



## Temperature transfer functions based on freshwater testate amoebae from China

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### Abstract

Globally, lakes and reservoirs ecosystems are integral parts of ecological processes. Nevertheless, global warming is rapidly changing their function and sustainability, especially in the populated area of the northern hemisphere. Here we examined testate amoebae community and 10 environmental variables from 51 lakes and reservoirs across China and developed testate amoebae transfer functions for temperature based on both abundance- and biomass-datasets. A total of 169 testate amoebae taxa were identified. Our partial CCA analyses revealed that water temperature explained 5.15% ( $P = 0.006$ ) and 5.57% ( $P = 0.008$ ) of the total variance in testate amoebae abundance and biomass, respectively. The WA-PLS was the best model in abundance-based temperature transfer function (RMSEP = 2.87 °C,  $R^2 = 0.60$ ), whereas the MAT proved to be the best model for biomass-based temperature transfer function (RMSEP = 3.34 °C,  $R^2 = 0.67$ ). The application of all models should be carried out with suitable precautions. Our results suggested that freshwater testate amoebae could contribute to a better understanding of the ecological integrity and its vulnerability in inland aquatic environments.

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**Keywords:** Abundance; Biomass; Freshwater; Testate amoebae; Transfer function; Water temperature

### Introduction

Lakes and reservoirs are essential components of biogeochemical and ecological processes (Messenger et al. 2016).

However, change in global or regional climate patterns represents a growing challenge to their integrity and function (Collen et al. 2014), and will strongly affect their biogeochemical cycles and mesoscale atmospheric circulation at lake-land boundaries (Desai et al. 2009). For example, the increase in temperatures will substantially change the ultraviolet exposure of aquatic organisms (Schindler 2009). Global warming is expected to affect species distribution and habitat associations in both aquatic and terrestrial ecosys-

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tems (Menéndez et al. 2014). In China, human-induced environmental changes have driven complex physical and environmental changes on inland waterbodies (Ji et al. 2017; Yang and Lu 2014). For example, the sensitivity of lake surface water to global warming was reported in Qinghai Lake (Xiao et al. 2013) and Taihu Lake (Liu et al. 2015). Therefore, exploring and understanding ecosystem development and dynamics using biotic proxies is advantageous for global and regional sustainable management (Marcisz et al. 2016).

Freshwater testate amoebae are a diverse and abundant polyphyletic group of shelled protozoa in freshwater lakes and reservoirs (Asioli et al. 1996; Patterson and Kumar 2000; Patterson et al. 1985, 2013). They largely feed on bacteria, algae and fungi present in the water (Han et al. 2011a; Ogden and Hedley 1980). Most of testate amoebae species can well be preserved in the lake sediments due to their decay-resistant shells (Beyens and Meisterfeld 2001; Patterson and Kumar 2002). Over the last three decades, freshwater testate amoebae assemblages have been successfully used as paleoenvironmental indicators (Asioli et al. 1996; Kumar and Patterson 2000; Patterson et al. 1985, 2002; Prentice et al. 2017). However, there have been few relevant studies in Chinese lakes and reservoirs (Qin et al. 2011).

Our previous study on freshwater testate amoebae across China revealed that altitudinal distribution of the lakes and reservoirs greatly affected testate amoebae species richness, plausibly because of water temperature effects (Ju et al. 2014). In the present study, we extended the investigation on relationship between temperature and testate amoebae. Water temperature is known to be responsible for the dynamics of freshwater testate and naked amoebae community in subtropical rivers and reservoirs of China (Ren et al. 2018); therefore, we expected to obtain the same or closer results for freshwater testate amoebae. The thermal conditions of the lakes and reservoirs were given more importance than altitudes because global warming alters the quality of water not the heights of the lakes (Ptak et al. 2018). We hypothesized that species composition of testate amoebae assemblages in warmer freshwater lakes will be dominated by thermophilic species because they grow better at higher than normal temperatures (Baumgartner et al. 2003). If water temperature affects the species composition of testate amoeba in lacustrine bottom sediments, then testate amoebae can be used as proxy for this environmental variable in paleolimnological reconstructions (Roe et al. 2010). Our transfer functions for temperatures were carefully developed to gain insight into the ecological integrity and vulnerability of freshwater ecosystems to global warming (Anderson 2000; Belyea 2007; Juggins 2013a; Payne et al. 2012).

The aims of this study were therefore to analyze the relationship between water temperature and freshwater testate

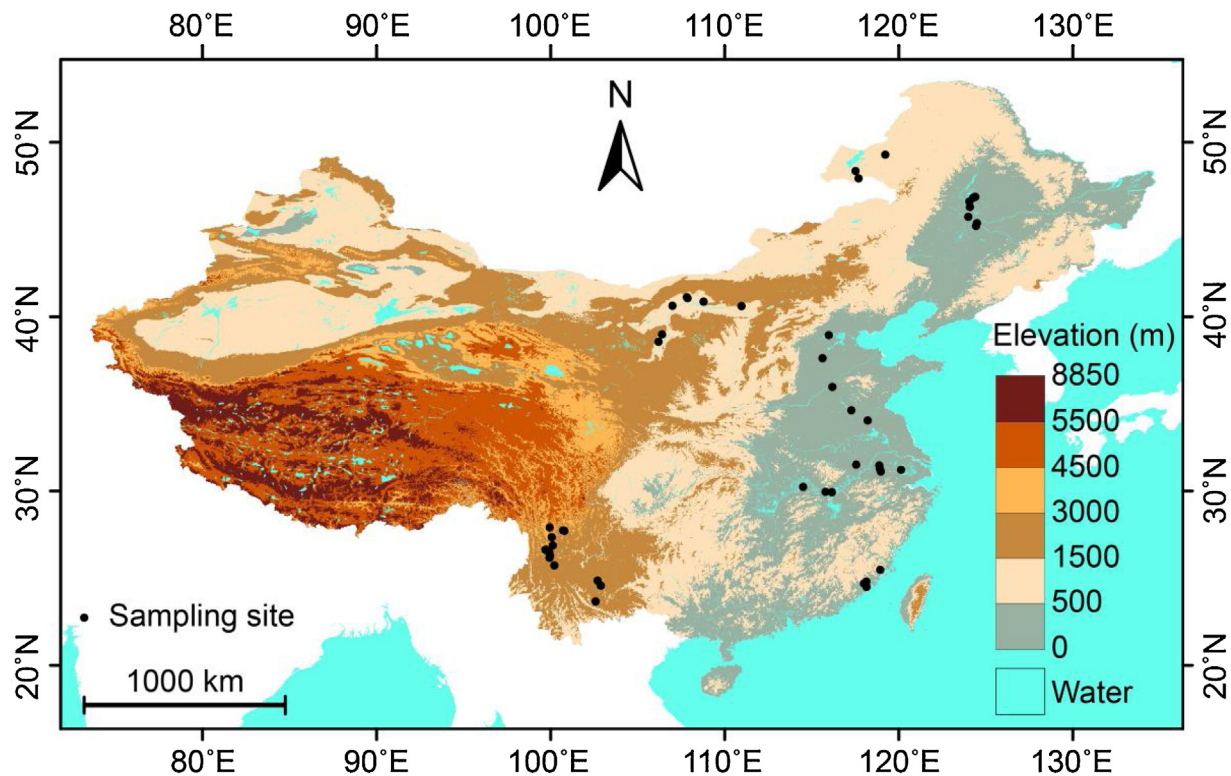
amoebae, and develop temperature transfer functions based on the testate amoebae abundance and biomass data.

