Environ Earth Sci (2016) 75:627
DOI 10.1007/s12665-016-5442-7

ORIGINAL ARTICLE

Testate amoebae as indicators of water quality and contamination in shallow lakes of the Middle and Lower Yangtze Plain

Yangmin Qin[1,2] · **Richard Payne**[3,4] · **Xiangdong Yang**[5] · **Min Yao**[5,6] · **Jiantao Xue**[1,2] · **Yansheng Gu**[2] · **Shucheng Xie**[1,2]

Received: 29 August 2015 / Accepted: 6 February 2016

Abstract Testate amoebae are micro-organisms characterized by an agglutinated or autogenous shell enclosing the cytoplasm. Testate amoebae have been widely proposed as valuable bioindicators in a range of ecosystems (such as soils, peatlands and lakes). The use of testate amoebae as bioindicators of water quality in aquatic ecosystems is much less developed than for other microorganisms and previous research is geographically restricted. We investigated a large range of environmental variables and their relations to testate amoeba communities from 37 shallow lakes in the middle and lower Yangtze River Plain of China. Multiple factor analysis, redundancy analysis (RDA) and a forward-selection approach were used to explore the overall community structure and the links between the environmental variables and testate amoeba composition. Results showed that testate amoebae are widely distributed in most of the lakes despite often high levels of pollution. Our data highlight some links between water quality variables and testate amoeba communities. In particular, our results suggest that heavy metals have a role in shaping testate amoeba assemblage structure, although correlations are comparatively weak, perhaps due to lagged responses. Our results support previous suggestions that testate amoebae may be useful bioindicators in aquatic ecosystems but emphasize the need for an improved mechanistic understanding.

Keywords Testate amoebae · Lakes · Indicator · Middle and Lower Yangtze Plain

Introduction

The middle and lower Yangtze reaches of southern China are known as the ‘country of a thousand lakes’ (Jin et al. 1990). This is one of only 17 regions recognized as both a Global Biodiversity Hotspot and a Global 200 Priority Ecoregion (Mittermeier and Mittermeier 1997; Olson and Dinerstein 1998). However, this region is also one of the most developed regions of China. With the development of extensive agriculture, fishing, farming and urbanization during recent decades, most lakes in this area have undergone eutrophication (Shu et al. 1996; Cheng and Li. 2006; Yang et al. 2005). The deterioration of drinking water due to cyanobacterial blooms in many lakes has become a serious socio-economic problem (Qin et al. 2004; Dong et al. 2008; Wang et al. 2012; Shi et al. 2015). A large amount of wastewater has been released into many of the lakes from industrial activities, residential areas and

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Electronic supplementary material The online version of this article (doi:10.1007/s12665-016-5442-7) contains supplementary material, which is available to authorized users.

✉ Yangmin Qin
qinyangmin2005@163.com

[1] Department of Geography, School of Earth Science, China University of Geosciences, 430074 Wuhan, China

[2] State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, 430074 Wuhan, China

[3] Environment, University of York, Heslington York YO105DD, UK

[4] Department of Zoology and Ecology, Penza State University, Krasnaya str. 40, 440026 Penza, Russia

[5] State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, China

[6] School of Environment and Planning, Liaocheng University, 252059 Liaocheng, China

agriculture. In addition, the building of dikes and dams since late twentieth century has isolated the lakes from the Yangtze River, changed the natural hydrological conditions and reduced the lake areas (Qin et al. 2009). Such human activities have seriously damaged the aquatic ecosystems leading to a decline in biodiversity, and threats to local human health (Dearing et al. 2012; Wang et al. 2012; Shi et al. 2015). Limnological investigations are essential to aid future long-term watershed management and sustainable development (Dong et al. 2008; Yang et al. 2008; Wang et al. 2012; Chen et al. 2014).

An increasing body of evidence suggests that microorganisms such as protozoa and diatoms are sensitive to a wide variety of environmental changes (Charman et al. 1998; Bobrov et al. 1999; Booth 2001; Beyens and Meisterfeld 2001; Mitchell et al. 2008; Mazei et al. 2012; Payne 2013; Lamentowicz et al. 2015; Li et al. 2015; Song et al. 2014). High species diversity, rapid responses to environmental change and cosmopolitan distribution mean that microorganisms can be useful bioindicators in ecological and palaeoecological studies (Qin et al. 2013; Payne 2013). Recent research has suggested that a group of protozoa with particular potential as bioindicators foidentifications were made with the aid of Penardr water quality is the testate amoebae. Testate amoebae have been suggested to respond to anthropogenic eutrophication, acidification and metal inputs in a number of studies worldwide (Asioli et al.1996; Mitchell et al. 1999; Patterson and Kumar 2000; Kauppila et al. 2006; Escobar et al.2008; Neville et al. 2010; Kihlman and Kauppila 2012; Ju et al. 2014; Macumber et al. 2014; Roe and Patterson 2014).

