

# Ecology of testate amoebae in Dajiuhu peatland of Shennongjia Mountains, China, in relation to hydrology

Yangmin QIN (✉)<sup>1,2</sup>, Richard J PAYNE<sup>3</sup>, Yansheng GU<sup>1</sup>, Xianyu HUANG<sup>4</sup>, Hongmei WANG<sup>1</sup>

<sup>1</sup> State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430074, China

<sup>2</sup> Key Laboratory of Wetland Ecology and Environment, Chinese Academy of Sciences, Changchun 130012, China

<sup>3</sup> School of Science and the Environment, Manchester Metropolitan University, Manchester M1 5GD, UK

<sup>4</sup> State Key Laboratory of Geological Processes and Mineral Resource, China University of Geosciences, Wuhan 430074, China

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2012

**Abstract** This study investigates the testate amoeba communities of a large peatland in Central China. The ecology and seasonal variability of testate amoeba communities were studied during 2009–2010. Investigation of environmental controls using ordination showed that the relationship between testate amoeba communities and depth to water table (DWT) and pH are extremely weak. The small proportion of variance explained by water table depth here (only 1.9% in the full data) shows that the hydrological control is weaker than we expected in this peatland, and weaker than any study we are aware of using a similar methodology. Attempts to develop species-environment (transfer function) models or identify indicator species for future palaeoecological studies were unsuccessful. Previous large-scale studies of peatland testate amoeba ecology have been largely restricted to Europe and North America and results have been relatively consistent among studies. Our results contrast with this consensus and suggest that at least in minerotrophic peatlands in China testate amoeba communities may be primarily controlled by different environmental variables. In China, testate amoebae have been relatively little studied but may prove to be valuable for a variety of applications in palaeoecology and biomonitoring and much further work is required.

**Keywords** testate amoebae, ecology, minerotrophic, Dajiuhu peatland, China

## 1 Introduction

Testate amoebae are a group of protozoa living in freshwater lakes, rivers, peatlands, mosses, and soils.

Testate amoebae are used in a variety of ecological and palaeoecological studies of *Sphagnum*-dominated peatlands, because they produce decay-resistant and morphologically distinct shells, or tests (Charman et al., 2000; Meisterfeld, 2002; Mitchell et al., 2008) and may indicate a variety of environmental changes. During the past 20 years research on the ecology of testate amoebae in peatlands around the world has indicated that wetness conditions are the most important environmental control on the composition of testate amoeba communities. Such studies have repeatedly suggested that the depth to water table (DWT) can be used as a robust and easily measurable proxy for the finer-scale wetness conditions (Charman, 1997; Woodland et al., 1998; Bobrov et al., 1999; Mitchell et al., 1999; Lamentowicz and Mitchell, 2005; Payne et al., 2006; Payne and Mitchell, 2007; Booth, 2001, 2007; Swindles et al., 2009; Markel et al., 2010). As the surface wetness of a peatland reflects climate, testate amoebae are therefore used as a useful tool to reconstruct changes in hydroclimate by application of hydrological transfer functions to palaeoecological records (Bobrov et al., 1999; Charman et al., 2004; Payne and Mitchell, 2007; Payne et al., 2008; Booth, 2011).

In China, and eastern Asia more generally, studies on testate amoebae are relatively few, however, over the past 100 years nearly 300 testate amoeba species and subspecies have been identified (Shen, 1983; Yang et al., 2004; Qin et al., 2011) including several new species described in recent years (Yang et al., 2005; Yang and Shen, 2005; Qin et al., 2008a, b). Further new species have been recently described from other areas of eastern Asia (Bobrov, 2001; Bobrov and Mazei, 2004). Testate amoebae from soils, mosses, and lakes have been investigated in terms of their ecology, distribution, diversity and biogeography (Shen, 1983; Ning and Shen, 1999; Qin et al., 2011). Recent studies have started using testate amoebae as bioindicators of environmental and paleoenvironmental

change in Chinese lakes and peatlands (Qin et al., 2007, 2009; Li et al., 2009).