## Material and Methods

### Study area and sampling design

Our study sites included 51 lakes and reservoirs located in six geographical regions of China: South-east China (5 reservoirs), the middle and lower reaches of the Yangtze River Valley (8 lakes), East-central China (5 lakes), North China (10 lakes), North-east China (7 lakes) and South-west China (16 lakes). They were selected and grouped based on their latitudes and longitudes (Fig. 1 and Supplementary Table S1).

In general, China is relatively cold and dry from November to February. In summer, it becomes hot and rainy. Consequently, the climates are largely different across the above-mentioned geographical regions. According to Wang and Dou (1998), South-east China is hot in summer and warm in winter. East-central China and Yangtze River Valley are hot in summer and cold in winter. South-west China and North-east China are temperate and cold, respectively. North China is cold and largely covered by ice in winter. From the south-east to north-east China, the mean annual temperature ranges from 21 to  $-1$  °C, respectively. The mean annual precipitation varies from 1600 to 300 mm, respectively. The mean air temperature seasonality among lakes (between July and January) varies from 53.0 to 9.5 °C, respectively (Ju et al. 2014). Recent studies showed that the best season to capture the greatest number of testate amoebae from the environment is summer, indicating testate amoebae prefer to live in warm condition (Davidova and Vasilev 2013; Han et al. 2011b). In that line, sampling was conducted once per lake or reservoir, from July to August 2012. A portable global positioning system (Jisibao G330, Beijing, China) was used to determine the latitude, longitude and altitude of the sampling sites. Water temperature, electrical conductivity, pH and dissolved oxygen of the epilimnion layers were measured using a multi-parameter water quality analyzer (YSI, Yellow Springs, OH, USA). The measurements were taken in every one meter from surface to bottom and the mean value was calculated. Water transparency was estimated with 30 cm diameter Secchi disc. Water depth was measured with a Speedtech SM-5 Depthmate portable sounder (Speedtech Instruments, Great Falls, VA, USA). The depth-integrated water samples in the epilimnion were taken into the laboratory and examined for the total nitrogen, total phosphorous and chlorophyll *a* following standards methods in Greenberg et al. (1992). Triplicate sediment samples within 10–50 m distance were collected from the deepest area using Kajak sediment corer (KC Denmark A/S, Silkeborg, Denmark). The upper 1 cm of sediments were retained for testate amoebae analysis (Beyens and Meisterfeld 2001). To avoid replication and low resolution in testate amoebae data (Payne and Mitchell 2009), triplicate surface sediments representing



**Fig. 1.** Study area map showing 51 lakes and reservoirs in China. The map was created by QGIS version 2.18 (QGIS Development Team 2016).

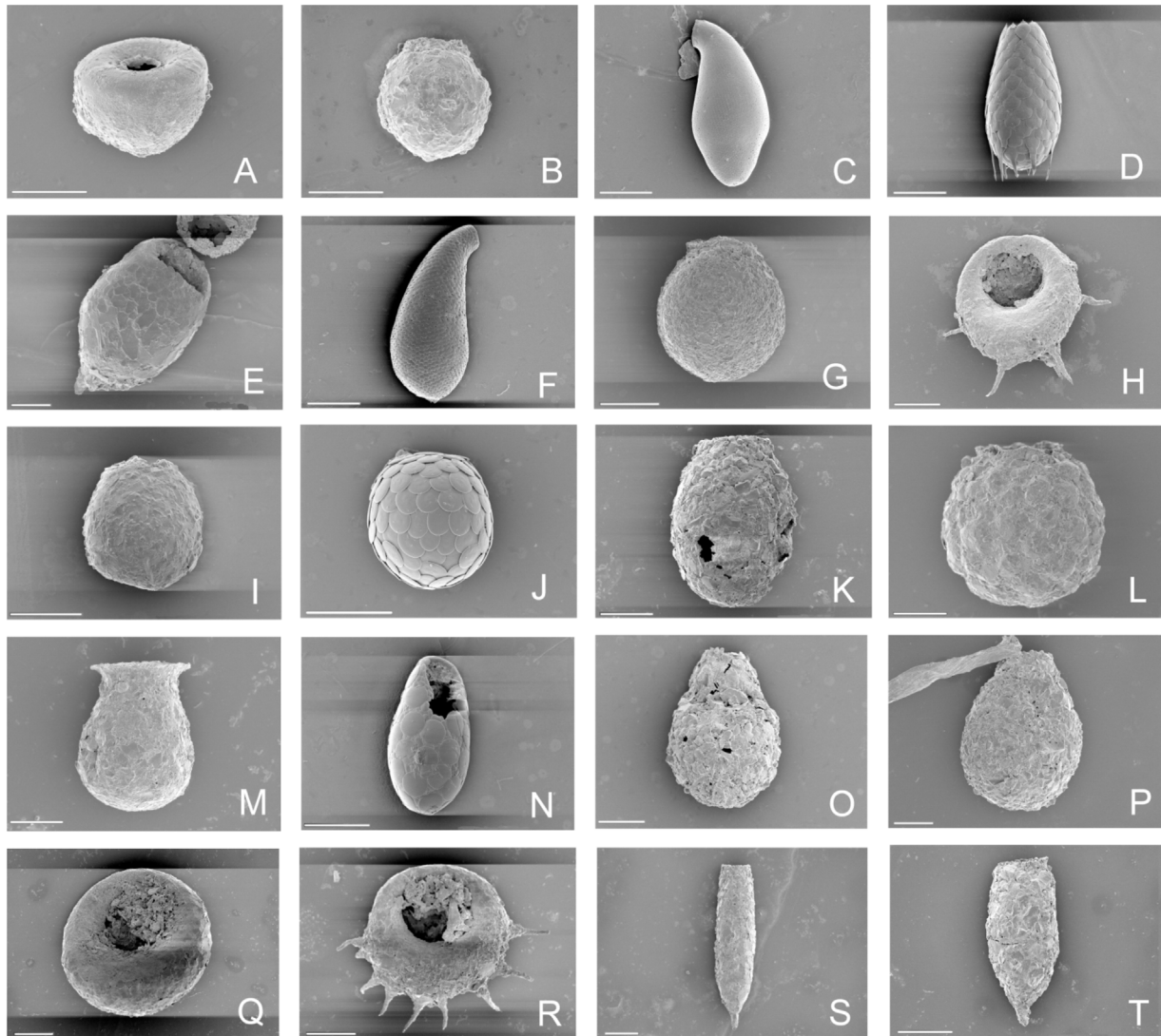
each lake or reservoir were mixed into one sample. Sediments were subsequently pooled and stored in polyethylene bags at 4 °C in the dark (Beyens and Meisterfeld 2001). After fieldwork, all samples were immediately taken into the laboratory and preserved in the refrigerator at 4 °C until further processing. The data for 10 environmental variables measured from 51 lakes and reservoirs are displayed in Supplementary Table S2.