Testate amoebae are micro-organisms characterized by an agglutinated or autogenous shell enclosing the cytoplasm (Mitchell et al. 2008). Their high abundance and responses to environmental stress which are both large-magnitude and distinctive, suggest considerable potential to indicate water conditions. Excellent preservation of tests in lacustrine sediments also shows potential for the reconstruction of past trajectories of change in water quality (Patterson et al. 2002; Tsugeki et al. 2003; Escobar et al. 2008; Roe et al. 2010; Qin et al. 2011). It is reasonable to expect that testate amoebae will respond to aquatic pollution by metals or nutrients but previous studies have been restricted in terms of their geographic location (particularly in North America) and the types of lakes and pollutants considered.

This study focuses on the water quality of lakes within the middle and lower Yangtze River plain and the potential role of testate amoebae as bioindicators. The aims are to investigate the testate amoeba community structure, test the physico-chemical controls on amoeba communities and to understand how the testate amoeba community responds to human activities in contaminated shallow lakes.

Materials and methods

Study sites and sampling

This study forms part of an ongoing research theme investigating the ecology and paleoecology of lakes in the middle and lower Yangtze River plain (Dong et al. 2008; Yang et al. 2008; Yao et al. 2011). The lakes were selected beside the Yangtze River and sampled beginning at Shanghai City in the estuary of Yangtze River to Wuhan, the middle reach of Yangtze River. In total we sampled 37 shallow lakes, with one sample from nearly the middle or the deepest point of each lake (Fig. 1). The lakes span a broad range of physical and chemical gradients. The lakes span more than 800 km and range in depth from 1.2 to 6.55 m. Samples from four of these lakes were analyzed for testate amoebae, but do not have a complete set of environmental data so these are only briefly discussed.

Water samples were taken seasonally from July 2007 to July 2008 and analyzed for water chemistry within 1 week of collection. Sediment samples were also collected on these sampling occasions but proved insufficiently large for metal and testate amoeba analysis. Additional sediment samples were therefore collected from each lake in October 2010 and the upper 0.5 cm sub-sampled for analysis. These sediment samples are likely to integrate conditions in the lake over several years so the 2-year gap between water and sediment sampling is unlikely to have a large influence on our results.

Water and sediment chemistry

Results of water quality analyses have been previously published (Yang et al. 2008; Yao et al. 2011). Chemical variables including total phosphorus (TP), total nitrogen (TN), nitrate (NO_3_N), nitrite (NO_2_N), ammonia (NH_4_N), chlorophyll-a (Chl-a), total iron, chemical oxygen demand (COD), SiO_2, dissolved inorganic phosphorus, CO^{3-}, and HCO^- were determined using standard techniques (Institute of Hydrogeography and Engineering Geology, MGMR 1990; Jin et al. 1990). K^+ and Na^+ concentrations in water were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES; Leeman-Labs Profile, Hudson, NH, USA.. Concentrations of Ca^{2+}, Mg^{2+}, and ${SO_4}^{2-}$ were analyzed using EDTA titrimetric methods. The argentometric method ($AgNO_3$) was used for Cl^- determination (Greenberg et al. 1992). Water pH and conductivity were measured in the field using a HI-214 conductivity meter and Hanna EC-214 pH meter (HANNA instruments, Canada). Data used in our analyses are means of the four seasonal samples.