The outstanding characteristic of China climate is dominance of the Asian Monsoon, which leads to dramatic seasonal variability in availability of water resources (An et al., 2000; Qin et al., 2011). It is extremely important to understand changes in the pattern and timing of the Asian Monsoon during the Holocene which allows future variability to be modeled. Existing proxy-climate records are primarily driven by temperature and are poorly suited to the task of studying monsoon variability. Testate amoeba palaeoecological records from peatlands reconstruct the past wetness of the site in ombrotrophic mires, which is a balance between meteoric water input and loss through evapotranspiration. The relative contribution of temperature and precipitation in forcing these changes varies between the studies which have been addressed before (Charman et al., 2004) with recent studies suggesting summer moisture deficit as a key control (Charman, 2007; Charman et al., 2009). By allowing the reconstruction of hydroclimatic change testate amoeba-based palaeoecological records quantified using transfer functions have the potential to add greatly to our knowledge of monsoon variability.

In this paper we investigate the diversity, ecology and seasonal variability of testate amoebae from a large peatland in Central China with a particular focus on the possible application of testate amoebae in palaeoecology.

## 2 Methods

### 2.1 Geography of sampling site

The Dajiuhe peatland is located in the Dajiuhe Basin of the Shennongjia Mountains in Central China (109°56'–110°11'E, 31°24'–31°33'N). The region is situated between the subtropical and warm temperate zones with climate controlled by the monsoon cycle (Zhu et al., 2009). The altitude of the peatland is 1700–1760 m a.s.l, with a total area of 16 km<sup>2</sup> and mean annual temperature of 7.4°C. Vegetation is dominated by *Carex* sp., *Sanquisorba officinalis*, *Juncus* sp., and *Gentiana pseudo-aquatica*. *Sphagnum* sp. is only found in the center of the site (Zhao et al., 2007; Qin et al., 2008b; Zhu et al., 2009).

Dajiuhe peatland is one of very few peatlands in Central China and is among few peatlands globally at such a low latitude. Dajiuhe peatland plays an important role in local biodiversity and water resources as the origin of several rivers. The site is in the central track of the Asian Monsoon and it is probable that a record of the long-term variability of the monsoon is preserved within the deep *Sphagnum* peats of the central peatland (Zhao et al., 2007; Zhu et al., 2009).

The Dajiuhe peatland has been damaged by human activities, particularly between the 1970s and 1990s.

*Sphagnum* was cut for sale to medical companies and crops were grown in drained areas. The area of peatland was dramatically reduced. Since the 1990s the National Reserve of Shennongjia Mountains has been designated to protect the local ecosystem, and the peatland is currently preserved.

### 2.2 Field sampling

Samples were collected in August 2009, July and September 2010. In 2009, we collected a total of 88 samples to investigate the diversity of testate amoebae in detail. In 2010 the same 25 points were sampled in both July and September to assess seasonal variability. Microhabitats within the peatland were sampled in an attempt to include the natural heterogeneity such as hummocks, hollows, and open waters. The portion of the *Sphagnum* stem from 3 to 5 cm was used in analyses as this portion contains greatest taxonomic diversity (Mitchell and Gilbert, 2004). For other mosses, such as *Polytrichum*, we used the entire upper 5 cm section for analysis.

At each sampling time, a shallow hole was drilled to measure the DWT and extract water to determine pH. We waited from 30 min to 12 h for equilibration of the water level in the hole (discussed further below). The DWT was measured from where the samples were collected to the surface of the water in the hole. Temperature and pH were measured on extracted water using a thermometer/thermocouple and a Eutech Instruments pH probe, respectively.

### 2.3 Laboratory processes

All extracted samples were refrigerated until analysis. Testate amoebae are usually isolated from *Sphagnum* using a sieving procedure (Hendon and Charman, 1997; Booth et al., 2010). Samples were boiled in water for 5 min and then sieved at 250 and 25 μm with the intermediate material retained. The use of a 25 μm sieve means that smaller taxa will be under-represented in our results (Payne and Mitchell, 2009); this sieving was considered necessary to remove abundant fine organic material in our samples. The material retained in the 25 μm sieve was washed into 50 mL centrifuge tubes and centrifuged at 3000 r/min for five minutes. The residues were stained with Safranin for microscopic identification. For each sample, we identified and counted at least 150 individual testate amoeba shells (Payne and Mitchell, 2009) with taxonomic references including Charman et al. (2000), Penard (1902) and Meisterfeld (2002).

