-1})	11.4
Tunnel spillway capacity (10^3 m^3 s^{-1})	1.7
Reservoir	
Average depth (m)	28.7
Maximum depth (m)	93
Surface area (km^2)	370
Normal volume (10^9 m^3)	10.8
Maximum volume (10^9 m^3)	13.9
Dead storage capacity (10^9 m^3)	4.31
Mean retention time (yr)	2
Annual inflow at the dam site (m^3 s^{-1})	192
Flood control level (m)	114–115
Power station	
Hydraulic head (m)	81
Installed capacity (10^6 W)	302
Average annual energy generation (10^{12} kWh)	0.99
Watershed	
Watershed area (km^2)	5734.0
Average annual rainfall (mm)	1974.7

References: Han and Dumont (2011); Cheng et al. (2015).

2.2. Sediment core chronology and multi-proxy

The sediment core was dated using ^{210}Pb method and one gram of dry sediment was adequate for this method (Lin et al., 2010; Sanchez-Cabeza and Ruiz-Fernández, 2012). Selected slices of the sediment core were measured for ^{210}Pb activity by gamma spectroscopy using an HPGeGWL-80,210-S well-type coaxial low background intrinsic germanium detector (Ortec, Oak Ridge, USA). The sediment was balanced into pre-weighed crucibles and dehumidified at 105 °C for 24 h. It was balanced again after getting cold in a desiccator. The sediment was then crushed, dried again at 105 °C for 12 h and re-weighed again after cooling in a desiccator. The dehumidified sediment was held in tubes which were sealed by epoxy. Each individual sample was counted for 24 h in a gamma counter and the total ^{210}Pb activity was computed. The excess ^{210}Pb was calculated as the difference between total ^{210}Pb and supported ^{210}Pb (or ^{226}Ra). The constant activity model was then applied to determine ^{210}Pb dates for different intervals of the core (Sanchez-Cabeza and Ruiz-Fernández, 2012). The elevated activities of ^{210}Pb have been identified in the atmosphere, hydrosphere and biosphere (Jia, 2013). Consequently, in sediments of <150 years old, ^{210}Pb may not be in equilibrium with ^{226}Ra probably due to the dry and wet depositions of ^{210}Pb derived from anthropogenic sources (Sanchez-Cabeza and Ruiz-Fernández, 2012). For that reason, anthropogenic ^{210}Pb in the sediment (excess ^{210}Pb) was investigated as an environmental contaminant (Jia, 2013; Sanchez-Cabeza and Ruiz-Fernández, 2012).

The dry bulk density of each sample was calculated as the mass of dry solids divided by total volume of the wet sample (Verstraeten and Poesen, 2001). The samples were weighed after being oven dried for 24 h at 105 °C and the sediment dry bulk density was calculated by dividing the dry sediment mass by its volume.

Heavy metal contents of the sediment were determined according to Bussan et al. (2019) using Agilent 725 inductively coupled plasma-optical emission spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Sample preparation, digestion and production of calibration curves and measurement are shown in supplementary material. The elements and wavelengths monitored were: aluminum: 308.215 nm; arsenic: 188.979 nm; beryllium: 234.861 nm; calcium: 317.933 nm; cadmium: 228.802 nm; cobalt: 228.616 nm; chromium: 283.563 nm; copper: 327.393 nm; iron: 238.204 nm; lithium: 670.784 nm; magnesium: 280.271 nm; manganese: 260.568 nm; molybdenum: 204.597

nm; sodium: 589.592 nm; nickel: 231.604 nm; lead: 220.353 nm; antimony: 206.836 nm; selenium: 196.026 nm; strontium: 407.771 nm; titanium: 190.801 nm; vanadium: 292.402 nm and zinc: 213.857 nm.

Total carbon, nitrogen and sulfur contents were also quantified (Karstens et al., 2016). Each sample (about 200 mg) was dried at 105 °C for 24 h, screened to <2 mm and subsequently crushed. Sediment organic matter was then quantified gravimetrically by loss-on-ignition in a muffle furnace at 550 °C for about 4 h. The carbon, nitrogen and sulfur contents were quantified by combustion in a Vario Max CNS elemental analyzer (Elementar, Hanau, Hesse, Germany). The carbon-to-nitrogen ratio was quantified as a ratio of the mass of carbon to the mass of nitrogen in each sample. This ratio served as an instrument for understanding the sources and limitation of sedimentary organic matter that could lead to information about the ecology of investigated microorganisms (Kaushal and Binford, 1999).

Organochlorine pesticides were measured according to Thevenon et al. (2013) using TSQ Quantum XLS Ultra triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Sample preparation and measurement are shown in supplementary material with details given in our previous study (Lin et al., 2010). Organochlorine pesticides found in sediment and analyzed by this work were hexachlorocyclo-hexane isomers alpha, beta, gamma and delta, 1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane and 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene. The results for hexachlorocyclo-hexane were given as the sum of the three isomers (alpha, beta and gamma) and dichlorodiphenyltrichloroethane (DDT) compounds as the sum of all metabolites.

2.3. Testate amoebae, weather and climate analyses

Twenty-nine homogenized sediment samples (volume = 1 cm^3) were put into clean glass beakers with distilled water and subsequently stirred gently for 5 min. The samples were then screened using 300 and 25 μm sieves to remove oversized and fine particles, respectively. The 25–300 μm sediment fractions were then washed into brown bottles and diluted to 50 ml with distilled water (Ndayishimiye et al., 2019). Observation of individuals with the fraction 25–300 μm was performed in the hydro-bios plankton chambers using a light microscope at 200–400× magnification (Supplementary Table 1). The identification of species followed the taxonomic references by Casper and Schönborn (1985), Mazei and Warren (2012, 2014, 2015), Ogunseitan (2005), Siemensma (2019) and Todorov and Bankov (2019). The statistically significant numbers of specimens were quantified using methods described in Patterson and Fishbein (1989). More than 150 individuals were counted for each sample (Supplementary Fig. 1), as a count of 150 or more individuals has been shown to be adequate for most samples (Payne and Mitchell, 2009). Species richness was calculated as a count of species per sample. The total abundance was measured based on counted testate amoebae individuals per 1 ml sediment sample. For biomass, we first measured the geometric shape of each species and calculated the volume using our measurements (shell length, breadth and diameter). The carbon biomass of each species was estimated from its shell geometric volume, with a carbon/volume conversion factor 1 $\mu m^3 = 1.1 \times 10^{-7}$ μg C (Weisse et al., 1990). The total biomass per sample was computed based on the sum of all testate amoebae individuals.