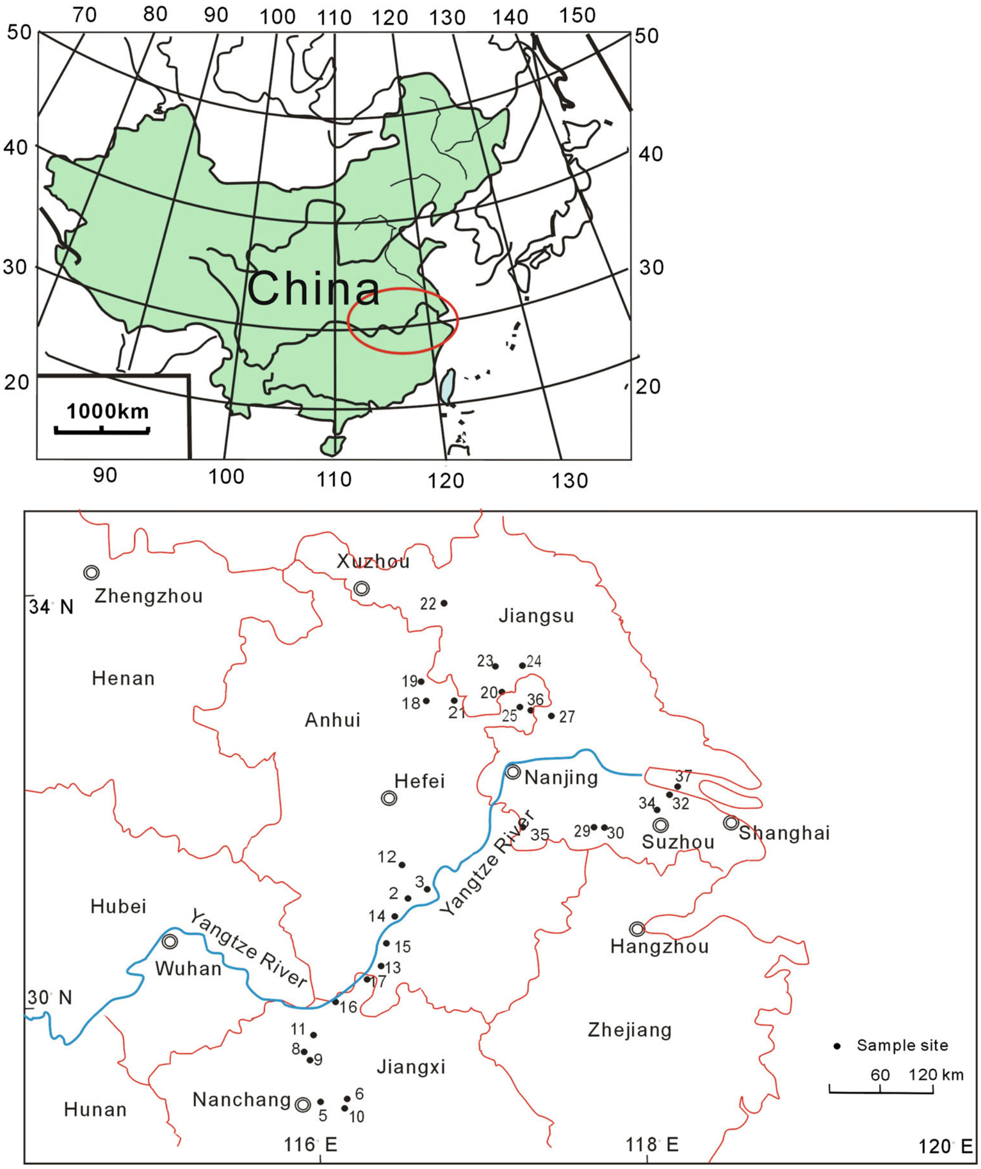

Fig. 1 Map showing the location of sample sites in the middle and lower reaches of Yangtze River. See Table 1 for names of the lakes

Metal concentrations in sediments were determined in the State Key Laboratory of Geological Processes and mineral Resource, China University of Geosciences (Wuhan). Sediment samples were dried, ground and passed through 180 μm mesh. Sieved 20 mg samples were acid digested and analyzed by ICP-MS to determine heavy metal concentrations in sediments (Supplementary Table 2). Metal oxide weight percentages were normalized to 100 % and corrected by the *ablation yield correction factor* (AYCF) (Moor et al. 2001; Liu et al. 2008).

Testate amoeba analysis

Wet subsamples of about 5 g of benthic sediment were analyzed for testate amoebae. Isolation of testate amoeba from the sediment samples using a modified version of the procedure of Patterson and Kumar (2000): sediment samples were washed through 500 and 35 μm sieves into pertri dishes to retain the testate amoeba shells. However, we note that sieve sizes used to retain testate amoeba shells have varied considerably, including sieve sizes larger and smaller than that used in this study and that the smallest tests may pass through the mesh (Charman et al. 1998; Patterson and Kumar 2000; Patterson et al. 2002; Payne and Mitchell 2009; Avel and Pensa 2013). As material and test concentration were limited we analyzed all tests in the dishes. Slides were prepared and examined using a stereomicroscope (Olympus SZX16) at 70–200× magnification for species identification. In counting we aimed for at least 100 shells, however, the abundance of testate amoebae was very low in some samples, with as few as 32 individuals. We acknowledge that these relatively low total are sub-

