Effects of natural and anthropogenic changes on testate amoebae communities in an alpine lake over the past 2500 years

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HIGHLIGHTS

- Three succession stages were identified in testate amoebae of Lake Lugu during past 2500 years.
- In the first stage, the community was dominated by *Centropyxis* and influenced by soil erosion.
- A shift in the dominant genera in early second stage was controlled by soil erosion and total nitrogen.
- In the third stage, *Difflugia*-rich community was driven by soil erosion and human activities.
- Community turn-over rate was the highest and lowest in the third and first stages, respectively.

GRAPHICAL ABSTRACT
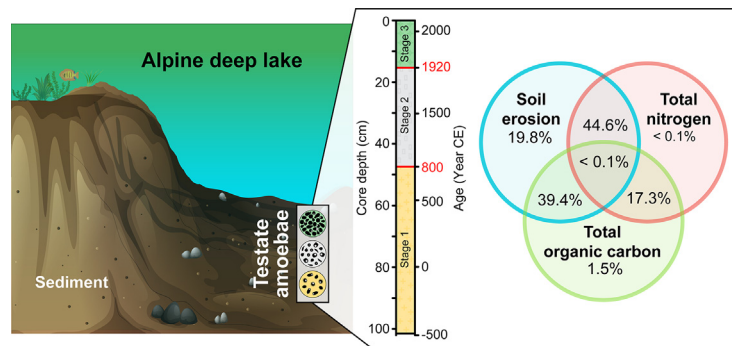
ABSTRACT

Deep high-altitude mountain lakes can act as a natural laboratory, and have the potential to contribute ecological data for understanding the way natural climate and anthropogenic changes that can affect the ecosystems. We present a multi-proxy record from a sediment core from such a lake (Lake Lugu) in southwest China with emphasis on the changes in the testate amoebae community, along with sedimentological data (magnetic susceptibility, total organic carbon/TOC and total nitrogen/TN) over the last 2500 years. In total, 29 testate amoebae species belonging to eight genera (*Arcella*, *Centropyxis*, *Cyphoderia*, *Difflugia*, *Netzelia*, *Phryganella*, *Pseudodifflugia* and *Zivkovicia*) were identified. Three stages were clearly defined for the lake based on testate amoebae community. The first stage dated to about 500 BCE–800 CE, with the testate amoebae community dominated by *Centropyxis* and influenced by soil erosion. The second stage (about 800–1920 CE) was characterized by a gradual increase of TOC and TN and an abrupt shift from *Centropyxis*-dominated to *Difflugia*-dominated communities. The third stage (about 1920–2010 CE) showed the pronounced impact of environmental change, high proliferation of *Difflugia* and a strong influence of human activities. Our results suggest that the testate amoebae assemblages in this high-altitude mountain lake are sensitive paleoenvironmental indicators that can help to monitor alpine lake ecosystem change and model lake succession under changing climate and environment. The potential causes of changes in the testate amoebae species composition and three stages of Lake Lugu succession were soil erosion and pollutants. The soil erosion led to the inwash of terrestrial particles and few testate amoebae species into Lake Lugu showing the importance of stochastic processes. The nutrient enrichment from soil erosion generated

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disturbances in the environment and species competition that led to the proliferation of some species and disappearance of others through niche based deterministic processes.

1. Introduction

The reconstruction of past conditions forms the basis for interpreting the long-term development of earth's climate systems and environmental issues (Berke, 2018; Li et al., 2016; Sheng et al., 2015; Xiao et al., 2014). The Yunnan Plateau is located in the southwest China and possesses many high-altitude mountain lakes (Chang et al., 2018; Shen, 2013; Yang et al., 2005, 2006). Over recent decades, the sediment records of Yunnan Plateau's lakes have been explored with the purpose of understanding the connections between regional climate variability and monsoons (Li et al., 2016; Sheng et al., 2015; Wang et al., 2010; Zheng et al., 2014), the impact of human activities on the lake ecosystems (Liu et al., 2019; Wu et al., 2008; Zhang et al., 2013) and global warming effects on the habitats and microbial communities (Wang et al., 2017; Yang et al., 2006). Nevertheless, there is a lack of work on developing paleolimnological tools to distinguish local and regional climatic conditions in Yunnan over multi-millennial time-scale (Chang et al., 2018; Li et al., 2016; Sheng et al., 2015; Wang et al., 2014; Xiao et al., 2014). This lack of a longer timescale perspective has the potential to limit the understanding of lake evolution in relation to variability in the Asian monsoon and human impacts on the lake ecosystem (Chang et al., 2018; Li et al., 2016; Sheng et al., 2015; Wang et al., 2010).

The testate amoebae (Amoebozoa: Arcellinida; Rhizaria: Euglyphida, Tectofilosida) are single-celled organisms characterized by the presence of a test (Adl et al., 2019; Ogden and Hedley, 1980; Todorov and Bankov, 2019). They are abundant in freshwater lakes and rivers worldwide (Asioli et al., 1996; Burdíková et al., 2012; Davidova and Vasilev, 2013; Ju et al., 2014; Patterson et al., 1985; Roe and Patterson, 2006; Yang et al., 2010) and play an important role in the lake ecosystem; for example in material cycling, energy flow and water purification (Han et al., 2011b; Ogden and Hedley, 1980). In addition, testate amoebae are abundant in terrestrial ecosystems (Ehrmann et al., 2012; Smith et al., 2008), and play a pivotal role for diverse ecological processes such as carbon, nitrogen and silicon cycling (Puppe et al., 2015; Schröter et al., 2003). The testate amoebae from lake sediment have been documented at species level and employed to reconstruct the past climate and environment (Asioli et al., 1996; Charman, 2001; Mitchell et al., 2008; Patterson et al., 1985; Prentice et al., 2018; Qin et al., 2009). In this context, testate amoebae are well-suited to act as bioindicators due to their sensitivity to environmental changes: First, the populations of testate amoebae can vary with natural climate variability (Burdíková et al., 2012; Han et al., 2011a; Ren et al., 2018; Yang et al., 2006). In the summer, *Difflugia* can grow better compared to *Arcella* and *Centropyxis* potentially due to higher temperatures and better food supply (Burdíková et al., 2012; Davidova and Vasilev, 2013; Han et al., 2011a). Second, the latitude and temperature control the spatial distribution of the testate amoebae, thus, more species are found to be more abundant in subtropical flood plains than in boreal or alpine lakes (Ju et al., 2014; Yang et al., 2010). Third, species surviving in the new environment are those with high capability of adaptation (Han et al., 2011b). The reconstruction of past climates and environments using testate amoebae proxies has focused on the water table, pH and nutrients and recently on the water temperature (Ndayishimiye et al., 2019). For the Yunnan Plateau's lakes, such reconstruction has been given a less attention (Ndayishimiye et al., 2019; Yang et al., 2005, 2006). Hence, the multi-proxy approach should be useful in the reconstruction of past changes in the plateau lakes.