The air temperature and precipitation data were collected from a weather station of Heyuan (Fig. 1a). These data indicated that January was the coldest month of the year with dry weather, changeable with numerous hazy days, but the temperature did not fall below freezing point. Heyuan has the highest temperature in July (summer) and severe flooding is often associated with this month (Fig. 2).

2.4. Data analyses

The relative abundance of testate amoebae species along with species richness, abundance and biomass were plotted against the age and depth of the sediment core using Tilia 2.0.b.4 (Grimm, 1992). Q-mode hierarchical cluster analysis was used to define the succession stages in the testate amoebae community (Fishbein and Patterson, 1993). The samples ($n = 29$) were clustered into different stages of reservoir succession (i.e., development stages) using incremental sum of squares (Grimm, 1992). The sample-stages were compared based on the Tukey's honest significant difference with a significant level ($P <$

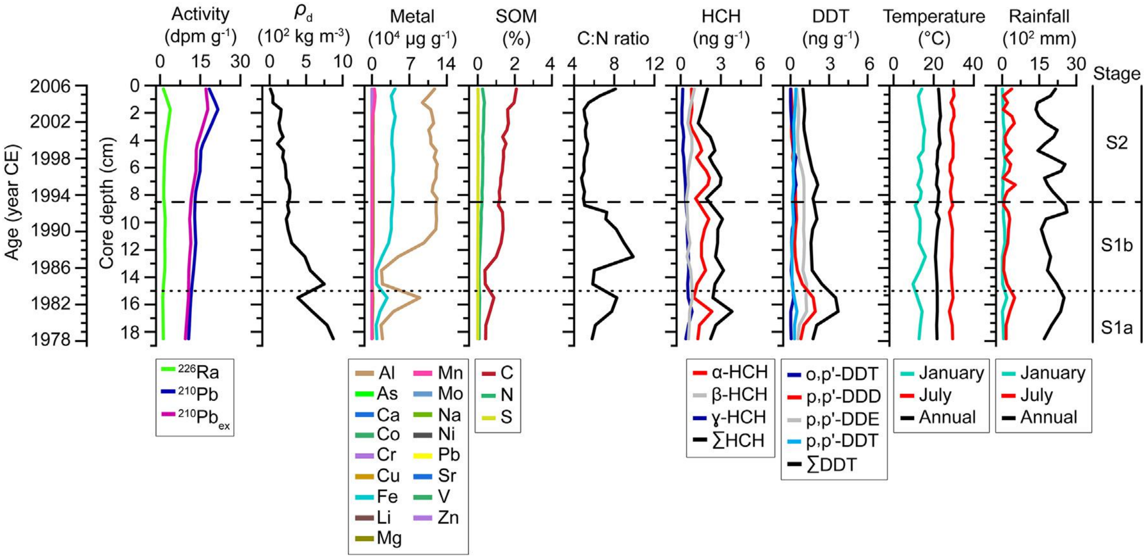

Fig. 2. Diagram of the down-core variation of measured environmental variables. Zones of major and minor compositional change in testate amoebae communities along the core are indicated by dashed and dotted lines, respectively. Abbreviations: ρ_d, sediment dry bulk density; Al, aluminum; As, arsenic; C, carbon; Ca, calcium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; Li, lithium; Mg, magnesium; Mn, manganese; Mo, molybdenum; N, nitrogen; Na, sodium; Ni, nickel; Pb, Lead; Ra, radium; Sr, strontium; S, sulfur; V, vanadium; Zn, zinc; Metal, residual metal concentration; SOM, sedimentary organic matter; C:N ratio, carbon-to-nitrogen ratio; HCH, hexachlorocyclo-hexane; α-HCH, alpha-hexachlorocyclo-hexane; β-HCH, beta-hexachlorocyclo-hexane; γ-HCH, gamma-hexachlorocyclo-hexane. Equations: $\sum HCH = \alpha\text{-}HCH + \beta\text{-}HCH + \gamma\text{-}HCH$; DDT, dichlorodiphenyltrichloroethane; o,p'-DDT, 1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane; p,p'-DDD, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane; p,p'-DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; p,p'-DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; $\sum DDT = o,p'\text{-}DDT + p,p'\text{-}DDD + p,p'\text{-}DDE + p,p'\text{-}DDT$. The sample sizes of the first (S1) and second (S2) stages are 12 and 17, respectively.

0.05). The testate amoebae diversity was measured using species richness and dominance indices. The dominance was calculated as one minus Simpson index with values 0 and 1 indicating that all species are equally present and one species dominates the community completely, respectively. The contribution of each species in the overall Bray-Curtis dissimilarity was determined by the similarity percentages using the abundance and biomass data, respectively.

Normality of the environmental variables was tested with the Anderson-Darling normality test using PAST version 3.13 (Hammer et al., 2001). Since most of the data did not fit the normal distribution, the species and environmental data were standardized using logarithmic transformations (McDonald, 2014). The species-environment relationships in testate amoebae abundance and biomass-based datasets were determined by multivariate redundancy analysis. After detrended correspondence analysis, results detected a unimodal response of the species data. Tests of significance of the main and all canonical axes were analyzed for the statistical assessment of the relation between testate amoebae and environmental variables (Monte Carlo test: 499 permutations). The stepwise forward selection of explanatory variables was employed in CANOCO version 5.0 (ter Braak and Šmilauer, 2012) (a detail on stepwise forward selection is shown in supplementary material). Four groups of environmental factors (physichochemistry, nutrient, organochlorine pesticides and weather) were formed based on explanatory variables with significant influence on the testate amoebae community at $P < 0.05$ (Supplementary Table 2). The variance explained by each environmental factor within the species data was determined by RDA-based variation partitioning analysis using CANOCO version 5.0 (ter Braak and Šmilauer, 2012). A partial least squares path modeling approach in R environment (R Core Team, 2019) was employed to quantify the interrelationship between environmental variables and testate amoebae using the abundance- and biomass-based datasets, respectively. We further employed multiple factor analysis to link five groups of descriptors (physichochemistry, nutrient, organochlorine pesticides, weather and testate amoebae). Multiple factor analysis was chosen because it permits the simultaneous coupling of numerous groups or subsets of variables defined on the same objects and to evaluate the general structure of the data (Abdi et al., 2013). The important steps used to perform multiple factor analysis with the package FactoMineR (Lê et al., 2008) in R environment (R Core Team, 2019) are summarized in supplementary material.