Table 1 Summary details for lakes studied

#	Site name	Depth	Richness	Shannon *H*	Count total
1	Qinglan	2.55	9	1.73	54
2	Baidang	1.7	15	2.49	78
3	Fengsha	1.325	12	2.06	155
4	Niuya	1.9	11	2.00	56
5	Jinxi	2.075	7	1.77	41
6	Yaohu	1.45	12	2.15	52
7	Qingshanhu	1.265	11	2.30	88
8	Banghu	1.9	9	1.58	132
9	Shahu	2.325	12	2.03	148
10	Junshan	2.3	11	2.02	52
11	Poyang	3.75	12	2.24	135
12	Huangbo	1.225	14	2.20	66
13	Huangni	1.8	13	2.28	106
14	Pogang	1.525	10	1.68	112
15	Shengjin	1.775	14	2.06	141
16	Taipo	2.425	17	2.47	129
17	Huayuan	2.3	5	1.02	87
18	Tuohu	2.35	5	1.32	56
19	Nvshan	6.55	5	1.05	34
20	Qili	3.2	4	1.11	32
21	Luoma	3.8	12	2.22	70
22	Hongze	3.9	9	2.02	37
23	Baima	1.325	7	1.62	81
24	Jinhu	2.6	8	1.52	68
25	Gaoyou	3.15	7	1.70	44
26	Shaobo	2.55	10	1.75	97
27	Shanghu	*3.175*	*14*	*2.56*	*42*
28	Tuanjiu	1.2	9	1.78	62
29	Dongjiu	1.35	11	1.76	131
30	Caohu	3.35	10	2.05	78
31	Kuncheng	2.15	7	1.16	98
32	Ezhendang	3	10	1.82	131
33	Shijiu	2.05	4	0.99	70
34	Yanghu	*2.35*	*7*	*1.16*	*103*
35	Nanhu	2.85	10	1.71	67
36	Huangmaotan	*1.875*	*13*	*2.36*	*75*
37	Xiujiu	*1.85*	*7*	*1.61*	*17*

Numbers correspond to locations shown on Fig. 1

Sites shown in italics have incomplete environmental data and are not included in statistical analyses

optimal, but given the lack of previous research believe that our results are still of value. Simulation studies suggest that even low totals can give valuable data on community structure (Payne and Mitchell 2009). Our results should be interpreted in the light of the count totals. Species identifications were made with the aid of Penard (1902), Ogden (1983), Shen (1983), Kumar and Dalby (1998).

Numerical analyses

We compiled the testate amoeba data and converted to percentages of the total count. To quantify sample diversity we determined the Shannon diversity index (SDI), calculated as follows:

$$S.D.I = -\sum_{i=1}^{S}\left(\frac{X_i}{N_i}\right) \times \ln\left(\frac{X_i}{N_i}\right)$$

where Xi is the abundance of each taxon in a sample, Ni is the total abundance of the sample, and S is the species richness of the sample (Shannon and Weaver 1949). The Shannon diversity index assumes that all individuals are represented in the sample and are randomly sampled from an ''infinitely large'' population (Shannon and Weaver 1949).

To explore the overall structure of the environmental and testate amoeba data we first conducted non-metric multi-dimensional scaling ordinations of both testate amoebae and environmental datasets (Clarke 1993). The species data was Hellinger transformed prior to all analyses (Legendre and Gallagher 2001). To identify groupings of samples with similar environmental or species characteristics we used cluster analysis based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean). We used IndVal (Dufrêne and Legendre 1997; Podani and Csányi 2010) to identify indicator species for sample groupings based on the first splits of the cluster analysis of environmental data.

To examine the links between species composition and environment we first simultaneously ordinated both the species and environmental data using multiple factor analysis (MFA: Escofier and Pages 1994). To quantitatively test the explanatory power of environmental variables we used redundancy analysis (RDA; Legendre and Legendre 2012). The environmental data consisted of 55 distinct environmental variables, compared to 33 sites and 23 taxa. To reduce the variables included in the redundancy analysis to a manageable number we used a forward-selection approach in which variables were successively added according to their explanatory power while accounting for variables already selected (999 Monte Carlo permutations, cut-off at $P < 0.05$). This approach allowed us to objectively select a minimum suite of explanatory variables but it is important to note that selection does not imply causation as many variables are strongly correlated (Supplementary Table 1).

Results

Water quality

All the studied lakes are quite shallow with depth ranges from 1.2 to 6.55 m, and weakly alkaline, with pH values varying between 7.6 and 8.3. Across the 37 studied lakes, pH ranged from 7.55 to 8.27, Ca^{2+} from 4.78 to 41.56 µg/L, Mg^{2+} from 2.45 to 15.57 µg/L, concentrations of TP ranged from 10 to 390 µg/L, TN ranged from 320 to 3320 µg/L, conductivity from 67.5 to 700.5 $\mu S/cm^2$ and chlorophyll a from 1.25 to 38.53 µg/L (Supplementary Table 2).

Many of the environmental variables are strongly correlated. Correlations are particularly strong between many of the heavier elements with atomic mass above 60 (Cu-Pb). In this range most correlations are significant ($P < 0.05$) and many very strong (Supplementary Table 1). Strong and significant correlations are also apparent between many variables associated with nutrient status such as TP, TN, NO_3_N, NH_3_N, etc. (Supplementary Table 1). These correlations most likely represent commonality of source.