Community composition, driven by physical and chemical factors, is shaped at least in part by deterministic processes (Gotelli and McGill, 2006). These processes are also known as niche-based processes (environmental filtering) producing the competitive patterns of species diversity and composition in community ecology (Gotelli and Graves, 1996; Nyirabuhoro et al., 2020). In contrast, community composition can also be controlled by random fluctuations in demographic processes (e.g., birth, death and migration) or shaped by stochastic/neutral processes (Sloan et al., 2006). Both stochastic and deterministic processes can simultaneously influence community assembly, but to different degrees which may vary from system to system (Chen et al., 2019; Nyirabuhoro et al., 2020). Thus, it is important to quantify the relative importance of these ecological processes in shaping testate amoebae community assembly.

In the current study, we present the record of testate amoebae along with magnetic susceptibility, total organic carbon (TOC) and total nitrogen (TN) from a sediment core of Lake Lugu, Yunnan Plateau. We hypothesized that: 1/ Lake Lugu may show different stages characterized by distinct testate amoebae community compositions. 2/ The testate amoebae community in different stages may exhibit distinct responses to the environmental factors and ecological processes. To test our hypotheses, we set the following objectives: (i) identification of the number of stages in the Lake Lugu succession over the past 2500 years; (ii) determination of the influence of environment and ecological processes on the testate amoebae community assemblage across different stages of Lake Lugu succession; (iii) investigation of the connections among testate amoebae community and watershed environmental changes by comparing our records with other fossil records.

2. Materials and methods

2.1. Study site and sampling

Lake Lugu (27°39′–27°45′N, 100°44′–100°50′E) is a high-altitude (2690 m a.s.l.) semi-closed deep lake located in the northwest Yunnan Plateau in southwest China (Fig. 1 and Table 1). Its watershed is located in the upper reaches of Yangtze River, the Jinsha River system. The lake is surrounded by fault cliffs, making the Lake Lugu landscape one of the most beautiful sceneries of China (Wang and Dou, 1998). The substratum of the watershed is made up of limestone, mudstone and sandstone (Zhang et al., 1997). The soils include Alfisols, Oxisols, Entisols and Mollisols (Bai et al., 2002). The terrestrial vegetation covers 47.6% of the total catchment area and is organized into different forests zones along an altitudinal gradient (Wang et al., 2015). Below 3000 m a.s.l., the vegetation is dominated by broad-leaved deciduous trees, *Cyclobalanopsis/Acer* forest and *Pinus yunnanensis*. At 3000–3500 m a.s.l., conifers dominate (*Pinus densata*, *P. yunnanensis*, *Picea* and *Abies*). Above 3500 m a.s.l., the forest is also coniferous, with *Picea*, *Abies* and *Pinus koraiensis* the most common tree species (Wang et al., 2015).

The coring location was identified using a portable global positioning system (Jisibao G330, Beijing, China). A 101 cm sediment core was collected from the deepest zone (water depth = 42.5 m) of the southeast basin of the lake (Fig. 1) in June 2012 using an Austrian UWITEC Gravity Corer. The core was subsampled in the field at 0.5 cm contiguous intervals. These subsamples were placed into numbered polyethylene bags, stored in a refrigerator with temperature at 4 °C and immediately returned into the laboratory for further analyses.

2.2. Sediment core chronology and multi-proxy

Six bulk sediment and terrestrial plant remains samples (Supplementary Table S1) were collected along the core (depth range: 19.5–91.0 cm) and freeze-dried for accelerator mass spectrometry radiocarbon dating. The radiocarbon ages were measured at the Beta
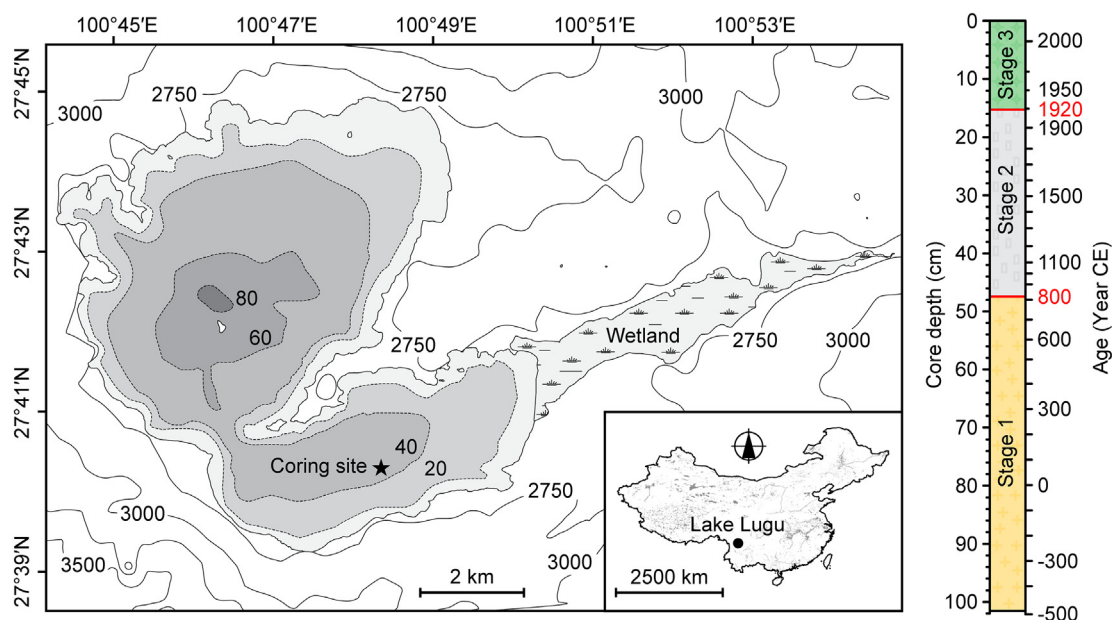
Fig. 1. The location of Lake Lugu in the northwest of the Yunnan Plateau in China. Three development stages revealed by testate amoebae are shown at right column. The contour lines in the map are expressed in meters. The map was created by QGIS version 3.10.0 (QGIS Development Team, 2019).

Radiocarbon Analytic Laboratory (Miami, FL, USA). We used six sediment samples because the sediment deposition rate in Lake Lugu was known to be low (<3 mm yr^{-1}, Wang et al., 2014) and relatively constant over time (Chang et al., 2018; Lin et al., 2017). The radiocarbon ages were calibrated to calendar years (0 yr BP = 1950 CE) using CALIB 7.1 (Stuiver et al., 2019) and the CalPal-Online (http://www.calpal-online.de/).