### 2.4 Data analysis

Ordination was used to investigate the environmental controls on testate amoeba communities in this site. Percentage data were square-root transformed and subjected to principal components analysis (PCA) and

(partial) redundancy analyses (RDA) using CANOCO vers. 4.53 (CANOCO for Windows 1997–2004, Biometris-Plant Research International, Wageningen). Three physical environmental parameters were included in the analysis: DWT, pH and temperature (for 2009 data only). Dummy variables were included for the three sampling times and for three plant types (*Sphagnum*-, *Polytrichum*- and Sedge-dominated). The importance of these variables was tested individually and in combination with significance testing by Monte Carlo permutation tests (999 random permutations). As an additional test, analyses of similarity (ANOSIM) between sampling times were conducted and similarity percentage (SIMPER) analyses were used to determine the contribution of species to Bray-Curtis dissimilarity (Clarke, 1993).

As DWT explained a significant proportion of the variance in the 2009 data (the 2010 data sets were too small for species environment-modeling), transfer functions for hydrology were developed using a standard suite of techniques: weighted average (WA), weighted average partial least squares (WAPLS) and maximum likelihood (Birks, 1995). Model performance was tested using bootstrap and jack-knife cross validation with three performance measures: the root mean squared error of prediction (RMSEP), the maximum bias along the hydrological gradient and  $R^2$ . Transfer function analyses were carried out using C2 vers 1.3 (Juggins, 2003).

As transfer functions performed poorly (see Sect. 3) we also attempted to identify indicator species of differing hydrological conditions. The identification of indicator species of known ecological preference formed the basis of most palaeoecological studies before the quantitative revolution of the late 1980s and 1990s. Modern techniques permit a more quantitative approach to the identification of indicator species which may allow more informed qualitative reconstruction of changing environmental conditions. We applied the IndVal approach of Dufrene and Legendre (1997) which attempts to identify indicators with both high specificity and high fidelity and uses permutation tests to assess their significance. We divided the hydrological gradient of the 2009 data into a nested sequence of sub-divisions and implemented IndVal using the program INDVAL vers. 2.0 with significance tested using 999 permutations and a cut-off at  $P = 0.01$ .

### 3 Results

#### 3.1 Species diversity and community

After making a number of taxonomic groupings to ensure consistency, a total of 34 testate amoeba taxa or 'types' were identified in the samples. Testate amoeba community composition of the Dajiuhu peatland is broadly similar to those found in European and Northern American peats, with most species being cosmopolitan in distribution. The

dominant taxa were *Assulina muscorum*, *Assulina seminulum*, and *Trinema-Corythion* type, which occur in almost all samples. Three testate amoeba taxa: *Hyalosphenia papilio*, *Nebela wailesi* and *Heleopera sphagni* are new records in China.

There are some common peatland testate amoeba species which are absent in our study, such as *Amphitrema wrightianum*. The absence of such species in our study may be due to either the minerotrophic characters of the Dajiuhu peatland, or the geographical barrier.

#### 3.2 Species-environment relationship

The measured environmental variables collectively explain around 15% of variance in the full data and around 30% of variance in the 2010 data sets (Table 1). There is only rather weak evidence that pH is an important environmental control on testate amoebae in this site, when other variables are accounted for a significant relationship. There is some evidence for the importance of wetness (DWT) as an environmental control on amoebae in this site. A significant relationship of DWT with amoeba community structure is observed with all data sets but not from that data of July 2010 alone. The proportion of variance in the testate amoebae data explained by DWT is modest, around 2% in the overall combined and 2009 data and over 4% in the combined 2010 data, although more notable in the (small) September 2010 data set (13%). Even using the crude approach we apply here (3 dummy variables for general community type) there is evidence for a relationship between testate amoeba communities and plant communities in the 2010 data.