Testate amoebae species composition

The testate amoeba community overall was comparatively species-poor with only 23 taxa identified (Tables 1, 2) and a mean taxon richness of 9.8. Shannon diversity varied from 0.99 to 2.56 supporting the low diversity of the community when considering evenness as well as richness. The most abundant taxa were *Centropyxis ecornis* (mean abundance 21.4 %), *Centropyxis cassis* (15.3 %), *Difflugia oblonga* (13.5 %) and *Centropyxis aculeata* (12.7 %). These are all taxa which are relatively frequently found in lakes and other wet habitats (Booth 2001; Warner et al. 2007; Payne et al. 2008; Qin et al. 2009; Swindles et al. 2009; Roe et al. 2010; Sullivan and Booth 2011; Patterson et al. 2013a; Turner et al. 2013; Lamentowicz et al. 2015). No taxa were present in all samples and some species were notably abundant in a few samples but comparatively rare overall (e.g., *Centropyxis platystoma* and *Pontigualsia compressa*). Overall the community composition is notable for the dominance of taxa with xenosome tests, and *Difflugia* species in particular (14 of 23 taxa).

Multiple factor analysis and ordination

The Non-metric Multidimensional Scaling (NMDS) ordination plot for environmental variables (Fig. 2a) shows strong clustering of around half the sites but with others strongly scattered along the two axes. Lakes Kuncheng, Caohu, Ezhendang and Qingshanhu have high scores on axis one and are distinguished from the other sites in the

Table 2 Details of testate amoeba communities of the sites

#	Taxon	*N*	Minimum %	Maximum %	Mean %
1	*Arcella discoides*	14.0	0.0	21.6	2.9
2	*Centropyxis aculeata*	20.0	0.0	67.1	12.7
3	*Centropyxis cassis*	29.0	0.0	50.0	15.3
4	*Centropyxis ecornis*	29.0	0.0	64.7	21.4
5	*Centropyxis platystoma*	11.0	0.0	41.1	2.6
6	*Cucurbitella tricuspis*	16.0	0.0	27.3	4.5
7	*Difflugia amphoralis*	2.0	0.0	2.3	0.1
8	*Difflugia acuminata*	13.0	0.0	7.3	1.2
9	*Difflugia acutissima*	19.0	0.0	16.1	2.6
10	*Difflugia bicornis*	4.0	0.0	3.9	0.2
11	*Difflugia biwae*	1.0	0.0	5.9	0.2
12	*Mediolus (Difflugia) corona*	1.0	0.0	14.8	0.8
13	*Difflugia elegans*	17.0	0.0	25.6	4.1
14	*Difflugia lanceolata*	11.0	0.0	24.3	1.4
15	*Difflugia mammillaris*	6.0	0.0	7.7	0.8
16	*Difflugia oblonga*	32.0	0.0	37.0	13.5
17	*Difflugia pulex*	18.0	0.0	27.3	4.3
18	*Difflugia urceolata*	20.0	0.0	8.2	1.7
19	*Difflugia smilion*	15.0	0.0	12.8	1.9
20	*Difflugia sp.1*	1.0	0.0	2.9	0.1
21	*Lesquereusia modesta*	4.0	0.0	5.4	0.3
22	*Pontigulasia compressa*	19.0	0.0	34.2	3.7
23	*Pontigulasia incisa*	17.0	0.0	17.3	3.3

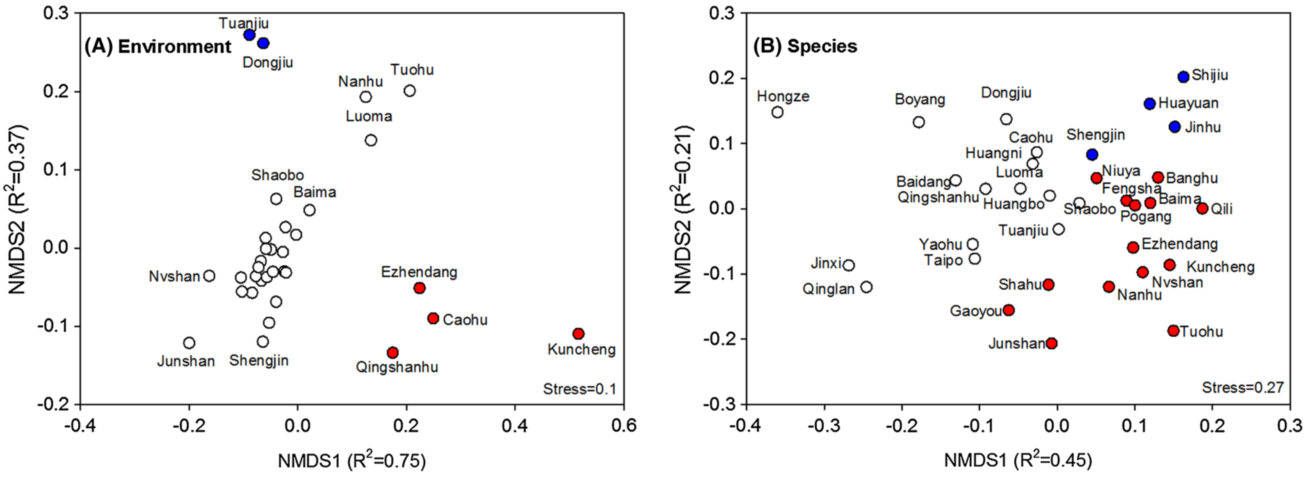

Fig. 2 Non-metric multidimensional scaling plots for **a** Environmental variables and **b** Hellinger transformed species data. Differences in colour represent groupings based on a UPGMA cluster analysis. Plot A omits some site names where points are tightly clustered. Note difference in stress between the two plots