For the magnetic susceptibility, TOC and TN, samples were taken at 0.5 cm intervals along the core for depths 0–10 cm, 1 cm for 10–30 cm, 2 cm for 30–70 cm and 4 cm interval of the core depths 70–101 cm. In total, 70 samples were analyzed. The magnetic susceptibility measurements were carried out on the freeze-dried sediments (mass = 0.1 g) using acid washed polystyrene pots (volume = 8 ml) and a Bartington MS2 magnetometer (Bartington Instruments Ltd., Oxon, England). The volume low- and high-field magnetic susceptibilities were measured at frequency of 0.46 and 4.6 kHZ, respectively. The mass-specific susceptibility of the sample was calculated as the volume susceptibility divided by the bulk density. These measurements were replicated at least twice per sample. The mean-values and percent frequency-dependent susceptibility (the difference between the mass-specific susceptibility readings at low and high frequencies divided by the mass-specific susceptibility reading at low frequency times 100) were computed in order to interpret the sediment deposition mechanisms. The mass-specific low-field magnetic susceptibility reflected the composition and concentration of iron oxides deposition. The percentage frequency-dependent susceptibility indicated the contribution of ultrafine-grained materials generated during the genesis of the sediment from the watershed (Jordanova et al., 2008; Thompson et al., 1975). Analysis of TOC and TN concentrations was performed on the freeze-dried sediments that had been gently homogenized, pretreated with 10% HCl, subsequently freeze-dried and burned using Euro EA3000 elemental analyzer (EurovectorSrl, Pavia, Italy).

2.3. Testate amoebae analysis

Seventy samples were processed for testate amoebae analysis (Supplementary Fig. S1), with sample preparation following Ndayishimiye et al. (2019). Counting focused on individuals with shell size 25–300 μm in the hydro-bios plankton chambers of a light microscope operating at 200–400× magnification. Species identification and classification followed Mazei and Tsyganov (2006), Mazei and Warren (2012, 2014, 2015), Ogden and Hedley (1980), Siemensma (2019), Todorov and Bankov (2019) and Tsyganov and Mazei (2006). We identified >150 individuals per sample (Supplementary Fig. S1), a number considered high enough to analyze community data statistically (Patterson and Fishbein, 1989; Payne and Mitchell, 2009). Species richness and abundance were calculated following Ndayishimiye et al. (2019). For biomass, we first determined the geometric shape of each species and calculated the volume using our measurements (shell length, breadth and depth and aperture diameter). The volume was then multiplied by the carbon/volume conversion factor (1 μm³ = 1.1×10^{-7} μg C) proposed by Weisse et al. (1990). The total biomass of testate amoebae per site was finally calculated as the sum of all counted individuals (μg C ml^{-1}).

2.4. Statistical analysis

Cluster analysis was used to define the succession stages in the testate amoebae community (Fishbein and Patterson, 1993). The relative abundance of testate amoebae at species level along with species richness, abundance and biomass at genus level were plotted against the

Table 1
Main characteristic of Lake Lugu and its watershed.

Parameter	Measurement
Lake	
Maximum length (km)	9.4
Maximum width (km)	5.2
Maximum depth (m)	93.5
Average depth (m)	40.3
Surface area (km²)	48.5
Water volume (m³)	19.5 x 10⁸
Water residence time (yr)	18.5
Watershed	
Watershed area (km²)	171.4
Evapotranspiration (mm)	1270
Mean annual precipitation (mm)	926
Mean temperature in winter (ºC)	5.2
Mean temperature in summer (ºC)	17.3
Mean annual temperature (ºC)	11.7

References: Bai et al. (2002), Chen et al. (2014), Wang and Dou (1998)

age and depth of the core using Tilia 2.0.b.4 (Grimm, 1992). The samples were clustered into different stages of Lake Lugu succession based on the incremental sum of squares (Grimm, 1992). The sample-stages were compared based on the Tukey's honest significant difference test, for five environmental variables, five dominant species (abundance and biomass) and eight genera (species richness, abundance and biomass) using PAST version 3.13 (Hammer et al., 2001). The six community diversity indices (species richness, abundance, dominance, Simpson, Shannon-Wiener and Pielou's evenness) were computed using PAST version 3.13 (Hammer et al., 2001), using Tukey's honest significant difference (with a conventional $P < 0.05$, considered significant).

The Anderson-Darling normality test was calculated using PAST version 3.13 (Hammer et al., 2001), and our results revealed the non-normal distributed testate amoebae abundance data ($n = 70$, $P < 0.01$) and great standard deviations among stages of Lake Lugu succession (72, 84 and 153 for the first, second and third stages, respectively). Therefore, we transformed the data using $\log (x + 1)$ to make the data fit the assumptions of parametric statistical tests and analyses (McDonald, 2014). The contribution of each species or genus in the overall Bray-Curtis dissimilarity was created by the similarity percentages using abundance data.

The lengths of the gradient in the testate amoebae species data were determined by detrended correspondence analysis. The longest gradient length was <4 standard deviation suggesting that redundancy analysis (RDA) was the appropriate ordination technique to analyze the relationship between species and environmental variables (ter Braak and Šmilauer, 2012). The significance of species-environment relationship was determined by Monte-Carlo tests with 999 permutations (Dale and Dale, 2002). The total explained variation within the testate amoebae species data was partitioned among the three groups of environmental variables (magnetic susceptibility, TOC and TN) using variation partitioning analysis (VPA) (Borcard et al., 1992). The member of each group was filtered by stepwise selection via testing the pure effect. The multivariate statistical analyses were performed in CANOCO version 5.0 (ter Braak and Šmilauer, 2012).

A time-lag regression analysis was used to quantify the rates and patterns of variability in the testate amoebae community (Collins et al., 2000). This technique can measure the community dissimilarity over increasing time lags. For a stable community, dissimilarity between samples does not change as time-lags increase, while for an unstable community, dissimilarity between samples increases or decreases over time, suggesting community is undergoing directional change or convergence, respectively (Collins et al., 2000). The time lags and community dissimilarity used for this study were the radiocarbon dates calibrated to calendar years and Bray-Curtis distance. The time-lag regression analysis was performed using PAST version 3.13 (Hammer et al., 2001).

The fit of the Sloan neutral community model to the testate amoebae data was used to assess the potential importance of stochastic process in community assembly (Sloan et al., 2006). This model takes into account of the occurrence frequency, and relative abundance of the species in their local and regional communities, respectively. The parameters N and m described the metacommunity size and immigration rate, respectively. The Nm value is the estimate of dispersal between communities, and it provides a description of the relationship between the occurrence frequency and mean relative abundance (Sloan et al., 2006). The best fit distribution curves of the models were determined using the least-square method (Ren et al., 2018), in R version 3.6.0 (R Core Team, 2019). Further, we used the null models to determine the effect of deterministic process on the community assemblage (Gotelli and McGill, 2006). For each analysis, we simulated 5000 random matrices and determined the difference between observed and simulated communities using the EcoSim Professional version 1.0 (Entsminger, 2014). C-score estimated the average number of the checkerboard units between possible pairs of species in the presence-absence matrix (Stone and Roberts, 1990). For each species pair, the checkerboard unit was estimated as $(r_i–S) (r_j–S)$, where r_i and r_j are the row totals for species i and j, respectively and S is the number of core samples containing both species i and j. The C-score was calculated based on SIM9 algorithm (Connor and Simberloff, 1979); observed C-score values greater than simulated values indicate interspecific competition. The strength of the deterministic process across stages of Lake Lugu succession was shown by the standardized effect size for the checkerboard scores (Gurevitch et al., 1992). The standardized effect size was computed as $(I_{obs} - I_{sim}) / S_{sim}$, where I_{obs} stands for the observed assemblages, I_{sim} stands for the simulated assemblages, and S_{sim} is the standard deviation of the null assemblage.