The testate amoebae community complexity (i.e., the number and size of populations and their interactions) and dynamics (i.e., how the members and their interactions change over time) were investigated using cohesion metric in R environment (R Core Team, 2019). This metric summed a total of the contribution of every species in the community considering that rare species were completely excluded. For a community complexity, scores vary between −1 and 0; while for community dynamics, scores fluctuate between 0 and 1 (Herren and McMahon, 2017). In addition, both neutral community model (Sloan et al., 2006) and null model for species co-occurrence (Gotelli and McGill, 2006) were used to analyze the relative importance of stochastic and deterministic processes that shaped testate amoebae community over time. The best fit distribution curve of the neutral community model was computed using the least square method in R version 3.6.0 (R Core Team, 2019). For the null model, 5000 random matrices were replicated and analyzed the difference between observed and simulated communities using the C-score index and SIM9 algorithm in EcoSim Professional version 1.0 (Entsminger, 2014). The strength of the stochastic and deterministic processes was illustrated using coefficients of determination and standardized effect size for the C-scores, respectively, with detail provided in supplementary material.

3. Results

3.1. Sediment dating, sedimentological and weather variables

The ^{210}Pb sediment radiochronology showed that the 20 cm sediment core dated from about 1978 to 2006 (Fig. 2). The sediment dry bulk density showed a slight decreasing trend over time with high values between 1978 and 1993 (mean and standard error $\mu \pm$ SE, 476.0 ± 67.1 kg m^{-3}, $n = 12$). Arsenic, calcium, chromium, cobalt, iron, lead and vanadium exhibited low concentrations (5–12,931 μg g^{-1}) between 1978 and 1986. The concentrations of aluminum and iron remained high and stable between 1990 and 2006 ($n = 21$, $114{,}763.3 \pm 1504.6$ and $38{,}995.8 \pm 429.1$ μg g^{-1}, respectively). The values of carbon-to-nitrogen ratio were high (5.05–9.89%) between 1984 and 1993. The organochlorine pesticides indicated a little decreasing trend between 1983 and 2006 except delta-hexachlorocyclo-hexane that was detected with 0.2 ng g^{-1} in one sample of 2003. The hexachlorocyclo-hexane reached its peak in 1981 with a sum of 3.8 ng g^{-1} for the three isomers (alpha, beta and gamma). The four metabolites of dichlorodiphenyltrichloroethane (DDT) reached their maximum in 1981 with a sum of 3.7 ng g^{-1}. The annual precipitation ($n = 29$) varied from 1362 to 2621 mm over time, with a mean value of 1979 ± 67 mm (Fig. 2). Changes in other sedimentological and weather variables are summarized in supplementary results.

3.2. Variability of testate amoebae community

A total of 27 testate amoebae species were observed from 29 samples along the sediment core (Fig. 3 and Supplementary Table 1). About 44% of species in the dataset belonged to the genus *Difflugia*. Other species were from genera *Arcella*, *Centropyxis*, *Cyclopyxis*, *Euglypha*, *Netzelia* and *Pseudodifflugia*. Species richness changed from 18 to 26 ($n = 29$, mean $= 22 \pm 0$ species). The total abundance varied from 616 to 825 ind. ml^{-1} ($n = 29$, mean $= 724 \pm 12$ ind. ml^{-1}) and was mainly contributed by *Centropyxis aculeata*, *Centropyxis aerophila*, *Difflugia globulosa*, *Difflugia mica*, *Difflugia penardi*, *Difflugia pristis*, *Netzelia gramen* and *Netzelia tuberspinifera*. The total biomass changed from 9.0 to 19.4 μg C ml^{-1} ($n = 29$, mean $= 14.0 \pm 0.5$ μg C ml^{-1}) and was largely contributed by *Centropyxis aculeata*, *Centropyxis sylivatica*, *Cyclopyxis eurystoma*, *Difflugia globulosa*, *Difflugia limnetica*, *Difflugia oblonga*, *Netzelia corona* and *Netzelia tuberspinifera* (Fig. 3).

The first and second stages of Xinfengjiang Reservoir spanned from roughly 1978 to 1993 and 1993 to 2006, respectively (Fig. 3). The two community diversity indices (species richness and dominance) showed a significant difference between stages 1 and 2 at $P < 0.05$ (Fig. 4a). The contributors to the overall community dissimilarity in abundance-based dataset were *Arcella arenaria*, *Arcella hemisphaerica*, *Arcella vulgaris*, *Centropyxis sphagnicola*, *Centropyxis sylivatica*, *Difflugia bryophila*, *Difflugia kabylica*, *Difflugia oblonga*, *Netzelia corona* and *Netzelia mulanensis*. The top ten contributors in biomass-based dataset were *Centropyxis aculeata*, *Centropyxis sylivatica*, *Cyclopyxis eurystoma*, *Cyclopyxis kahli*, *Difflugia acuminata*, *Difflugia globulosa*, *Difflugia penardi*, *Difflugia pyriformis*, *Euglypha tuberculata* and *Netzelia corona* (Fig. 4b).

3.3. Testate amoebae-environment relationships

The species-environment relationships of the two stages were different, and both abundance-based data and biomass-data showed similar results (Fig. 5 and Supplementary Table 2). In stage 1, few environmental variables significantly influenced testate amoebae community at $P < 0.05$ (three for abundance data, three for biomass data, Supplementary Table 2). In the abundance-based dataset, nitrogen was generally negatively correlated with *Arcella* and *Netzelia*. The percentage of community variation explained by pure nutrient and the correlation between nutrient and testate amoebae were the highest and