first split of the cluster analysis. These sites are noted by high abundance of several heavy metals including Zn (up to 789 ppm) and Cr (up to 319 ppm) and TN (up to 3.3 mg/L, all in Kuncheng Lake). The NMDS results highlight Kuncheng Lake as a clear extreme outlier in terms of these variables and this site is assigned its own grouping in further cluster analysis splits. On axis 2, sites Tuanjiu, Dongjiu, Nanhu, Luoma and Tuohu have notably high scores and Tuanjiu and Dongjiu form a distinct group in the cluster analysis. This gradient is weaker but appears to be associated with base richness with these sites typically having higher values for variables such as Mg^{2+}, Ca^{2+} and Na^{+}. The NMDS plot for species (Fig. 2b) fits the data less well but suggests less extreme scattering of sites. Cluster analysis first separates species with low and high scores on axis 1 and then splits samples with higher axis 1 scores along axis 2. There is little obvious similarity between the two NMDS plots. The sites which have notably high scores on particularly axis one in Fig. 2a (e.g., Kuncheng Lake) are not distinguished on Fig. 2b. We found no significant IndVal indicator species ($P < 0.05$) for UPGMA clusters based on environmental data.

The Multiple Factor Analysis (MFA) plot (Fig. 3) suggests some possible links between species and environmental variables. Axis 1 is positively correlated with environmental variables including TN, conductivity and some metals, and negatively correlated with some moderately abundant taxa such as *Difflugia pulex* and *Pontigulasia incisa*. Two of the most abundant taxa *C. aculeata* and *C. ecornis* are negatively correlated with axis 2; many of the lanthanide metals are positively correlated with this axis.

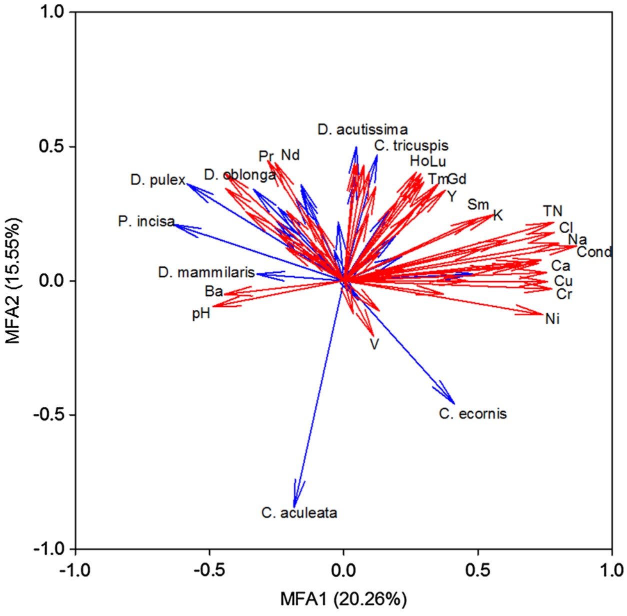

Fig. 3 Multiple factor analysis plot of testate amoeba species (*blue*) and environmental data (*red*). Only variables with more extreme axis scores are labelled

The first selected variable in RDA forward selection is Praseodymium (Pr), a rare earth metal explaining 7.3 % of variability. This illustrates the problem of forward selection when environmental data includes numerous correlated variables. Pr is a very rare element and found at low concentrations in our samples (mean 10.6 ppm) but is strongly correlated with many other heavy metals (Supplementary Table 1). Our result clearly indicates a relationship between the testate amoeba community and a suite of heavy metals rather than Pr *per se*. Other variables

Table 3 Variables identified in RDA forward selection and explanatory power as sole environmental variable, listed in order of selection

Variable	Variance explained (%)	*P*
Pr	7.3	0.03
Conductivity	*5.8*	*0.08*
Chlorophyll a	*5.0*	*0.12*
NO_3_N	*5.8*	*0.08*
SiO_2	*6.3*	*0.06*
Mg	*3.6*	*0.3*
All above variables:	36.4	

Note that not all variables are significant at $P < 0.05$ by this test (these shown in italics)