Our measurements (Supplementary Table S2) revealed that the lakes and reservoirs located at elevated altitudes (e.g., South-west China) were deep, transparent and cool compared to those located at low altitudes (e.g., Yangtze River Valley). In addition, most of them presented lower concentrations of nutrients (total nitrogen and total phosphorus), phytoplankton and water dissolved minerals than their counterparts. Few lakes yielded odd results compared to the measurements taken from other lakes located within the same geographical region. For example, Donghaizi Lake recorded an elevated electrical conductivity ( $8051 \mu\text{S cm}^{-1}$ ) and low chlorophyll *a* concentration ( $0.23 \mu\text{g L}^{-1}$ ) compared to Bei'er Lake ( $380 \mu\text{S cm}^{-1}$  and  $8.26 \mu\text{g L}^{-1}$ , respectively) or Hasuhai Lake ( $1050 \mu\text{S cm}^{-1}$  and  $21.73 \mu\text{g L}^{-1}$ , respectively).

### Testate amoebae analysis

With the aid of clean glass beakers,  $1 \text{ cm}^3$  of homogenized surface sediments were dissolved with distilled water

and subsequently stirred gently for 5 min. The samples were then screened using 300 (Beyens and Meisterfeld 2001) and 25  $\mu\text{m}$  sieves (Wall et al. 2010) to eliminate large and fine particles, respectively. The 25–300  $\mu\text{m}$  sediment fractions were washed into brown bottles and diluted to 50 ml with distilled water. Testate amoebae were examined in Hydro-Bios plankton chambers under a microscope, at 200–400 $\times$  magnification. The shell length, breadth, depth and diameter of aperture were measured using an ocular micrometer. Species were identified following taxonomic literature in Cash and Hopkinson (1905, 1909), Cash et al. (1915, 1919), Leidy (1879), Mazei and Tsyganov (2006), Meisterfeld (2002a,b), Ogden and Hedley (1980), Penard (1902), Shen (1983), Yang and Shen (2005), and Yang et al. (2005a). The statistically significant numbers of specimens were calculated based on the guidelines in Patterson and Fishbein (1989). Generally,  $\geq 150$  individuals were counted for each sample (Supplementary Fig. S1), and a count of 150 or more individuals are sufficient for most samples (Payne and Mitchell 2009). The abundance was computed based on counted testate amoebae individuals per 1 ml sediment sample (Beyens and Meisterfeld 2001; Magurran 1988). The carbon biomass of each species identified was estimated from its shell geometric volume, with a carbon/volume conversion factor  $1 \mu\text{m}^{-3} = 1.1 \times 10^{-7} \mu\text{g C}$  (Weisse et al. 1990). The total biomass per lake or reservoir was calculated based on the sum of all testate amoebae individuals (Gilbert et al. 1998). To record and help identify poorly described species, selected tests were examined



**Fig. 2.** Scanning electron micrographs of selected testate amoebae from the studied lakes and reservoirs. A—*Arcella hemisphaerica*. B—*Diffflugia kabylica*. C—*Cyphoderia trochus*. D—*Euglypha acanthophora*. E—*Centropyxis platystoma armata*. F—*Cyphoderia ampulla*. G—*Diffflugia limnetica*. H—*Centropyxis aculeata*. I—*Diffflugia gramen globulosa*. J—*Sphenoderia lenta*. K—*Diffflugia lithophila*. L—*Diffflugia tuberspinifera*. M—*Diffflugia mulanensis*. N—*Trinema enchelys*. O—*Pontigulasia incisa*. P—*Lesquereusia modesta*. Q—*Centropyxis ecornis*. R—*Centropyxis aculeata grandis*. S—*Diffflugia smilion*. T—*Diffflugia acuminata*. A–P Scale bars = 30  $\mu\text{m}$ , Q–T Scale bars = 50  $\mu\text{m}$ .

using scanning electron microscope (Model S-4800, Hitachi, Japan) (Fig. 2).

### Statistical analysis

In further analyses, species with low abundance or small number of occurrences were not removed (Charman et al. 2007) and all environmental variables were  $\log(x + 1)$  transformed except for pH. Both R-mode and Q-mode cluster analyses (Fishbein and Patterson 1993) created by the package ‘pheatmap’ (Kolde 2018) in R version 3.4.3 (R Core Team 2017) were used to determine particular assemblages and similar populations, respectively (Roe et al. 2010). Gradient in the assemblages was described using detrended correspon-

dence analysis (DCA) (ter Braak and Šmilauer 2012). The direct and indirect effects of environmental variables on testate amoebae abundance and biomass were assessed using the partial least squares path modeling (PLS-PM) (Sanchez 2013; Wetzels et al. 2009) by the package plspm (V0.4.7) in R version 3.4.3 (R Core Team 2017). The latent variables were: temperature (water temperature), chemical variables (total nitrogen, total phosphorus, dissolved oxygen and pH), primary producers (chlorophyll *a*) and testate amoebae community parameters (abundance and biomass). Reliability of our models was assessed by comparing obtained goodness-of-fit values with the baselines (Wetzels et al. 2009).