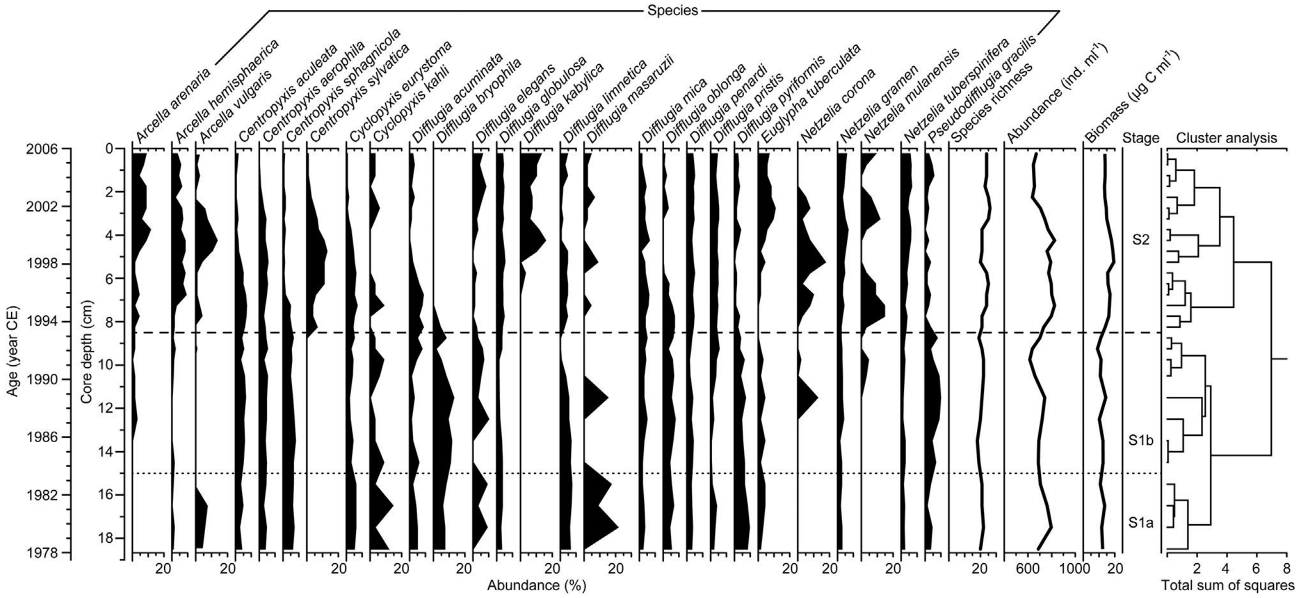

Fig. 3. Percentage of different species of testate amoebae along the sediment core from Xinfengjiang Reservoir. Zones of different developmental stages are indicated by dashed and dotted lines, respectively. The total species richness, abundance and biomass are shown (right). The sample sizes of the first (S1) and second (S2) stages are 12 and 17, respectively.

negative, respectively (e.g., 15.5% and −0.778 in abundance-based dataset of Fig. 5 and Table 2, respectively). In stage 2, high percentage of community variation explained by pure organochlorine pesticides (42.8%–55.6%). *Centropyxis* was the highest in both abundance- and biomass-based datasets and *Difflugia* species with shell lengths 60–300 μm (e.g., *D. acuminata*, *D. bryophila* and *D. oblonga*) were generally negatively correlated with physicochemistry. Further, six and seven environmental variables were significantly correlated with abundance-based and biomass-based communities, respectively (Supplementary Table 2). The percentage of community variation explained by pure organochlorine pesticides and total effects linked with pesticides-testate amoebae interrelationship were the greatest and positive, respectively (Fig. 5 and Table 2).

The relationship between samples and five groups of variables (physichochemistry, nutrient, organochlorine pesticides, weather and testate amoebae) explained the patterns of the testate amoebae community structure across time and stages and a split of stage 1 into two sub-stages (approximately 1978–1983 for sub-stage 1a

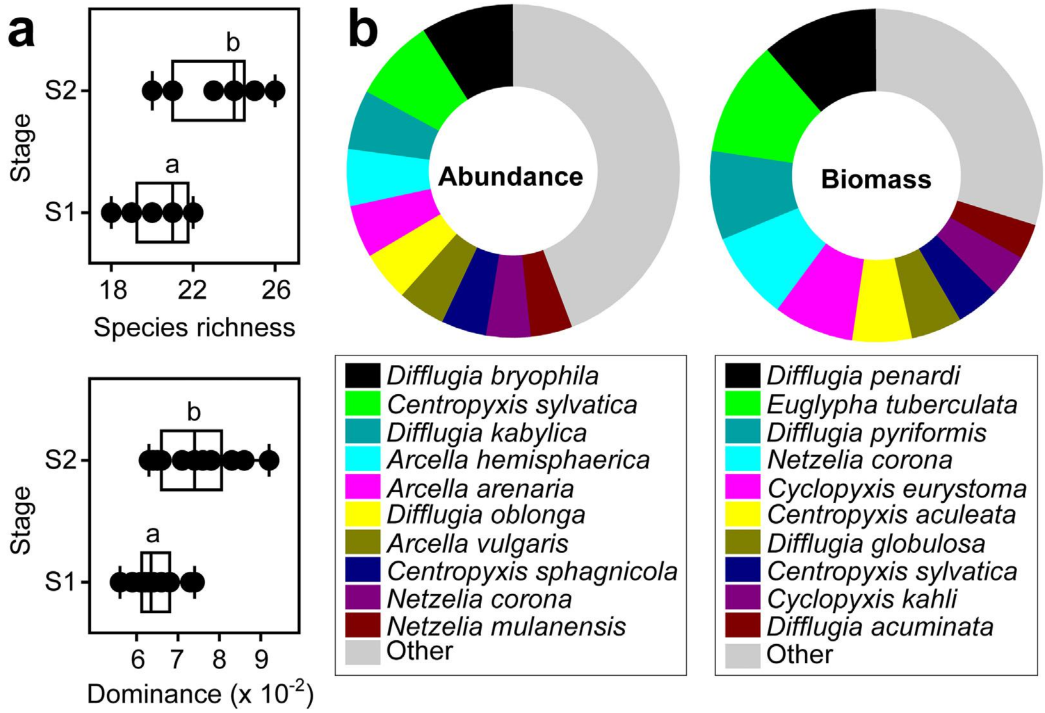

Fig. 4. Testate amoebae diversity and community dissimilarity in the two developmental stages of Xinfengjiang Reservoir. (a) Boxplots showing species richness and dominance (0 and 1 indicate that all species are equally present and one species dominates the community completely, respectively). The different letters (a and b) indicate significant difference between groups at $P < 0.05$. S1, stage 1; S2, stage 2. (b) The average contributions of each species to the average overall Bray-Curtis dissimilarity. The contributions of 17 low-abundance species (other) are not separately shown for simplicity.