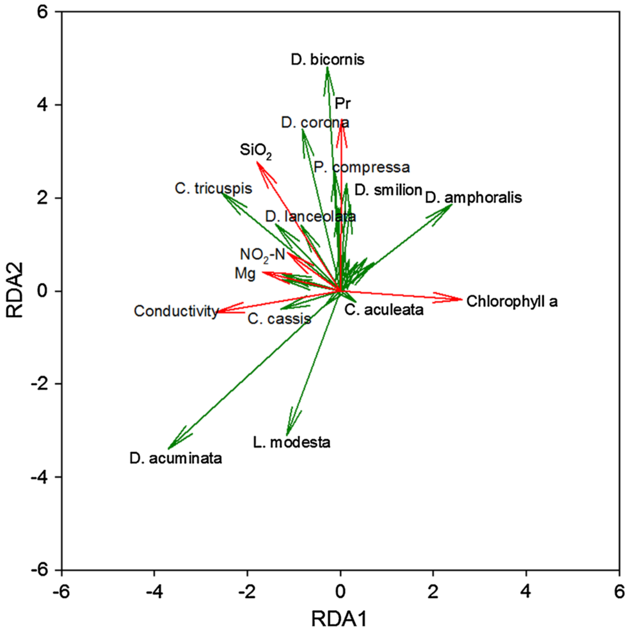

Fig. 4 RDA results based on forward selection of environmental variables. Environmental vectors exaggerated 5× for clarity and some taxa with short vectors unlabelled

identified as important by forward selection include (in order of selection): conductivity, chlorophyll a, NO_3_N, SiO_2 and Mg. A total of 36.4 % of variability is explained by these variables, although all but Pr are non-significant when tested independently (Table 3). Our results therefore imply that the testate amoeba community is affected by heavy metals and also by nutrient availability (albeit weakly). The ordination plot (Fig. 4) suggests that heavy metals (typified by Pr) are positively correlated with three comparatively rare taxa: *D. bicornis, Mediolus (Difflugia) corona* and *P. compressa* and negatively correlated with *D. acuminata* and *L. modesta*. *C. tricuspis* is positively correlated with SiO_2 and NO_2_N.

Discussion

Water quality

According to the OECD (1982) nutrient classification system (Ryding and Rast 1989), 20 of these lakes can be classed as eutrophic (TP: 35–100 μg/L) and 13 hypereutrophic (TP > 100 μg/L). Three lakes are mesotrophic ((TP < 35 μg/L)) while only one lake (Lake Baidang) has TP less than 10 μg/L. The concentrations of heavy metals are relatively high indicating that the water in most lakes is heavily polluted. These indications of high pollutant loadings can be attributed to high inputs from adjacent industry since the early twentieth century (Hu et al. 2012) with levels of heavy metals sufficient to be toxic to aquatic organisms (Yang et al. 2011). Previous limnological and palaeolimnological investigations in the lakes of this region suggest that the increasing concentrations of heavy metals and nutrients including TP are primarily linked to the rapid increase in intensity of human activities since the early to middle 20th century (Dong et al. 2008; Yang et al. 2008; Qin et al. 2009; Dearing et al. 2012; Wang et al. 2012). This has resulted in serious eutrophication and loss of biodiversity. The reduction in water quality is acknowledged to threaten the security of drinking-water resources and the stability of ecosystems and is an acknowledged issue for environmental management and policy in the region (Qin et al. 2004; Shi et al. 2015).

Testate amoeba community composition

Shannon diversity of these samples was low, falling between 0.99 and 2.56 (although note the low counts used to derive these figures). Previous aquatic testate amoeba studies have suggested that 'healthy' lakes have SDI values >2.5 (Patterson and Kumar 2000; Roe and Patterson 2006; Patterson et al. 2012). Our results suggest that these lakes have impoverished faunas, most likely due to the impacts of pollution.

In previous testate amoeba studies from lakes in China, several unusual taxa such as *Diffilugia biwae, D. tuberspinifera, D. mulanensis* and *Pentagonia zhangduensis* have been identified which are endemic to eastern Asia (Tsugeki et al. 2003; Yang et al. 2004, 2005; Qin et al. 2008, 2011; Gomaa et al. 2015). The community of these samples, however, is primarily composed of ubiquitous species which are abundant in lakes around the world (Reinhardt et al. 1998; Patterson et al. 2002; Escobar et al.2008; Neville et al. 2010; Roe et al. 2010; Patterson et al. 2013b). *D. biwae* was firstly detected in the sediments of Lake Biwa in an early phase when the water was oligotrophic (Kawamura 1918). Palaeoecological evidence shows that the abundance of *D. biwae* decreased rapidly due to declining water quality after the middle 1960s (Tsugeki et al. 2003). A similar decline was also found in Lake Zhangdu where the abundance of *D. biwae* declined with increasing eutrophication and hydrological modification since 1970s (Qin et al. 2009). *D. biwae* can thus be considered as an indicator of oligotrophic conditions. In the 37 lakes of this study, *D. biwae* was only found in Lake Poyang, the largest freshwater lake in China connecting to the Yangtze River and a lake with relatively good water quality. It seems probable that all of the other lakes we consider have become too eutrophic for *D.biwae*. Other regional endemics (*D. tuberspinifera, D. mulanensis* and *Pentagonia zhangduensis*) are also believed to be present primarily in oligotrophic conditions and were not detected in our samples. It is therefore probable that pollution has caused a loss of these species; palaeolimnological studies would be valuable to confirm this. It is possible that pollution may be a systemic threat to the endemic testate amoeba species of China.