Because the longest length of the gradient was >4 standard deviation in DCA (ter Braak and Šmilauer 2012), the

relationships between the assemblages of species and their environments were examined using canonical correspondence analyses (CCA) (ter Braak and Šmilauer 2012). Rare species were down-weighted to avoid their statistical effects on the ordination. The proportions of variance explained by measured environmental variables were calculated using partial canonical correspondence analysis (pCCA) (Borcard et al. 1992). The significance of ordination was tested by Monte-Carlo test with 999 permutations (Dale and Dale 2002). All DCA and CCA analyses on the abundance-based and biomass-based datasets were performed using CANOCO version 5.0 (ter Braak and Šmilauer 2012). The strength of relationship between pairs of significant environmental variables was estimated using Spearman correlation coefficients in SPSS version 22.0 (IBM Corp, Armonk, NY, USA).

## Transfer functions

Temperature transfer functions were developed using four standard models: partial least squares (PLS), weighted averaging (WA), weighted averaging with partial least squares (WA-PLS) and modern analogue technique (MAT) (Birks 1998; ter Braak and Juggins 1993). These models were chosen because they have been successfully used to develop transfer functions for water table and phosphorus from testate amoeba data in wetlands (Booth 2002; Charman et al. 2007; Patterson et al. 2012). Jack-Knifing approach was used in the cross-validation. The performance of the models and model cross-validation was assessed using coefficient of determination ( $R^2$ ) between observed and predicted values, root mean square error of prediction (RMSEP), and the bias between estimated and observed values (including maximum bias and average bias). Normally, a model with the combination of high  $R^2$ , low RMSEP and low bias in cross-validation is considered to be the best model and is preferred in model selection (Birks 1998). The transfer function models were further improved by data screening to remove samples with residuals greater than 20% of the range of temperature gradient following previous studies (Charman et al. 2007; Lamentowicz et al. 2008; Payne and Mitchell 2007). Calibration models were constructed using the package rioja (version 0.8–5) (Juggins 2013b). The optimal temperature and tolerance range of each dominant species were calculated using the package Hmisc (version 4.1-1) (Harrell Jr et al., 2018) in R version 3.4.3 (R Core Team 2017). In addition, the relationships between testate amoebae tolerance and optima for temperature were explored with Spearman correlation coefficients using SPSS version 22.0 (IBM Corp, Armonk, NY, USA).

Uneven sampling along the environmental gradient may result in biased RMSEP (Telford and Birks 2011). This was a potential limitation in our study as there were only a few samples from cold lakes with the greater numbers from the warmer end of the temperature gradient (Supplementary Table S2). Therefore, we applied the segment-wise

RMSEP approach proposed by Telford and Birks (2011) to correct the possible bias generated by our uneven sampling. The environmental gradient was divided into ten equal segments, and the RMSEP in each segment was calculated. The segment-wise RMSEP value was obtained based on the ten segment RMSEP values. This analysis was performed using STATISTICA version 6.0 (StatSoft, Inc., Tulsa, OK, USA).

## Results

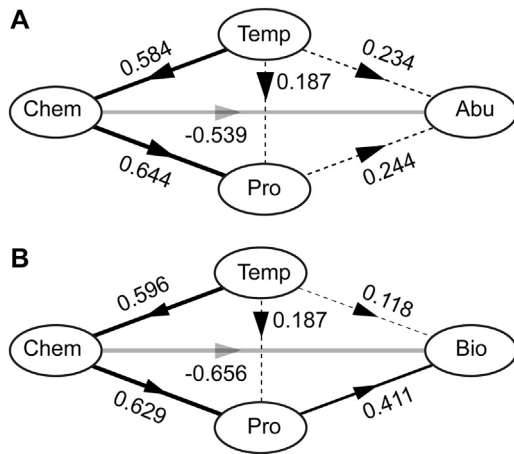
### Testate amoebae abundance and biomass

A total of 169 testate amoebae species were identified from 51 lakes and reservoirs (Supplementary Table S3). The species richness varied from 7 to 46 with a mean of 31 species per sample. The individuals with shell length  $>100 \mu\text{m}$  occurred in  $<5\%$  samples in this study (Supplementary Fig. S1). The testate amoebae abundance varied from 536 to  $10,850 \text{ ind ml}^{-1}$  (Supplementary Table S4). The carbon biomass ranged from 6.03 to  $192.53 \mu\text{g C ml}^{-1}$ .

The R-mode and Q-mode cluster analyses demonstrated that species with shell length  $>100 \mu\text{m}$  (e.g., *Centropyxis aculeata*, *Diffugia acuminata* and *Lesquereusia modesta*) and those restricted to Asia (e.g., *Diffugia mulanensis* and *Diffugia tuberspinifera*) clustered with a great number of samples mostly collected from the lakes and reservoirs located in the South-east China, the middle and lower reaches of the Yangtze River Valley, and the East-central China (Supplementary Fig. S2–S3). Moreover, their abundance and biomass were relatively high in all assemblages because they contributed approximately 75% of the total abundance or biomass. DCA ordination analysis revealed substantial overlap in the assemblages from the middle and the lower reaches of Yangtze, South-east, South-west, East-central, North-east and North of China (Supplementary Fig. S4–S5). Estimated testate amoebae diversity metrics in each lake or reservoir showed that some stations yielded low species richness that resulted in low abundance and biomass (e.g., Donghaizi Lake), while some lakes exhibited high diversity at relatively low abundance and biomass in the dataset (e.g., Wulanpao and Weishan lakes). The specific cases are demonstrated by lines of constant values on charts presented in Supplementary Figs. S4–S7.