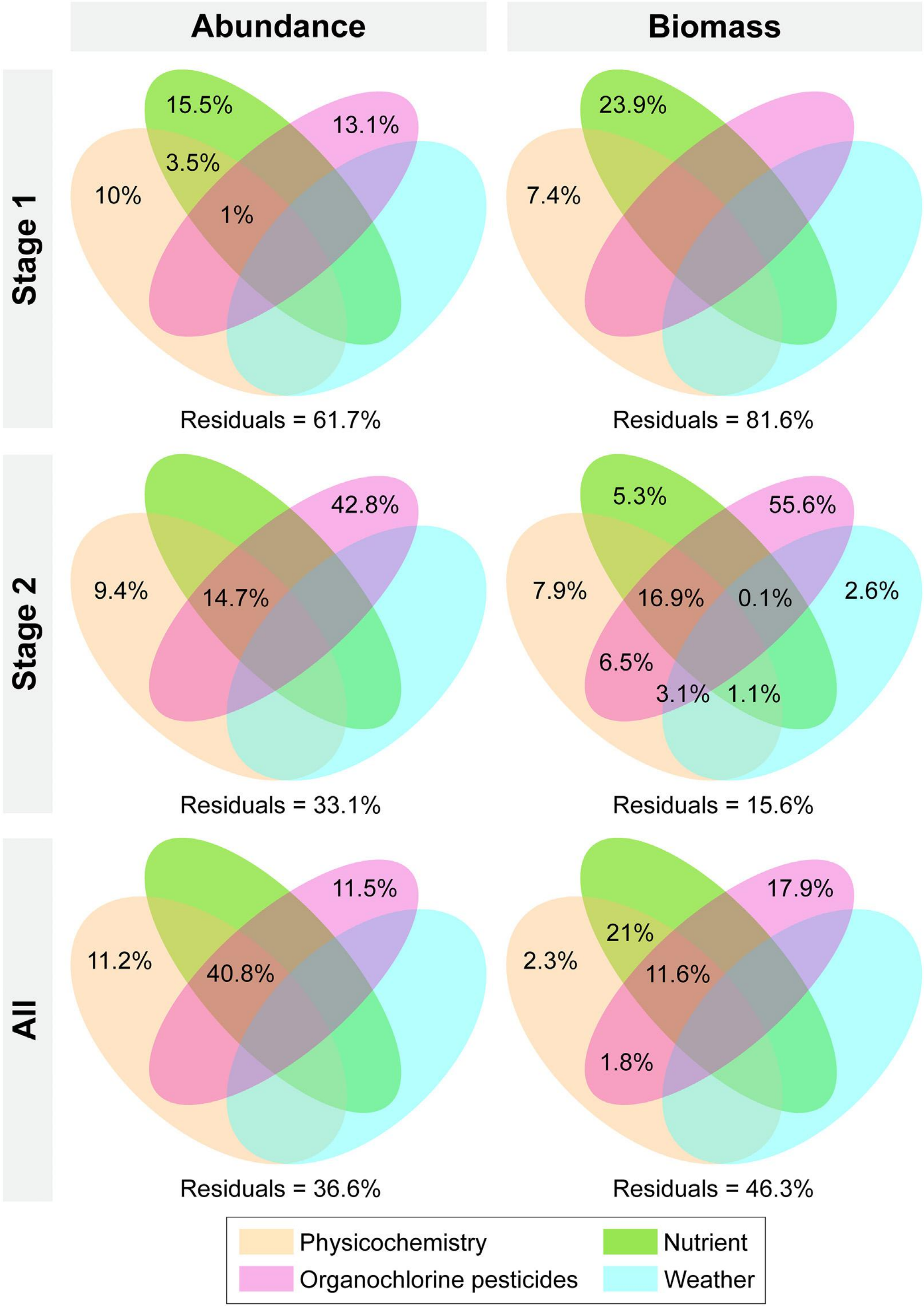

Fig. 5. Testate amoebae-environment relationship in Xinfengjiang Reservoir. Explained variances <1% are not shown for simplicity. Variation partitioning analysis results for environmental conditions (physicochemistry, nutrient, organochlorine pesticides and weather) were calculated using explanatory variables with $P < 0.05$ in redundancy analysis results. Significant explanatory variables: dry bulk density; arsenic; calcium; cobalt; chromium; iron; lead; vanadium; nitrogen; sulfur; carbon-to-nitrogen ratio; alpha-hexachlorocyclo-hexane; beta-hexachlorocyclo-hexane; gamma-hexachlorocyclo-hexane; 1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane; 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane; 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; rainfall. The sample sizes of stages 1 and 2 are 12 and 17, respectively.

and 1983–1993 for sub-stage 1b) (Fig. 6a and Supplementary Fig. 2). Testate amoebae abundance and biomass were generally negatively correlated to physichochemistry (e.g., sediment dry bulk density) and nutrient (e.g., carbon-to-nitrogen ratio) (Fig. 6b and Supplementary Fig. 2).

3.4. Stochastic and deterministic processes shaping the testate amoebae community

The testate amoebae community underwent positive directional change from 1978 to 2006 (Fig. 7). Lower (0.29 ± 0.01) and higher

Table 2
Relationship between environmental variables and testate amoebae based on partial least squares path modeling.

Environmental variable	Stage 1		Stage 2		All stages	
	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass
Physicochemistry	−0.413	−0.430	−0.839	−0.704	−0.085	0.303
Nutrient	−0.778	−1		0.749		0.709
Organochlorine pesticides	0.179		0.433	0.022	−0.266	0.534
Weather				0.032		

Note that the environmental variables are explanatory variables with $P < 0.05$ in redundancy analysis results (See Fig. 5).
Significant explanatory variables: dry bulk density; arsenic; calcium; cobalt; chromium; iron; lead; vanadium; nitrogen; sulfur; carbon-to-nitrogen ratio; alpha-hexachlorocyclo-hexane; beta-hexachlorocyclo-hexane; gamma-hexachlorocyclo-hexane; 1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane; 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane; 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; rainfall.
The dimensionless quantities in table (total effects) are the sum of direct effects and indirect effects.
The sample sizes of stages 1 and 2 are 12 and 17, respectively.