3. Results

3.1. Radiocarbon chronology and environmental proxies

The radiocarbon dates results indicated that our 101 cm sediment core spanned from approximately 500 BCE to 2010 CE (Supplementary Table S1). The relationship between age and depth fitted a smooth-spline model.

The five sediment proxies represented a wide range of environmental conditions in relation to the testate amoebae community succession stages (see below, Figs. 2 and 3 and Supplementary Fig. S2). Between 500 BCE– 800 CE, magnetic susceptibility values were low and concentrations of TOC and TN were strongly correlated ($n = 20$, $r = 0.995$, $P < 0.001$). Between 800–1920 CE, magnetic susceptibility remained relatively stable. The concentrations of TOC and TN increased steadily (means and standard errors $\mu \pm$ SE were $9.5 \pm 0.2\%$ and 0.9 ± 0.0 mg g^{-1}, respectively) and were strongly correlated ($n = 25$, $r = 0.939$, $P < 0.001$). Between 1920–2010 CE, magnetic susceptibility peaked while concentrations of TOC and TN decreased gradually ($n = 25$, $\mu \pm$ SE $= 7.2 \pm 0.3\%$ and 0.8 ± 0.0 mg g^{-1}, respectively).

3.2. Change in testate amoebae community among three stages

We observed 29 testate amoebae species belonging into eight genera ($n = 70$, Supplementary Table S2). The genera with more than two species in the dataset were *Difflugia*, *Centropyxis*, *Arcella* and *Netzelia*. Species richness varied from 10 to 27 ($n = 70$, $\mu \pm$ SE $= 21 \pm 1$ species). The total abundance ranged from 270 to 906 ind. ml^{-1} ($n = 70$, $\mu \pm$ SE $= 519 \pm 18$ ind. ml^{-1}) and was largely contributed by *Arcella hemisphaerica*, *Centropyxis aerophila*, *Centropyxis cassis*, *Centropyxis ecornis* and *Netzelia gramen*. The total biomass ranged between 5.6 and 14.9 µg C ml^{-1} ($n = 70$, $\mu \pm$ SE $= 10.3 \pm 0.2$ µg C ml^{-1}) and was largely comprised of *Centropyxis ecornis*, *Centropyxis hemisphaerica*, *Centropyxis marsupiformis*, *Difflugia acuminata* and *Netzelia tuberculata* (Fig. 3).

The first (S1), second (S2) and third (S2) evolution stages of Lake Lugu dated about 500 BCE– 800 CE, 800–1920 CE and 1920–2010 CE, respectively (Fig. 2). The important genera of the testate amoebae community were *Centropyxis* and *Difflugia* peaking about 114 and 1996 CE, respectively (Figs. 2 and 3 and Supplementary Fig. S2). Changes in species richness, abundance and biomass of *Centropyxis* and *Difflugia* occurred in S2 (*Centropyxis* declined, while *Difflugia* increased) (Fig. 3). The species richness, abundance, dominance, Shannon-Wiener and Simpson indices showed a significant difference among the three stages ($P < 0.05$, Supplementary Fig. S3). The main contributors to the overall community dissimilarity were *Netzelia gramen* (13.8%) at species level and *Difflugia* (31.9%) at genus level (Fig. 4).

3.3. Testate amoebae-environment relationships

The species-environment relations of the three stages were almost the same except that the dominant genera were different (Fig. 5 and Supplementary Table S3). In the first stage, percent frequency-
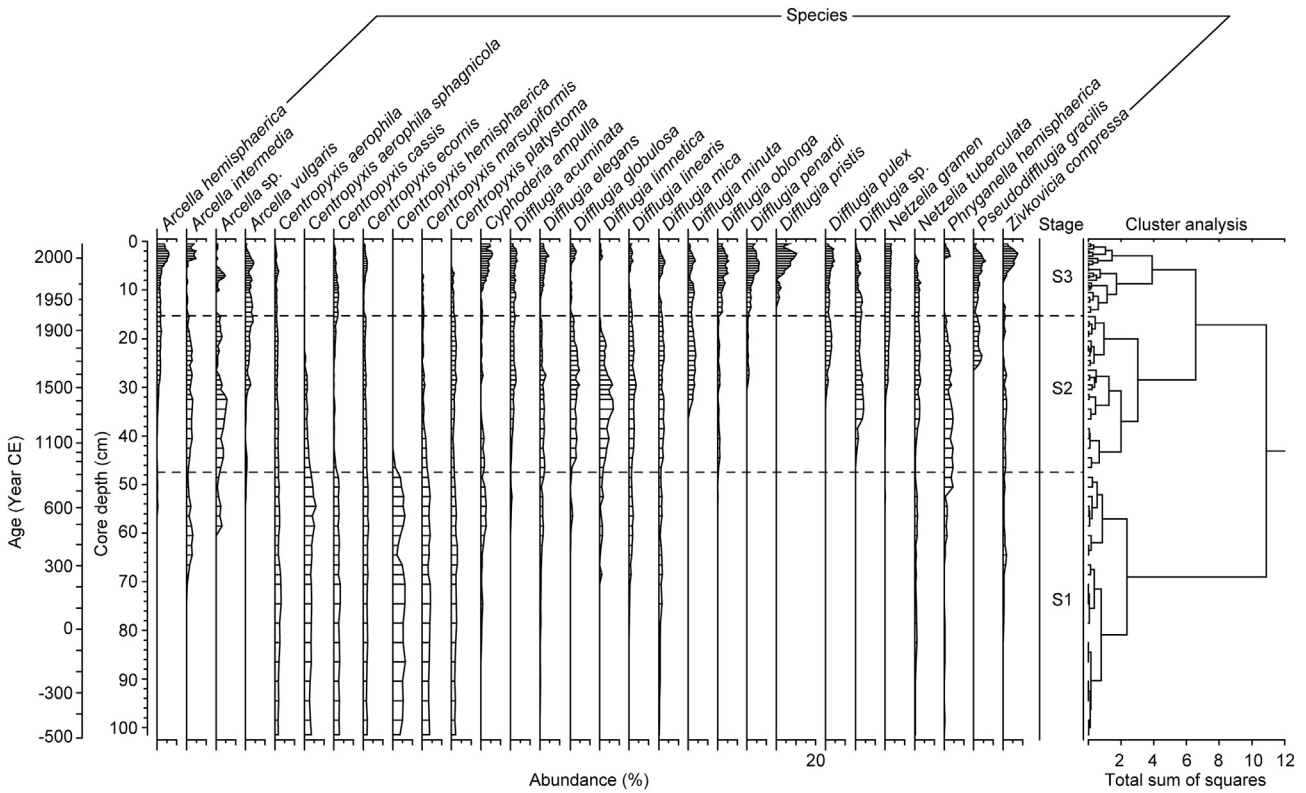
Fig. 2. Percentage of testate amoebae plotted against the sediment core depth in Lake Lugu. The testate amoebae stages and results of biostratigraphically constrained cluster analysis are shown on the right. The sample sizes of the first (S1), second (S2) and third (S3) stages are 20, 25 and 25, respectively.