### Water temperature as key variable correlated with testate amoebae community composition

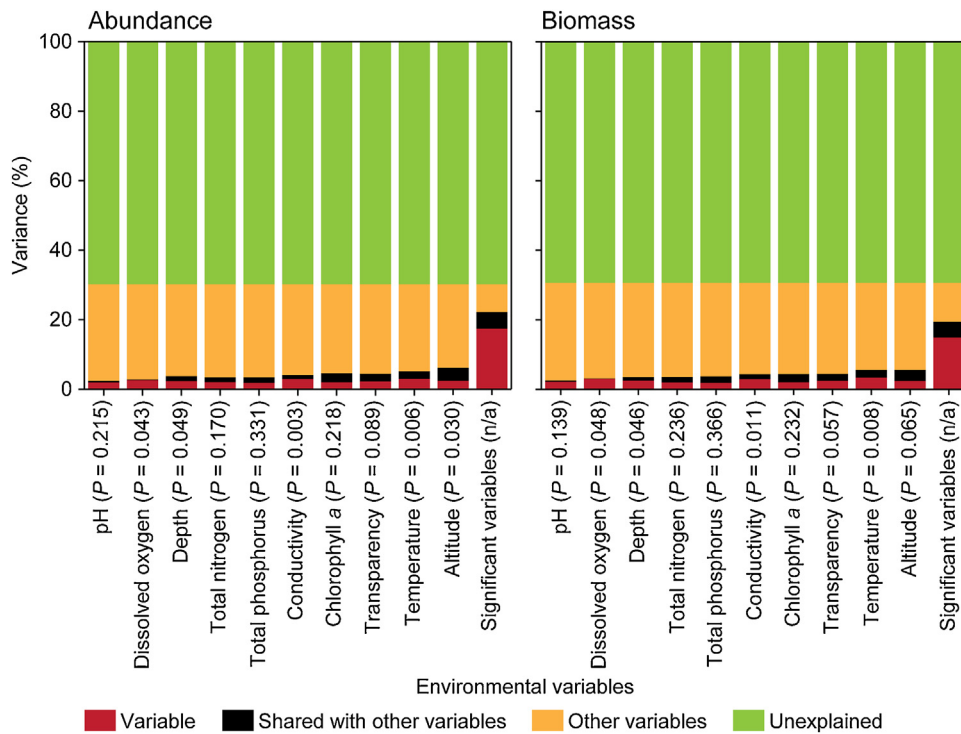
The water temperature measured from the epilimnion layers varied from  $7.1^\circ\text{C}$  (Habahuang Lake) to  $33.04^\circ\text{C}$  (Taibai Lake) (Supplementary Table S2). The partial least squares structural equation modeling demonstrated that the water temperature significantly correlated with total nitrogen, total phosphorus, dissolved oxygen, pH, chlorophyll *a*, as well as, testate amoebae abundance (GoF = 0.406) and biomass



**Fig. 3.** PLS-PM structural equations showing direct and indirect effects of lake surface water temperature on testate amoebae (A) abundance and (B) biomass. Correlations coefficients between latent variables are indicated alongside the path directions. Goodness-of-fit for testate amoebae abundance and biomass are 0.406 and 0.415, respectively. Black and grey lines stand for positive and negative correlations, respectively. Solid and dashed lines represent significant ( $P < 0.05$ ) and non-significant ( $P > 0.05$ ) relationships, respectively. Temp, water temperature; Chem, chemical properties; Pro, primary producers, Abu, abundance; Bio, biomass.

(GoF = 0.415) (Fig. 3). The relationships between the assemblages of species (e.g., testate amoebae abundance data) and their environment (e.g., ten environmental variables) showed that the first axis (Eigenvalue = 0.183) and second

axis (Eigenvalue = 0.125) explained 12.5% of the total variation in species data and 41.3% of the species–environment relationship (Supplementary Fig. S6). The main axis of species variation correlated strongly with water temperature ( $r = -0.632$ ,  $P < 0.001$ ) (Supplementary Table S5). All measured environmental variables together explained 19.1% of the total variation in species data and 63.0% of the species–environment relationship. The Monte-Carlo permutation tests showed that the first, second, third and fourth axes were significant at  $P < 0.001$ ,  $P = 0.004$ ,  $P = 0.117$  and  $P = 0.314$ , respectively. In biomass-based dataset, the first (Eigenvalue = 0.314) and second (Eigenvalue = 0.197) axes explained 12.5% of the total variance in species data and 40.7% of the species–environment relationship (Supplementary Fig. S7). The Monte-Carlo permutation tests revealed that the first, second, third and fourth axes were significant at  $P < 0.001$ ,  $P = 0.007$ ,  $P = 0.107$  and  $P = 0.306$ , respectively. The pCCAs indicated that altitude and water temperature explained approximately equal variances (Fig. 4). In the abundance-based dataset, altitude and water temperature explained 6.20% and 5.15% of the total community variance, respectively. However, water temperature was more significant ( $P = 0.006$ ) than altitude ( $P = 0.030$ ) because it explained more variance on its own (3.00%) and shared small variance with other variables



**Fig. 4.** Partial canonical correspondence analysis (pCCA) results showing the percentage variance in testate amoebae dataset explained by ten measured environmental variables. Data were log (x + 1) transformed except pH. P-value was indicated for each variable.

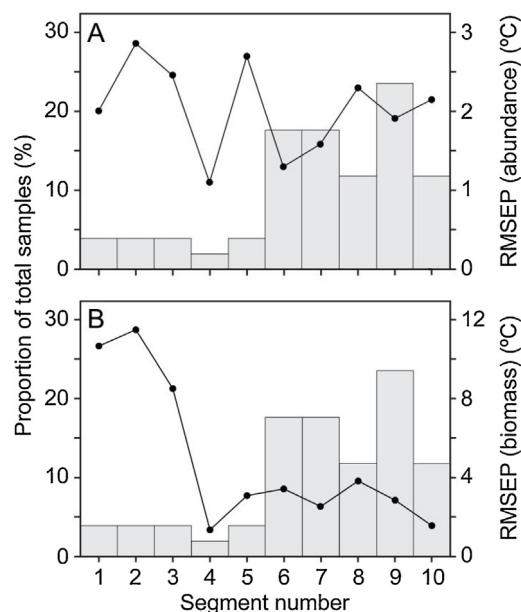
(2.15%). In the biomass-based dataset, those two important variables explained 5.60% and 5.57% of the total community variance, respectively. Again, water temperature accounted for more variance on its own (3.35%) than other variables. Using Spearman's correlation analysis, we found that those two variables were inter-correlated with very strong negative relationship ( $n=51$ ,  $\rho=-0.831$ ,  $P<0.001$ ), suggesting that water temperature decreasing with the elevation. The correlation coefficients between pairs of variables and their significance levels are summarized in Supplementary Table S6.

### Temperature transfer functions based on abundance and biomass data

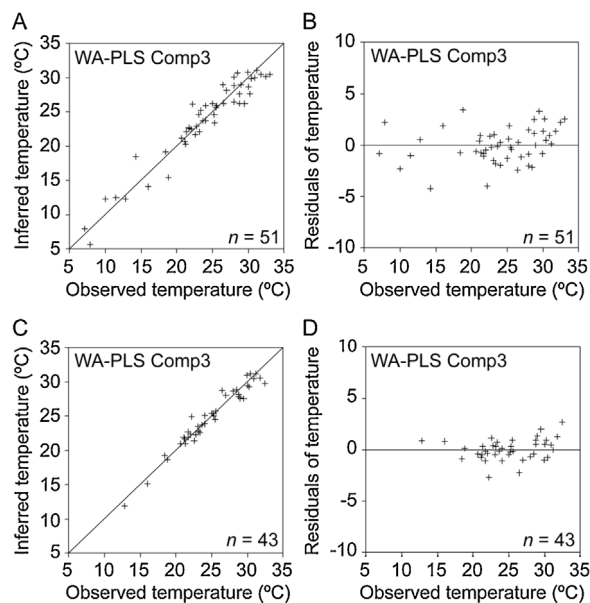
Both abundance and biomass data of testate amoebae displayed a significant relationship with water temperature (Figs. 3–4 and Supplementary Figs. S6–S7). This gives a good basis for examination of individual species niches in terms of the optimal values along the temperature gradient. Therefore, transfer functions for temperature based on testate amoebae abundance and biomass could be developed, respectively.