(0.35 ± 0) positive cohesion values of stages 1 and 2, demonstrated a strong positive directional change of the testate amoebae community over time (community dynamics). In contrast, different negative cohesion values of −0.32 ± 0 and –0.36 ± 0.01, have shown a strong negative directional change of the testate amoebae community over time (community complexity). Both stochastic and deterministic mechanisms shaped the testate amoebae community between 1978 and 2006 (Fig. 7). A change in the relative abundance of different species was connected to random demographic dynamics (neutral processes) and the neutral processes were important in both stages 1 and 2 (R^2 values were 0.759 and 0.779 in Sloan neutral community model, respectively). A close relationship between environmental conditions and testate amoebae species has shown better growing in eutrophic and warm conditions in stage 2 which was linked with non-random patterns in the biotic neighborhoods of species (deterministic processes) explained by standardized effect size >2 in the null model (Fig. 7).

4. Discussion

Testate amoebae are sensitive to different forms of environmental changes that are of scientific, protection or policy interest, with environmental pollution as the most frequent target (Patterson et al., 1996; Qin et al., 2009; Wang et al., 2020). In Xinfengjiang Reservoir, testate amoebae communities exhibited two distinct succession stages (Fig. 3), corresponding with shifts shown by earlier studies of long-term testate amoebae dynamics highlighting patterns of community development along with reservoir formation or evolution (Gurvich, 1975). These shifts might be driven by human-caused pollution due to rapid urbanization, agriculture development and industrial activity (Ndayishimiye et al., 2020a; Patterson et al., 1996; Qin et al., 2009).

4.1. Response of testate amoebae to natural change and human activity

High concentration of small particles (e.g., Al-/Fe-(hydr) oxides) in sediment of a lake can affect the sediment grain sizes and mineralogy, and testate amoebae (e.g., *Difflugia* and *Centropyxis*) can use these particles or grains to construct their shells (Armynot du Châtelet et al., 2010; Ndayishimiye et al., 2020b). This study shows that a long-term change in mineralogy of Xinfengjiang Reservoir impacted the testate amoebae community (Fig. 5 and Supplementary Table 2). A strong and negative correlation between physicochemistry and testate amoebae (e.g., *Arcella* and *Netzelia*) in stage 1 was possibly connected to declined iron and vanadium concentrations in sediment. A strong and negative correlation between physicochemistry and testate amoebae (e.g., *Centropyxis* and *Cyclopyxis*) in stage 2 was possibly linked with reduced calcium concentration in sediment. Higher sediment dry bulk density values in stage 1 than in stage 2 (Fig. 2) may indicate that the stage 1 was an environment favored by *Centropyxis*, *Cyclopyxis* and large size *Difflugia* species, while stage 2 was more suitable for *Arcella*, *Netzelia* and small size *Difflugia* species.

Reservoirs always receive organic carbon input from different sources (Kaushal and Binford, 1999) and this can influence the testate amoebae community through nutrient-enrichment process (Ren et al., 2018). It was clearly shown that shifts in the testate amoebae community were correlated with the eutrophication history of Xinfengjiang Reservoir because the carbon-to-nitrogen ratios were low (4.7 to 9.9) and the values constantly low after 1993 (Fig. 2). In general, the proportions of total organic carbon to total nitrogen in sediment <10 suggests a pronounced change in total nitrogen concentrations possibly due to algal growth in water (Meyers, 1997). Previous work revealed that the trophic state indices had considerably increased due to an increase in primary production of phytoplankton associated with watershed human activities (Han and Dumont, 2011). Hence, it is likely that a rapid decline in *Centropyxis* and occurrence of small size *Difflugia* species and *Netzelia* in more eutrophic and warm environment (stage 2) was possibly linked with changes in primary production and suitable food supply (Ndayishimiye et al., 2019, 2020a).

The use of organochlorine pesticides has been officially prohibited in China since 1983, although they are still utilized in some manufacturing processes (Jiang et al., 2009; Lin et al., 2010; Qian et al., 2020; Qiu et al., 2004). Hence, an obvious decrease in concentration of organochlorine pesticides was very clear after 1983 (Fig. 2). Our results evidently showed a close connection between weather and organochlorine pesticides in Xinfengjiang Reservoir across two stages (Fig. 6b and Supplementary Table 3), suggesting that organochlorine pesticides were not transported by runoff from the reservoir watershed (Cindoruk and Ozturk, 2016; Qiu et al., 2004). Instead, it is believed to be strongly linked with air dust deposition (Lin et al., 2010). The two important reasons for this air dust deposition could be: first, organochlorine pesticides can enter into the air by way of regional atmospheric transport from contaminated areas such as metropolitan cities (Fang et al., 2017; Qiu et al., 2004). This is likely to occur because Xinfengjiang Reservoir is located in the vicinity of a metropolitan area consisting of Guangzhou, Shenzhen, Hong Kong, Macau and Zhuhai cities. Second, atmospheric deposition of organochlorine pesticides in a large inland water body (lake or reservoir) can be achieved by wet deposition of contaminants (Cindoruk and Ozturk, 2016; Qiu et al., 2004).

High-level exposure to organochlorine pesticides can have a direct poisonous or beneficial effect on microbial communities including testate amoebae (Malik et al., 2019; Petz and Foissner, 1989). Previous field experiments demonstrated that contamination of populations of testate amoebae species (e.g., *Trinema lineare*, *Corythion dubium* and *Schoenbornia humicola*) may occur mostly through eating plankton (food) contaminated with organochlorine pesticides (Petz and Foissner, 1989). A lack of field experiment and data on plankton and pollutant-tolerance testate amoebae species of Xinfengjiang Reservoir may place a limitation on the given impact of organochlorine pesticides on the testate amoebae community. However, our data suggest that organochlorine pesticides significantly influenced testate amoebae community with a stronger effect in stage 2 (Fig. 5 and Supplementary Table 2). They also demonstrate that a slight decrease in species richness,