dependent susceptibility and TOC significantly influenced the testate amoebae community at $P < 0.01$. The dominant genus (*Centropyxis*) strongly correlated with TOC. In the second stage, mass-specific low-field magnetic susceptibility and TN influenced the community at $P < 0.01$. There was no significant correlation between *Centropyxis* species and TOC, and the community was characterized by balanced dominance between *Centropyxis* and *Difflugia*. In the third stage, mass-specific high-field magnetic susceptibility, TOC and TN influenced the community at $P < 0.05$. The dominant genus (*Difflugia*) strongly correlated with magnetic susceptibility.
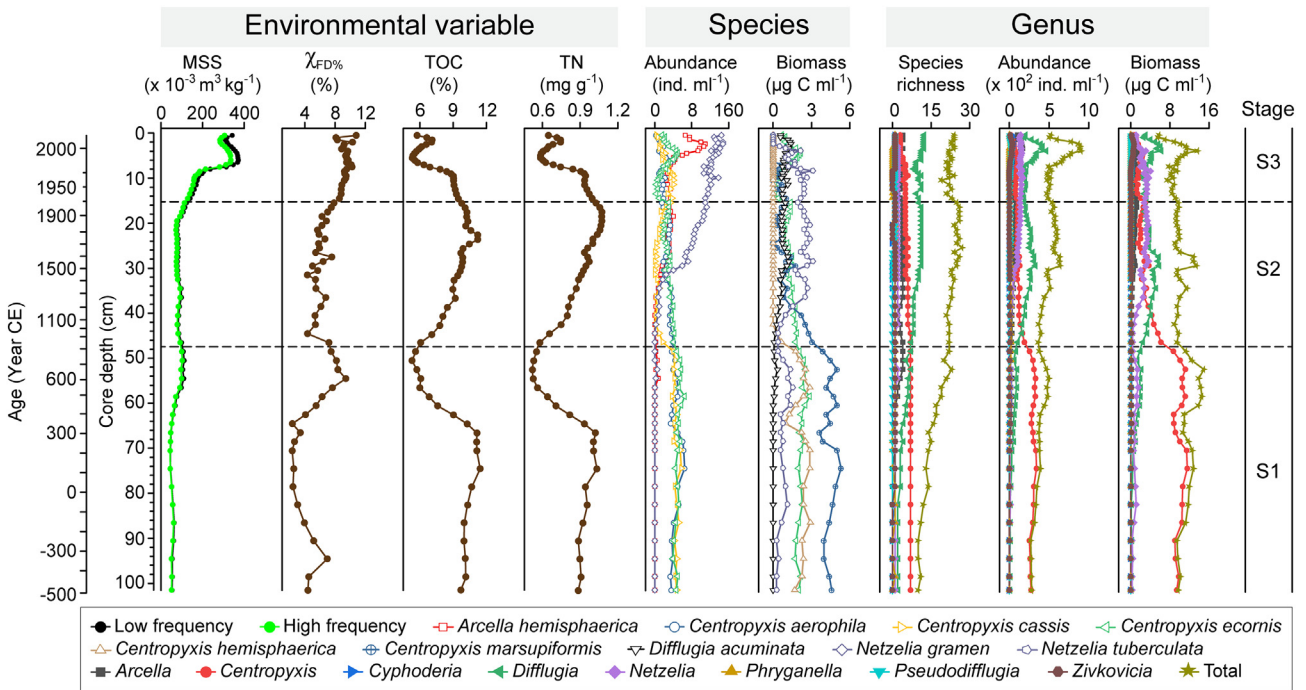

Fig. 3. Properties of the sediments and testate amoebae from Lake Lugu. At species level, only the top five dominant species in abundance and biomass are shown. At genus level, all genera of testate amoebae are shown. MSS, mass-specific susceptibility; $\chi_{FD\%}$, percent frequency-dependent susceptibility; TOC, total organic carbon; TN, total nitrogen. The samples of the first (S1), second (S2) and third (S3) stages are 20, 25 and 25, respectively.
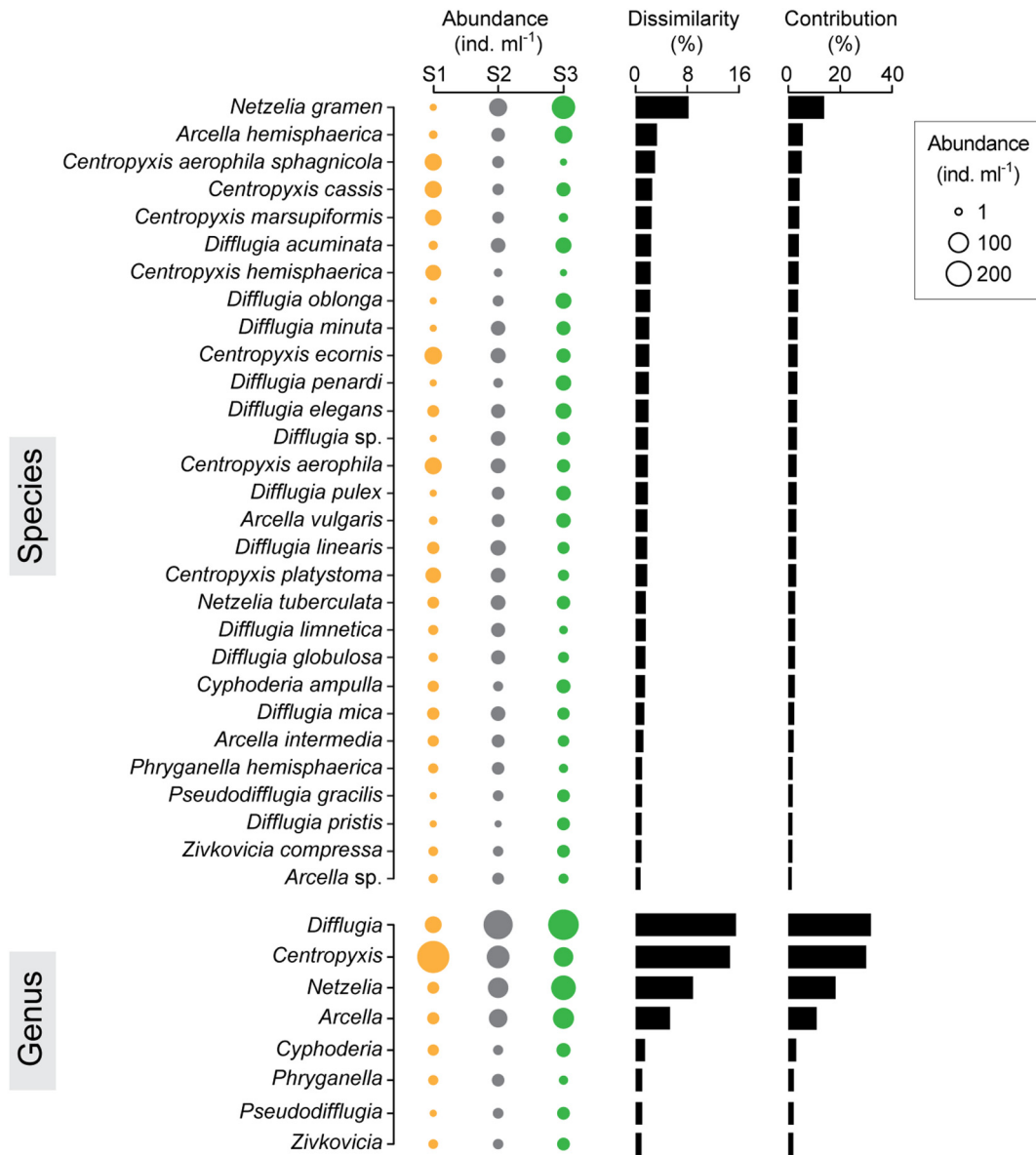
Fig. 4. The dissimilarity and contributions counted on the basis of statistically significant testate amoebae species and genera among three development stages of Lake Lugu, respectively. The balloon plots show the mean abundances. The samples of the first (S1), second (S2) and third (S3) stages are 20, 25 and 25, respectively.