The performance of several models for temperature based on abundance was very similar, with three-component weighted average partial least squares (WAPLS-Comp3) model being the best performing (root mean square error of prediction RMSEP = 4.73 °C,  $R^2=0.45$ ) (Table 1). And compared to a total temperature gradient range of 25.94 °C and standard deviation of 6.35 °C, the performance of these models for temperature was relatively good. However, segment-wise RMSEP (sRMSEP) was slightly higher (2.11 °C) than the original RMSEP (1.95 °C), and RMSEP values were higher at the poorly sampled cold end of the gradient except segment 4 which had only one sample (Fig. 5A). Comparisons of the observed and predicted water temperature indicated that the performance of this model was particularly poor for few samples with very low temperature (Fig. 6). These samples were removed following screening of the samples with residuals greater than 20% of the full range (5.19 °C) (Supplementary Table S7). The improved dataset ( $n=43$ ) resulted in an improved RMSEP of 2.87 °C with an  $R^2$  of 0.60 for the WAPLS three-component model (Table 1).

Transfer functions for water temperature based on testate amoeba biomass were also developed and tested (Table 2). The performance of modern analogue techniques (MAT) was significantly better than the three other approaches which were more routinely used. The MAT model yielded an RMSEP of 5.20 °C and  $R^2$  of 0.52. Similarly, sRMSEP based on biomass data was also higher (6.13 °C) than the original RMSEP (4.50 °C), and RMSEP values were higher at cold end of the gradient except segment 4 (Fig. 5B). The improved MAT model performance (RMSEP = 3.34 °C,  $R^2=0.67$ ) suggested that the reconstruction of temperature was also possible using biomass dataset. In screening results,



**Fig. 5.** Segment-wise RMSEP results showing sample numbers (proportion of total) and RMSEP values for each of ten even segments of the lake surface water temperature gradient. (A) Abundance-based and (B) biomass-based testate amoebae communities. Column charts show the temperature gradient was divided into ten equal segments, and the line graphs show RMSEP values of every segment.



**Fig. 6.** Observed vs. model estimated lake surface water temperature based on abundance data of testate amoebae. (A) Original data, (B) original data residuals, (C) dataset after removal of outliers (improved model), and (D) residuals of improved model.

most of the samples removed were of extreme low temperature samples (Supplementary Table S7). Thus, the inferred values were highly underestimated at this end of temperature gradient (Fig. 7).

**Table 1.** The performance statistics of temperature transfer functions for abundance-based freshwater testate amoebae communities.

Total dataset ( $n = 51$ )	WA.inv	WA.cla	WA.inv.tol	WA.cla.tol	WAPLS Comp3	WAPLS Comp4	WAPLS Comp5	PLS Comp3	MAT N05
Temperature ( $^{\circ}\text{C}$ )									
Crossval RMSEP	5.23	6.01	5.15	6.23	<b>4.73</b>	4.75	4.92	5.18	5.98
Crossval $R^2$	0.32	0.34	0.38	0.39	<b>0.45</b>	0.45	0.45	0.47	0.30
Crossval average bias	-0.31	-0.50	-0.65	-0.98	<b>-0.46</b>	-0.44	-0.	0.05	-1.66
Crossval maximum bias	11.09	9.81	12.08	9.53	<b>10.51</b>	10.20	9.78	9.50	14.62
Dataset after screening ( $n = 43$ )	WA.inv	WA.cla	WA.inv.tol	WA.cla.tol	WAPLS Comp3	WAPLS Comp4	WAPLS Comp5	PLS Comp3	MAT N05
Temperature ( $^{\circ}\text{C}$ )									
Crossval RMSEP	3.35	3.48	3.40	3.81	2.87	2.94	2.96	3.69	4.19
Crossval $R^2$	0.44	0.46	0.48	0.49	0.60	0.58	0.58	0.48	0.34
Crossval average bias	-0.34	-0.47	-0.43	-0.56	-0.28	-0.37	-0.43	0.14	-1.13
Crossval maximum bias	9.08	7.40	10.02	9.23	4.96	5.31	4.05	4.47	12.13

WA.inv, weighted averaging with inverse deshrinking; WA.cla, weighted averaging with classical deshrinking; WA.inv.tol, weighted averaging-tolerance downweighted with inverse deshrinking; WA.cla.tol, weighted averaging-tolerance downweighted with classical deshrinking; WAPLS, weighted averaging partial least squares (with component number); PLS, partial least squares; MAT, modern analogue technique. RMSEP, root mean square error of prediction. Values for the best performing model (WAPLS Comp3) are in bold.

Before performing statistics of temperature transfer functions, data were  $\log(x + 1)$  transformed.

**Table 2.** The performance statistics of temperature transfer functions for biomass-based testate amoebae communities.

Total dataset ( $n = 51$ )	WA.inv	WA.cla	WA.inv. tol	WA.cla. tol	WAPLS Comp2	PLS Comp1	MAT N03	MATN04	MAT N03.wm	MAT N04.wm
Temperature ( $^{\circ}\text{C}$ )										
Crossval RMSEP	5.36	6.13	5.15	5.85	5.10	5.80	5.39	5.27	5.36	<b>5.20</b>
Crossval $R^2$	0.31	0.32	0.37	0.38	0.37	0.28	0.52	0.53	0.51	<b>0.52</b>
Crossval average bias	-0.39	-0.59	-0.77	-1.09	-0.30	0.22	0.16	0.08	0.19	<b>0.14</b>
Crossval maximum bias	12.20	9.76	13.26	11.70	12.48	11.83	10.95	11.00	10.89	<b>10.86</b>
Dataset after screening ( $n = 42$ )	WA.inv	WA.cla	WA.inv. tol	WA.cla. tol	WAPLS Comp2	PLS Comp1	MAT N03	MAT N04	MAT N03.wm	MAT N04.wm
Temperature ( $^{\circ}\text{C}$ )										
Crossval RMSEP	3.19	3.54	3.19	3.41	3.03	4.09	3.54	3.41	3.49	3.34
Crossval $R^2$	0.43	0.45	0.44	0.46	0.50	0.33	0.68	0.66	0.68	0.67
Crossval average bias	-0.32	-0.47	-0.47	-0.64	-0.27	0.53	0.30	0.22	0.29	0.21
Crossval maximum bias	5.73	3.59	5.59	4.29	3.94	10.21	5.87	6.08	5.86	6.08

For model abbreviations see Table 1.