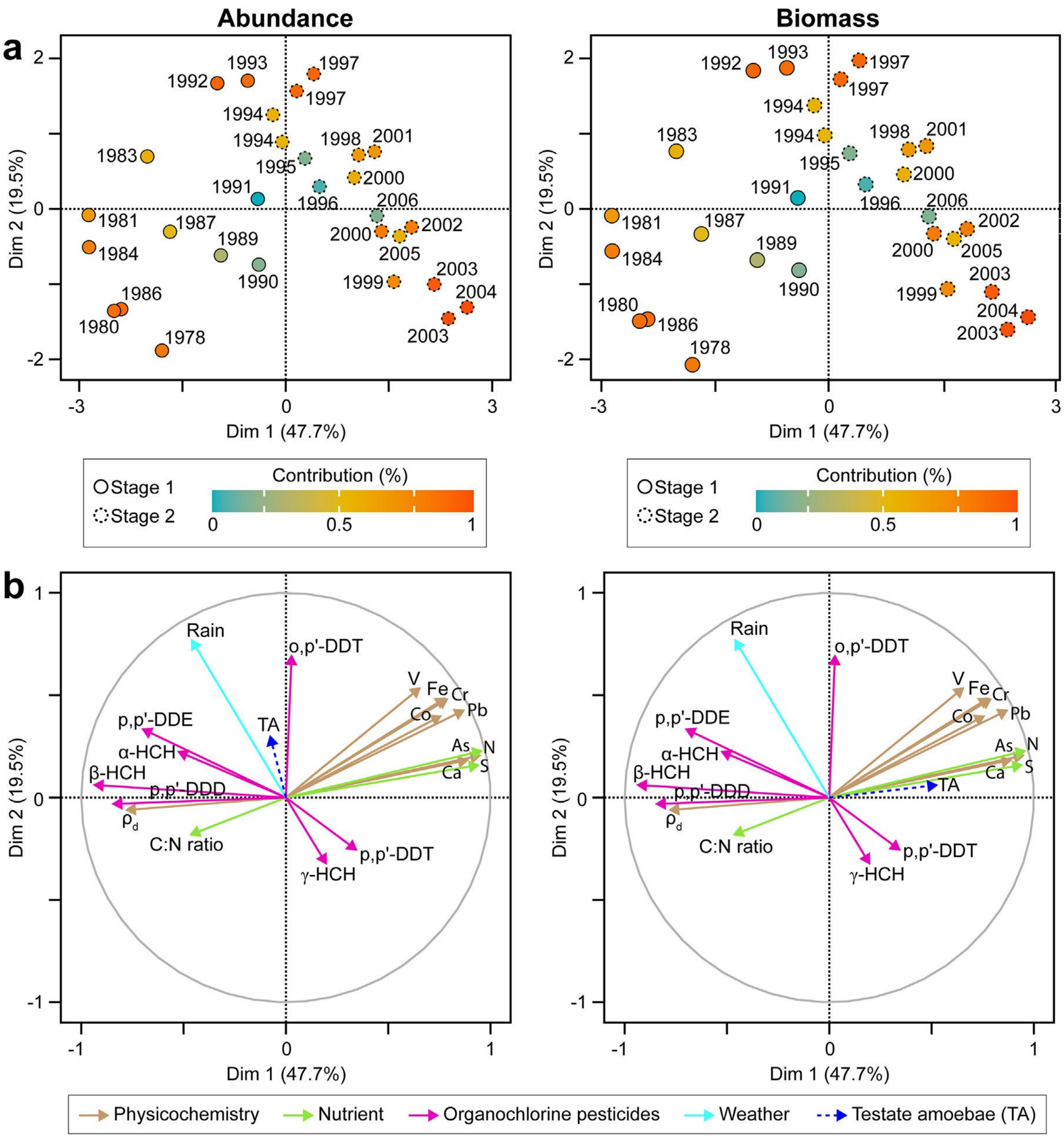

Fig. 6. Multiple factor analysis (MFA) of the environmental variables (physicochemistry, nutrient, organochlorine pesticides and weather) and testate amoebae (abundance- or bimass-based datasets). Biplot axes 1 and 2 are significant at $P < 0.01$. (a) MFA results for testate amoebae communities and environmental variables. Samples with similar profiles are close to each other on the factor map (stages 1 and 2). Samples of stage 1 are slightly separated into two subgroups. Arabic numbers from 1978 to 2006 show sediment-producing years. (b) Projection of environmental variables onto the plane defined by the first two MFA principal components. The radius of a grey circle represents the maximum length of a partial standardized axis. The coordinates of each variable are the correlation coefficients with the two first MFA principal components. The variables are better represented in this plane when arrow points are close to the circle. Explanatory variables with significant influence on the testate amoebae community: ρ_d, sediment dry bulk density; As, arsenic; Ca, calcium; Co, cobalt; Cr, chromium; Fe, iron; Pb, lead; V, vanadium; N, nitrogen; S, sulfur; C:N ratio, carbon-to-nitrogen ratio; α-HCH, alpha-hexachlorocyclo-hexane; β-HCH, beta-hexachlorocyclo-hexane; γ-HCH, gamma-hexachlorocyclo-hexane; o,p'-DDT, 1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane; p,p'-DDD, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane; p,p'-DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; p,p'-DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane.

abundance and biomass over time (Fig. 3) were linked with a decline in highly sensitive species (e.g., *Difflugia acuminata*, *D. bryophila* and *D. oblonga*) by environmental change (Ndayishimiye et al., 2020a; Patterson et al., 1996; Qin et al., 2009). Strong and positive correlation-based structural equations between organochlorine pesticides and testate amoebae community (Table 2) indicate that shifts in pesticides are synchrony with shifts in the testate amoebae community over time (Figs. 2 and 3). Variance partitioning analysis, as an alternative approach, can make things easier by attributing less importance to organochlorine pesticides in stage 1 because abundances of *Arcella*, *Netzelia*, *Centropyxis* (e.g., *C. sylvatica*) and *Difflugia* (e.g., *D. kabylica*) were low, and more importance on organochlorine pesticides in stage 2 through decline in *Centropyxis*, *Cyclopyxis* and *Difflugia* (such as *D. acuminata*, *D. bryophila* and *D. oblonga*) (Fig. 5). This may demonstrate that most important shift in the testate amoebae community was closely associated with changes in environmental variables specifically those driven by human-caused pollution.