Response of testate amoebae to pollution

The Multiple Factor Analysis (MFA) plot (Fig. 3) suggests axis 1 is more related to taxa such as *Difflugia mammillaris*, *D. pulex* and *Pontigulasia incisa* while axis 2 is negatively correlated with two of the most abundant taxa, *C. aculeata* and *C. ecornis*. Axis 1 is correlated with many general water quality indicators, including TN and conductivity, Ca, Cu, Cr, Cl and Na, while axis 2 is primarily correlated with many lanthanide series metals, suggesting specific responses of different species to differing aspects of pollution. Pr is the most significant correlate to testate amoeba community composition in redundancy analysis. Pr is a trace element but widely used in several industrial fields like petrochemicals and metallurgy, including the manufacture of electric motors. Our results cannot show that Pr *per se* has an influence on testate amoebae but do imply that heavy metal concentrations have an influence on assemblage structure.

In previous studies on testate amoebae ecology in lakes in the world it has been considered that most *Difflugia* and *Pontigulasia* taxa are more abundant in mesotrophic to eutrophic conditions or with high pH (Asioli et al. 1996; Patterson et al. 1996; Beyens and Meisterfeld 2001; Kihlman and Kauppila 2012; Macumber et al. 2014; Roe and Patterson 2014). While most *Centropyxis* taxa are opportunistic and capable of existing in oligotrophic water, low organic content, high concentration metal contaminated waters (Patterson et al. 1996; Kauppila et al. 2006; Kihlman and Kauppila 2009, 2012). However, they also recently reported can also tolerate to mesotrophic and highly eutrophic conditions (Patterson et al. 2013b). In this study the community of these polluted lakes was dominated by both *Difflugia* and *Centropyxis* taxa with no clear shift in dominance between these groups along pollution gradients. Given the limited previous research on the influence of heavy metals on testate amoebae the mechanisms underlying species responses are difficult to explain and are likely to be complex.

Potential for bioindication

Overall our results highlight how little is known of testate amoeba ecology in aquatic systems in general, and particularly in highly polluted lakes. Our environmental dataset includes a very large number of variables, including most that would be expected to be important determinants of testate amoeba community composition. However, the proportion of variance explained by these variables is unexpectedly low. Our strongest evidence is for impacts of heavy metals, but the results also provide some suggestion of links to nutrient status. Further work will be required to confirm the potential of testate amoebae as bioindicators in these systems. These lakes have unique histories of human impact, with some impacted by early industrial development while others have been comparatively undisturbed until recently. The sediment samples we analyse are likely to integrate testate amoebae over a comparatively long period of time. It may be that the relatively weak correlations we detect are because the testate amoeba community is still adapting to a changed environment. It is also possible that the gap between water and sediment sampling or other, unmeasured, variables are also important. The comparatively low count totals may also have impaired our ability to detect significant correlations. As a group of protists which are abundant and perform important functional roles in aquatic ecosystems the potential of testate amoebae as bioindicators in highly polluted lakes deserves further detailed attention.

Conclusion

The use of testate amoebae as bioindicators of pollution in aquatic ecosystems is much less developed than for other micoorganisms, particularly diatoms. Previous studies demonstrate considerable potential for testate amoebae to indicate various aspects of anthropogenic impact on lakes but these studies have been geographically restricted. Our study considers an unprecedentedly large range of environmental variables and their relations to testate amoebae communities from lakes in China. Results show that these lakes—many of which are highly disturbed—do, nevertheless, host testate amoebae but that communities are species poor and lack characteristic regional endemics. We found links between testate amoebae and their environment but disentangling precise environmental controls is complicated by correlations between many variables. Our data provides the strongest support for impacts of heavy metals but also suggest a role for nutrient status in shaping testate amoeba assemblages. Our results support previous suggestions that testate amoebae may be useful bioindicators but also show that controls may be complex and involve many pathways of impact. Clearly further research will be required.

Acknowledgments This work was supported by 973 program (2011CB808800), the National Natural Science Foundation of China (No. 41530753, 41330103, 41502167), and the 111 project (B08030) and. RJP was supported by the Russian Scientific Fund, Grant 14-14-00891.

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