Values for the best performing model (MAT N04.wm) are in bold.

Before performing statistics of temperature transfer functions, data were  $\log(x + 1)$  transformed.

## Optima and tolerances of temperature

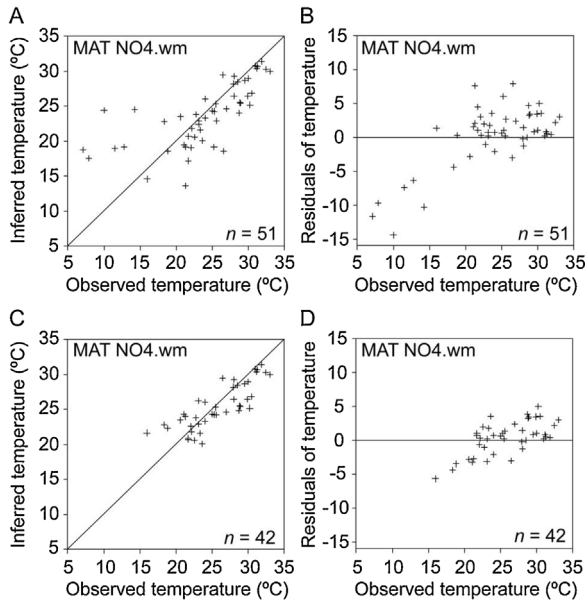
There were some minor differences in the relative positions of some species between abundance- and biomass-based optima estimates, but the general trends for both data were similar or identical (Fig. 8). In this study, the species occurring in 5 or more lakes and reservoirs were chosen to calculate their optima and tolerance of water temperature.

The water temperature preferences of 58 species (based on abundance) are presented in Fig. 8A. The temperature optima ranged from  $15.98 \pm 5.56^{\circ}\text{C}$  for *Diffflugia stoutii* to  $30.26 \pm 1.86^{\circ}\text{C}$  for *D. mulanensis*. For biomass-based tes-

tate amoebae data, the optimal values and tolerance ranges for the 47 species are illustrated in Fig. 8B. The temperature optima values ranged from  $20.38 \pm 2.49^{\circ}\text{C}$  for *Diffflugia amphoralis* to  $30.72 \pm 1.51^{\circ}\text{C}$  for *D. mulanensis*.

When comparing the optimal value of the same species inferred from abundance- and biomass-based data, similar trends for optimal values were found. The predicted optimal value was slightly higher for biomass than abundance, except for *Arcella hemisphaerica*, *Centropyxis orbicularis*, *Diffflugia kabylica*, *Diffflugia gramen globulosa* and *Diffflugia lanceolata*. Furthermore, unlike the findings for temperature based on abundance, there was no significant relationship





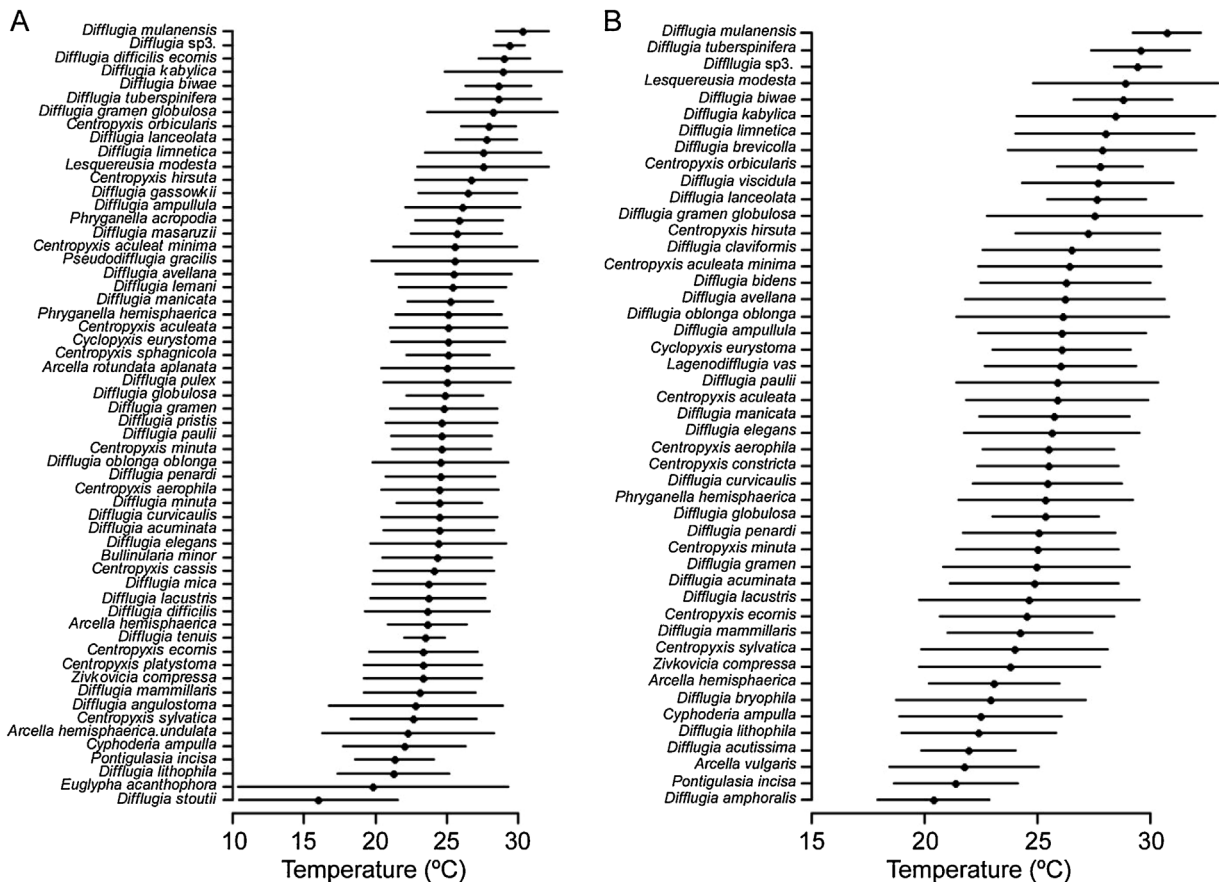
**Fig. 7.** Observed vs. model estimated lake surface water temperature based on biomass data of testate amoebae. (A) Original data, (B) original data residuals, (C) dataset after removal of outliers (improved model), and (D) residuals of improved model.

between the optima and the tolerance of species based on biomass.