It is an essential goal in ecology to quantify the relative importance of deterministic and stochastic processes for the assembly of microbial communities (Dini-Andreote et al., 2015; Zhou and Ning, 2017). Generally, both deterministic and stochastic processes can occur synchronously in the assembly of local communities (Chen et al., 2019; Nyirabuhoro et al., 2020; Ren et al., 2018; Wang et al., 2020). The ecological selection, imposed by both abiotic and biotic variables

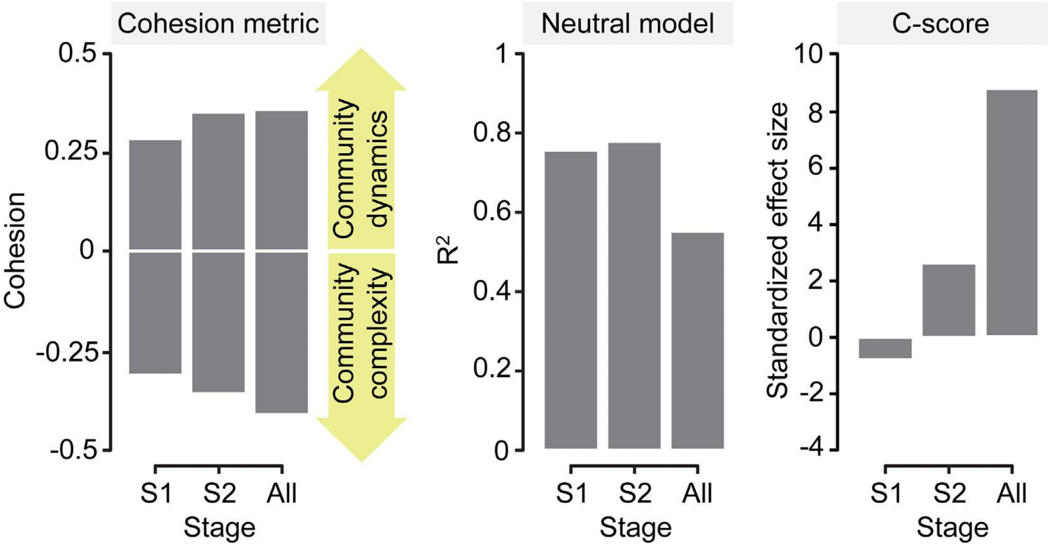

Fig. 7. Testate amoebae community change and assembly mechanisms shaping species composition in Xinfengjiang Reservoir. The community dynamics and complexity are shown by positive and negative cohesions, respectively (left column). Potential interpretations of results are demonstrated by arrows adjacent to each panel. The influences of stochastic and deterministic processes are shown by Sloan neutral community model (middle column) and null model (right column), respectively. S1, stage 1; S2, stage 2. The sample sizes of stages 1 and 2 are 12 and 17, respectively.

(deterministic processes), can influence organismal fitness and determine the community composition and relative abundance of species; consequently, the environmental variables can explain part of the variation in testate amoebae community (Wang et al., 2020). This study clearly indicates that both stochastic and deterministic processes shaped the testate amoebae community assembly over the past three decades, but deterministic processes were more important in eutrophic and warm environment. A fit to Sloan neutral community model with high R^2-values in the two stages (Fig. 7) evidently suggest that stochastic processes that can influence the testate amoebae species composition over time (Ren et al., 2018; Wang et al., 2020). The null model of stage 2 with high standardized effect sizes (in general value is >2 for species segregations and deterministic processes) might be evidence for the testate amoebae community shifting from species characteristic of relatively oligotrophic waters (e.g. *Netzelia tuberspinifera*) to species more common to mesotrophic and eutrophic ecosystems (e.g. *Netzelia corona*) (Qin et al., 2009).

4.2. Ecological impact and implications for future research

Reservoirs contain an important percentage of inland freshwater that contribute supporting, provisioning, regulation and cultural ecosystem services (Han and Dumont, 2011; Thevenon et al., 2013). However, landscape change and atmospheric pollution associated with human activity around these reservoirs may lead to biodiversity changes and significant disruption of ecosystems in complex ways (Hayes et al., 2017). Managing these reservoirs requires the assessment of the impact of both natural and human actions on the ecosystems and a substantial attention ecologically (Han and Dumont, 2011; Wang et al., 2020; Yang et al., 2016). In Xinfengjiang Reservoir, the testate amoebae community was impacted by both natural and anthropogenic changes over the past three decades, but at different degrees. Atmospheric pollutants linked with human activities (e.g., organochlorine pesticides) are correlated with a large fraction in variability of testate amoebae communities in this study. Because of the well-known biological activity of these chemicals it seems likely that this is not only a correlation but a causal relationship. The two important developmental stages of Xinfengjiang Reservoir may reflect a stronger influence of human disturbance than natural change over time. Restoration and protection of the important reservoirs should be a main concern because persistent environmental contaminants that enter a reservoir are retained in the sediment and sometime become more concentrated through food-chain with time (Lin et al., 2010; Thevenon et al., 2013). Regular monitoring of the testate amoebae community of Xinfengjiang Reservoir and suitable actions should be done to prevent spreading of pollutants and its influences. The priority areas for any further investigation must take into consideration of more reservoirs and anthropogenic pollutants to sustain a successful scale-up of our conclusion across a wide array of reservoirs. This will certainly suggest a sustainable management practice that can slow the reservoir degeneration, thereby help to keep reservoirs sustainable, healthy and beautiful.

5. Conclusions

This work presents a 30-year multi-proxy record of testate amoebae from a large subtropical reservoir (Xinfengjiang Reservoir) in south China. The testate amoebae community clearly indicated two important developmental stages (roughly 1978–1993 for stage 1 and 1993–2006 for stage 2). The relationships between environmental conditions (physicochemistry, nutrient, organochlorine pesticides and weather) and testate amoebae suggested a pronounced impact of human disturbance than natural change on the testate amoebae community variation over time. The results indicate that the testate amoebae community can be employed to monitor the conditions of continuously changing ecosystems in subtropical reservoirs.

The atmospheric deposition of organochlorine pesticides might be responsible for the dynamics of testate amoebae communities. The eutrophication facilitated the reproduction of testate amoebae which grow better in the slightly eutrophic conditions and a decline in the populations of several other sensitive species. In general, testate amoebae community dynamics were also shaped by stochastic processes, which were important in both stages 1 and 2, and strong community-environment relationship in stage 2 connected to non-random patterns in the biotic neighborhoods of species (deterministic processes). This study highlights the linkage between succession of microbial communities in large reservoirs and anthropogenic disturbance and natural change at decadal scale.

CRediT authorship contribution statement

Jean Claude Ndayishimiye: Investigation, Formal analysis, Writing original draft, Validation, Writing - review & editing. **Tian Lin**: Sampling, Data curation, Validation, Writing review & editing. **Pascaline Nyirabuhoro**: Formal analysis, Writing - original draft, Validation, Writing- review & editing. **Gan Zhang**: Sampling, Data curation, Validation, Writing review & editing. **Wenjing Zhang**: Writing review & editing. **Yuri Mazei**: Writing review & editing. **Hossein Ganjidoust**: Writing review & editing. **Jun Yang**: Conceptualization, Methodology, Resources, Formal analysis, Writing - original draft, Validation, Writing review & editing.

Declaration of competing interest

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147026.

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