The influences of magnetic susceptibility and TN on testate amoebae community were greatest in stages 1 and 2 (16.2 and 6.8% based on pure percentages of community variation in VPA, respectively). In stage 3, magnetic susceptibility, TOC and TN influenced the testate amoebae community, but at low values of percentage community variation (Fig. 5).

3.4. Stochastic and deterministic processes shaping the testate amoebae community

The testate amoebae community showed a directional change in each of three stages (Fig. 6 and Supplementary Fig. S4). A close relationship between magnetic susceptibility and testate amoebae species washed from the watershed soil into Lake Lugu (neutral process) was great in stages 1 and 3. A close connection between TN and testate amoebae species growing better in eutrophic environment (deterministic process) was more significant in stages 2 and 3 (Figs. 5 and 6 and Supplementary Fig. S4).

4. Discussion

4.1. Multi-proxy indicators of lake evolution over the past 2500 years

Magnetic susceptibility has been demonstrated to be a useful proxy for detailing past soil erosion (Oldfield and Richardson, 1990; Oldfield et al., 2003; Thompson et al., 1975). In southwest China, where the regional climate is characterized by the monsoons, magnetic susceptibility has been utilized to link the intensity or strength of precipitation with soil degradation in the Lake Lugu watershed (Chang et al., 2018; Liu et al., 2019; Wang et al., 2014, 2018). The falling raindrops can generate erosion by moving around small particles in the upper soil layer (Jordanova et al., 2008), and those particles may be found in the sediment of Lake Lugu as clay and fine silt (Chang et al., 2018; Liu et al., 2019). During intense rains (e.g., monsoonal precipitation), runoff transported soil particles, leaves and potential pollutants into Lake Lugu (Liu et al., 2019); recorded as increasing values of magnetic susceptibility of the sediment (Chang et al., 2018). This was apparent in about 300–1100 CE (Fig. 3). The paleoecological evidences from the
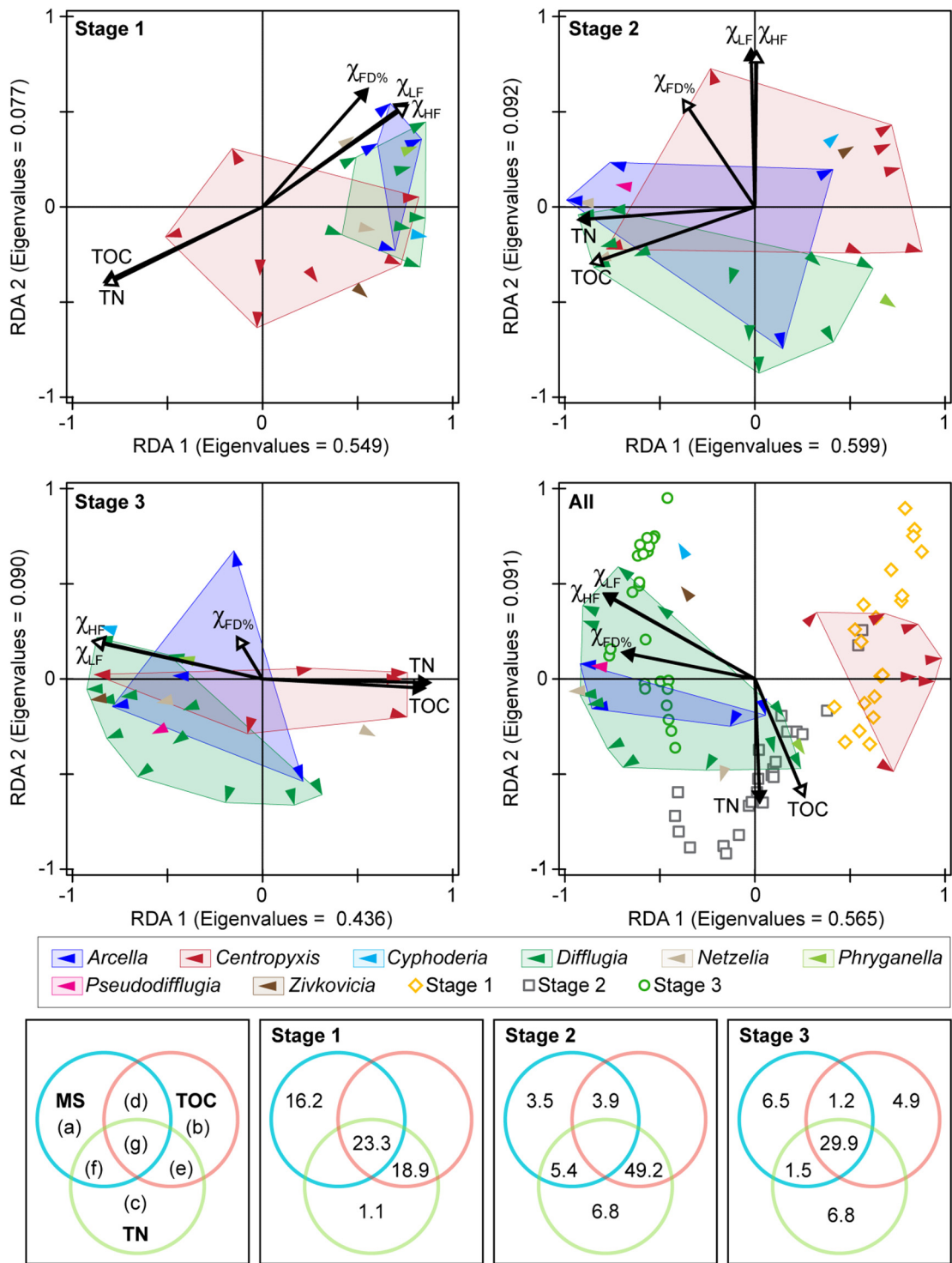
Fig. 5. Redundancy analysis (RDA) showing the relationships between testate amoebae species and the environmental variables. The diamond, square and circle symbols indicate samples in the first, second and third stages, respectively. The colored arrowheads indicate the direction of species in ordination space. The variables that remained significant ($P < 0.05$) after forward selection are shown with black filled arrowheads. The Venn diagrams indicate the variation partitioning analysis (VPA) results for the three groups of environmental variables (MS, TOC and TN). Pure and shared percentages of community variation are shown by letters (a, b and c) and (d, e, f and g), respectively. Note that the unexplained variance and explanations <1% are not shown for simplicity. The data are log $(x + 1)$ transformed. The sample sizes of stages 1, 2 and 3 are 20, 25 and 25, respectively. χ_{LF}, mass-specific low-field magnetic susceptibility; χ_{HF}, mass-specific high-field magnetic susceptibility; $\chi_{FD\%}$, percent frequency-dependent susceptibility; MS, magnetic susceptibility; TOC, total organic carbon; TN, total nitrogen.