## Discussion

This study examined the relationship between water temperature and freshwater testate amoebae and developed abundance- and biomass-based transfer functions for temperature. The sampling sites were selected from different locations and climate zones of China. Our datasets were relatively small in comparison with the datasets used to build other transfer functions (Bellen et al. 2014; Charman et al. 2007; Lamarre et al. 2013; Roe et al. 2010), but three replicates in each lake or reservoir were mixed up to generate one sample. Most of observed species were small and cosmopolitan (Bobrov et al. 2012; Smith et al. 2008; Yang et al. 2010). The estimated species richness (range: 7–46) was generally in the normal range compared with previous studies in China (Bobrov et al. 2012; Yang et al. 2005b, 2006).

Our structural equation models confirmed the significant role played by water temperature in the modification of lake water chemistry (Bridge and Demicco 2008; Mazumder et al. 1990), suitable food for testate amoebae (Dryden and Wright



**Fig. 8.** Optimal temperature and tolerance ranges for testate amoebae species appeared in at least five lakes and reservoirs. (A) Abundance-based dataset for 58 species, and (B) biomass-based dataset for the 47 species. Note that the species composition in the improved abundance- and biomass-based datasets of Chinese lakes and reservoirs is different.

1987; Han et al. 2011a; Ogden and Hedley 1980) and distribution of freshwater testate amoebae (Han et al. 2011b; Ren et al. 2018). In both abundance and biomass-based datasets, water temperature explained more variance on its own than other variables suggesting its strong effect on freshwater testate amoebae distribution.

Among the abundance-based transfer functions, WA-PLS models had the best performance (Table 1). WA-PLS regression method improved the predictive ability compared with the simple weighted averaging model. Partial least squares manifested very similar performance to WAPLS-Comp3, but this model did not yield good results in palaeoecological study using freshwater testate amoebae (Roe et al. 2010). WA-PLS models were unhelpful in explaining the significance of water temperature effect on testate amoebae specifically in the range of 20–30 °C. This was regarded as a drawback in the performance of our models because water temperature can affect testate amoebae indirectly (Fig. 3). The good performance of MAT models for water temperature transfer functions based on biomass data was a surprising finding (Table 2). However, this model has been seldom used in testate amoebae and paleolimnology and there are potential problems of autocorrelation in MAT approaches (Charman et al. 2007). Overall, MAT models can be used to promote the accuracy of paleoenvironmental reconstruction although these models have not been extensively tested in testate amoebae studies.

The relationships between temperature and the occurrence of certain testate amoebae species gained great attention in palaeoecology (Beyens and Meisterfeld 2001; Patterson et al. 1985; Yang et al. 2006). However, there was still no integrated report about their optimal tolerance to water temperature. In this study, the sampling of wider environmental gradients than those usually associated with only one lake or relative small areas has allowed wider modern analogues to be established for testate amoebae in lakes and reservoirs across China. More importantly, the good performance of testate amoebae abundance- and biomass-inferred temperature transfer functions successfully made the examination of water temperature optimum-tolerance values possible. However, some scholars stated that even where a statistically significant model is developed for water temperature, its application over long time periods requires caution, since the inferred temperatures may be merely artifacts of changing lake water chemistry (Anderson 2000; Juggins 2013a). It should, however, be noted that the temperature gradient in this study was sampled unevenly, with a small number of samples from the very cold and more samples from warm sites (Supplementary Table S2). This is likely to have skewed apparent model performance as RMSEP values are biased by the uneven sampling (Telford and Birks 2011) resulting in fact that all the models losing predictive ability on the extreme cold side of the temperature gradient. However, sRMSEP results were still less than the standard deviation and within the normal range of previous results, suggesting that the models have predictive power (Payne et al. 2012). Furthermore,

the surface sediment samples analyzed by this study were not dated. The environmental variables were only measured on the day of sampling and did not take seasonal fluctuations into account, and water temperature varied greatly in summer and winter especially in higher latitudes, which leading to the variables falling along relative environmental gradients. The models constructed for other environment variables (depth to water table, moisture and pH) are also an instantaneous value in previous works (Booth 2002; Charman et al. 2007).

Overall, our findings provided an insight into freshwater testate amoebae and their relationship to summer water temperature, as well as the applications and limitations of testate amoebae abundance- and biomass-based transfer functions for temperature. The influence of environmental variables on freshwater testate amoebae was discussed in depth. This is an important achievement because existing knowledge on ecology of freshwater testate amoebae could not resolve an artifact created by dependent variables such as altitude and water temperature (Ju et al. 2014). There are some potential limitations that merit further discussion. First, our testate amoeba biomass data can serve to down-weight small species relative to larger species. This will tend to reduce the influence of filose species with idiosome shells which are bacterivorous and increase the influence of lobose species with broader feeding preferences (the larger species). The current biomass calculation based on geometric shapes was first introduced for testate amoebae by Gilbert et al. (1998), while the carbon/volume conversion factor in their study was for ciliates (Weisse et al. 1990). Moreover, it is well-known that an amoeba may only occupy a small proportion of its total shell volume. However, there is no doubt that the proportion is different for different species. In order to improve the performance of the models, samples with high residuals were removed from the training sets in this study. Although it had been done frequently in the past (Charman et al. 2007; Lamentowicz et al. 2008), this process could lead to a reduction of the training set and temperature gradient. Second, the use of 25–300 µm sieves to recover testate amoebae from samples (Beyens and Meisterfeld 2001; Wall et al. 2010) may lead to the loss of testate amoebae individuals that are smaller than 25 µm and larger than 300 µm, thus influencing the accuracy and interpretation of testate amoebae communities.

## Conclusions

This is the first large-scale study using testate amoebae to develop transfer functions for water temperature in lakes and reservoirs. Our results showed that both abundance- and biomass-based testate amoeba data displayed the strongest response to water temperature, indicating that testate amoebae have considerable potential in the quantitative reconstruction of past temperature variability. The performance of the transfer functions developed for temperature were generally good, with WA-PLS to be the best model

in testate amoebae abundance-based temperature transfer functions, and MAT in the biomass-based temperature transfer functions. Although RMSEPs were quite large and the models were less predictive on the extreme cold side of temperature gradient, our results demonstrated the potential for using testate amoebae to the better understanding of the ecological integrity and its vulnerability in aquatic environments. Further studies about the applicability of temperature transfer functions based on freshwater testate amoebae in areas without altitudinal effects are recommended.

## Author contributions

JY conceived the idea and designed the research. LJ performed the laboratory experiments. JY contributed the new reagents and the analytic tools. JCN, LJ and JY analyzed the data and wrote the manuscript. All authors discussed the interpretation of the results and have approved the final manuscript.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ejop.2019.03.003>.

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