central Yunnan, southwest China, indicate that the early human settlement history in Yunnan is still poorly documented due to the lack of reliable dating results (Chen et al., 2014; Wu et al., 2015); and such evidences are still limited around Lake Lugu. Instead, recent studies showed that human occupation on the Yunnan Plateau escalated in 1450–1700 CE (Chang et al., 2018; Wang et al., 2017; Whitmore et al., 1994). On the one hand, this implies that the accelerated erosion that took place between 300–1100 CE was probably caused by natural climate variability (Chang et al., 2018). On the other hand, the last 100 years may indicate the impact of human activities generated by
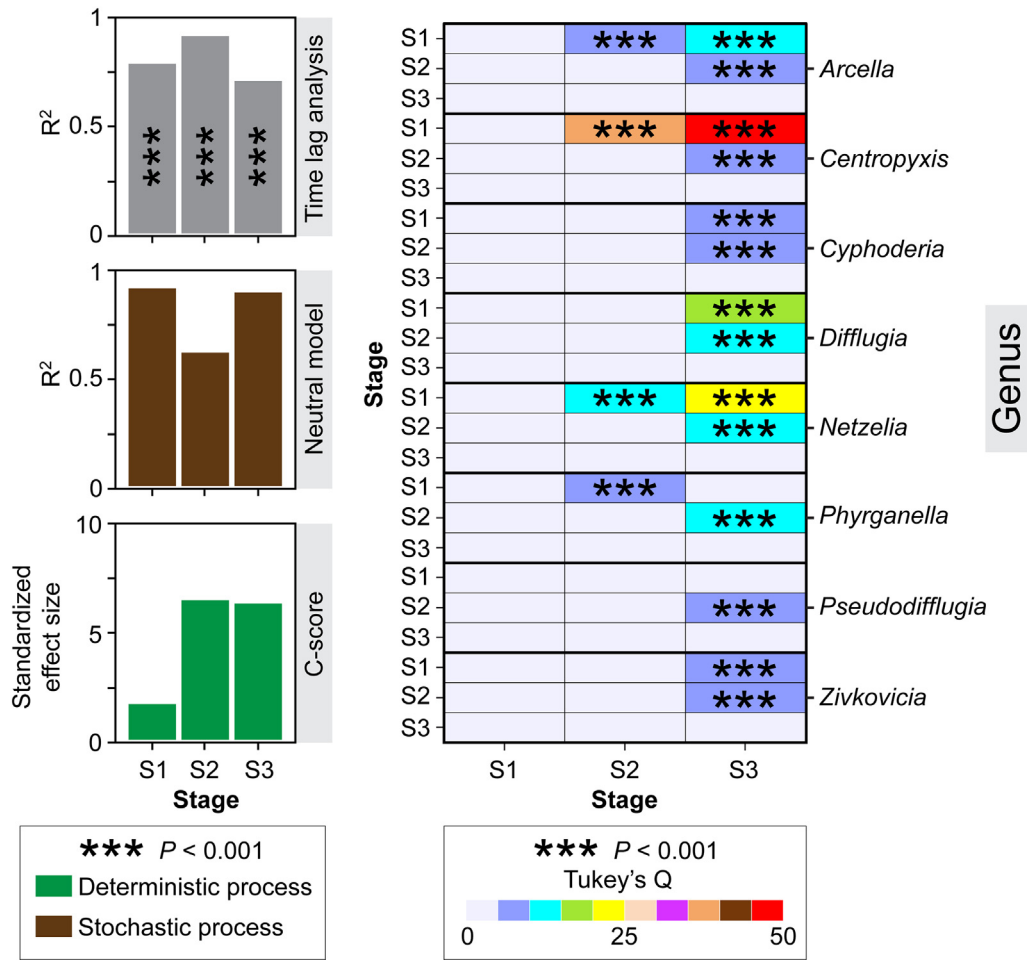
Fig. 6. Comparison of three development stages of Lake Lugu based on the mechanisms shaping the testate amoebae community composition. Instability and positive directional changes in the communities are shown by the coefficient of determinations created by time lag regression analysis. The strengths of stochastic and deterministic processes are indicated by the Sloan's neutral community model and C-score calculated using null model, respectively. The heatmap compares the mean abundances of testate amoebae at genus level. Data are log (x + 1) transformed.

deforestation in the catchment area of Lake Lugu (Liu et al., 2019; Zhang et al., 2013). This was evident based on high magnetic susceptibility values and low concentrations of TOC and TN measured from the top layers of the sediment core (about 1983–2010 CE, Fig. 3).

4.2. Responses of testate amoebae to watershed soil erosion and human activity

Although the response of testate amoebae to landscape degradation and lake succession in the high-altitude mountains is poorly studied, recent work has showed that soil erosion and human activities can disturb the freshwater testate amoebae community in low-altitude lakes and wetlands (Qin et al., 2009; Steele et al., 2018; Wanner et al., 2020). The current study also showed synchronized changes in the testate amoebae assemblage and sedimentological records suggesting an important role for soil erosion and human impacts (Fig. 3). The soil erosion that occurred between 300–1100 CE likely caused a shift in the dominant genera of the testate amoebae (from Centropyxis to Difflugia). The decline in the abundance and biomass of Centropyxis between 1920–2010 CE (Figs. 3 and 5) might be caused by low-oxygenation of the lake due to factors such as expanded sedimentation rates, deforestation and agriculture (Prentice et al., 2018; Wanner et al., 2020). The clay, fine silt and water pollutants generated from Lake Lugu watershed contributed to a reduced dissolved oxygen in water (Lin et al., 2017; Liu et al., 2019; Wu et al., 2008; Zhang et al., 2013), consequently, the metabolic activities of some testate amoebae species (e.g., Centropyxis) may

have been affected (Ren et al., 2018). In addition, the clay and fine silt and water pollutants may have impacted the primary producers containing the chlorophyll a and thus food supply for testate amoebae (Ndayishimiye et al., 2019). This may have led to food competition among species including cannibalism (Han et al., 2011b). Thus, we found the testate amoebae community structured deterministically between 800–2010 CE with intensive erosion and human impacts. The runoff in high-altitude mountains is the potential pathway utilized by testate amoebae species to migrate from watershed soils into lakes (Roe and Patterson, 2006). Many terrestrial testate amoebae species can survive in freshwater lakes (Prentice et al., 2018; Qin et al., 2009; Wanner et al., 2020); hence, the stochastic process shaped the testate amoebae species composition across the three stages of Lake Lugu succession. Therefore, we found that both stochastic and deterministic processes influenced the testate amoebae community assembly, but at different degrees largely depending on environmental conditions (Fig. 6 and Supplementary Fig. S4).

In lake ecosystems, the fluctuation of environmental factors can drive the population dynamics of the testate amoebae and changes in the community composition (Ren et al., 2018). Likewise, our results showed synchronized changes of environmental variables and abundance of testate amoebae over the past 2500 years, despite the decline in Centropyxis from the community (Fig. 3). To explain this more clearly and in relation to five measured environmental variables and human activities we can look at the trends of the total species richness and abundance at stage level (Supplementary Fig. S2). The shift in the dominant

genera of the testate amoebae (*Centropyxis* and *Difflugia*) was remarkable and associated with increased input of terrestrial inorganic particles and low organic matter in Lake Lugu (300–1100 CE). Similar change was repeated in and after 1920 CE, but with the abrupt increase of the testate amoebae species growing well in warmer conditions. Both *Difflugia* and *Netzelia* species were the examples of testate amoebae that can thrive at relatively high temperature and in more eutrophic environment (Ndayishimiye et al., 2019; Qin et al., 2009). In the first stage, the total species richness and abundance were largely dominated by *Centropyxis* under the influence of variables largely linked with natural climate variability. In the second stage, we showed a decline in species richness and abundance of *Centropyxis*, however, this did not affect the total values because the species occurrence and abundance of *Difflugia* were increased (Fig. 3). The dominance of *Difflugia* from around 1920 CE was shaped by environment (Fig. 5 and Supplementary Table S3), thus, leading to high total values of species richness and abundance in the third stage (Fig. 3). This was probably due to the ability of *Difflugia* to survive in the more eutrophic environment caused by human activities (Prentice et al., 2018).

4.3. Comparison of testate amoebae with other proxies

This study compared Lake Lugu records with local and regional climate reconstruction in Chang et al. (2018). Asian summer monsoon precipitation induced significant change in magnetic susceptibility, TOC and TN of Lake Lugu between 300–1100 CE (Chang et al., 2018). Similarly, our findings showed that the sedimentological erosion indicators (i.e., magnetic susceptibility) lead to the shift in the dominant genera of the testate amoebae (from *Centropyxis* to *Difflugia*) between 300–1100 CE. Chang et al. (2018) argued that after 900 CE, the increase in TOC and TN was generated by the forest growth in the Lake Lugu watershed because of wet conditions. Thus, our results aligned with Chang et al.'s (2018) findings and highlighted that the human activities impacted the landscape and microbial communities after 1920 CE.

4.4. Ecological and management implications

The high-altitude mountain lakes are ecologically fragile being sensitive to natural climate variability and human activities (Chang et al., 2018; Liu et al., 2019). Watershed erosion may lead to the variation of their biodiversity. Furthermore, the landscape changes and water pollution linked to human activities around high-altitude mountain lakes is deemed the major concern that needs attention ecologically and with management measures (Chang et al., 2018; Liu et al., 2019). In Lake Lugu, the natural processes such as natural climate variability and soil erosion showed a great impact on the testate amoebae community before 800 CE. Later, the testate amoebae community was found to be influenced by both natural processes and human activities such as deforestation, agriculture and untreated sewage (Wu et al., 2008). The three distinct stages of Lake Lugu succession based on the testate amoebae over the past 2500 years confirmed the long-lasting change of Lake Lugu ecosystem. Our findings suggest the need for regular monitoring of the testate amoebae community of the high-altitude mountain lakes and appropriate measures to manage or prevent the soil erosion and untreated pollutants discharged into Lake Lugu. Increased eutrophication means more algae and suitable food for the testate amoebae growing well in the eutrophic and warm conditions (Ndayishimiye et al., 2019; Yang et al., 2006). This is because the agricultural activities in the lake watersheds (e.g., fertilizer runoff), tourism and small-scale industrial development alongside the shores of high-altitude mountain lakes contribute pollutants and nutrients in the waters and sediments (Hundey et al., 2016; Lin et al., 2017; Liu et al., 2019).

Due to the fact that only one sediment core was taken in this study, a key priority for any future study is that it should be conducted on a larger spatial scale (more sampling cores in one or more deep lakes), so allowing us to generalize our conclusion across a wide range of lakes.

5. Conclusions

This study presents a multi-proxy record with particular attention to the testate amoebae from an alpine deep lake of Yunnan Plateau (Lake Lugu), southwest China covering the last 2500 years. The testate amoebae community clearly showed three distinct stages: 500 BCE– 800 CE, 800–1920 CE, and 1920–2010 CE. The close relationships between magnetic susceptibility and testate amoebae indicated the influence of soil erosion and human impact on the community composition specifically during the first and third stages. Our results suggest that the testate amoebae community in Lake Lugu acts as sensitive paleoenvironmental indicator that can be used to monitor the alpine lake ecosystem change and model the lake succession over time. The probable causes of changes in the testate amoebae community and three stages of Lake Lugu succession were the soil erosion and plant-soil nutrient input. The soil erosion led to the transportation of terrestrial matters and few testate amoebae species into Lake Lugu (stochastic process). The nutrient change resulted in temporary change in environmental conditions and species competition that led to the rapid growth of some species and disappearance of others (deterministic process). Further studies utilizing more environmental factors are required in order to distinguish the effects of soil erosion and human impact on the testate amoebae community changes of alpine lakes across space and time under changing climate and environment.

CRediT authorship contribution statement

Jean Claude Ndayishimiye: Investigation, Formal analysis, Writing - original draft, Validation, Writing - review & editing. **Pascaline Nyirabuhoro:** Formal analysis, Writing - original draft, Validation, Writing - review & editing. **Qian Wang:** Data curation, Validation, Writing - review & editing. **Xiangdong Yang:** Data curation, Validation, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137684.

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