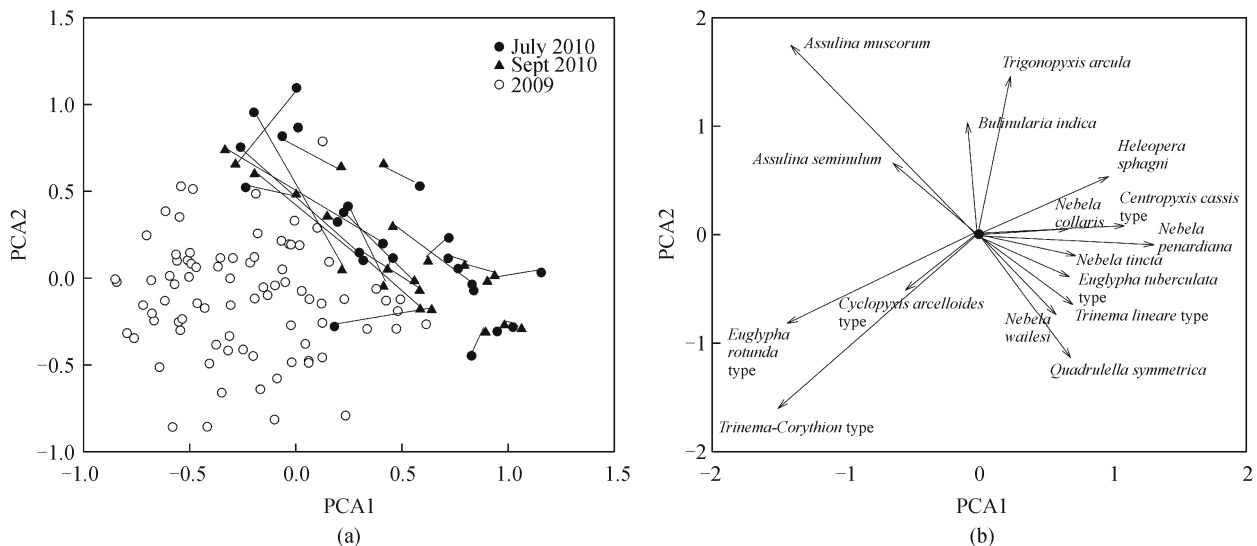
One of the most distinct findings is the difference between the 2009 and 2010 samples, explaining a large proportion of the variance and emerging very distinctly in the PCA plot with 2010 samples having higher scores on PCA1 and PCA2 (Table 1, Fig. 1). The difference between the two sets of samples from 2010 is less immediately apparent with considerable overlap in ordination space, although a nominal variable for sampling time does explain significant variance in the combined data set. Comparing the position of samples from the same locations in summer and autumn 2010 there is limited consistent change. Locations showing the largest changes generally show higher scores on PCA1 and lower scores on PCA2, but this pattern is by no means ubiquitous. ANOSIM finds significant differences between 2009 and 2010 samples ( $P < 0.001$ ) but no difference between the 2010 sampling times, and SIMPER identifies the most important contributors to the differences between the 2009 and 2010 samples as *Assulina muscorum*, *Trinema-Corythion* type and *Centropyxis cassis* type. Such differences are also apparent by comparison to the PCA species plot (Fig. 1).

As there was a significant (though weak) relationship between DWT and testate amoeba communities for the

**Table 1** Results of redundancy analysis of square root-transformed testate amoeba data, showing % variance explained by environmental variables and *P*-value determined by 999 Monte Carlo permutations. Analyses were conducted using five different combinations of samples from the three sampling times

Data	Explanatory variables*	Co-variables	% variance explained	P
All	DWT, pH, Time	–	14.7	0.001
All	DWT	Time, pH	1.9	0.004
All	pH	DWT, Time	(1.0)	ns
All	Time	DWT, pH	9.6	0.001
2009	DWT, pH	–	6.5	0.001
2009	DWT	pH	2.4	0.023
2009	pH	DWT	4.0	0.001
Jul-2010	DWT, pH, Plants	–	31.7	0.019
Jul-2010	DWT	Plants, pH	(3.7)	ns
Jul-2010	pH	DWT, Plants	(3.8)	ns
Jul-2010	Plants	DWT, pH	19.7	0.015
Sep-2010	DWT, pH, Plants	–	29.7	0.008
Sep-2010	DWT	Plants, pH	13.0	0.007
Sep-2010	pH	DWT, Plants	(6.5)	ns
Sep-2010	Plants	DWT, pH	(13.1)	ns
All 2010	DWT, pH, Plants, Time	–	27.0	0.001
All 2010	DWT	Plants, pH, Time	4.6	0.01
All 2010	pH	DWT, Plants, Time	(1.8)	ns
All 2010	Plants	DWT, pH, Time	16.2	0.001
All 2010	Time	Plants, pH, DWT	5.4	0.005

Note: \* temperature did not explain independent variance and was not included in any of the presented analyses



**Fig. 1** PCA ordination based on full data set. Plot (a) shows samples and plot (b) shows selected major species (> 1% total) well-fitted by the ordination

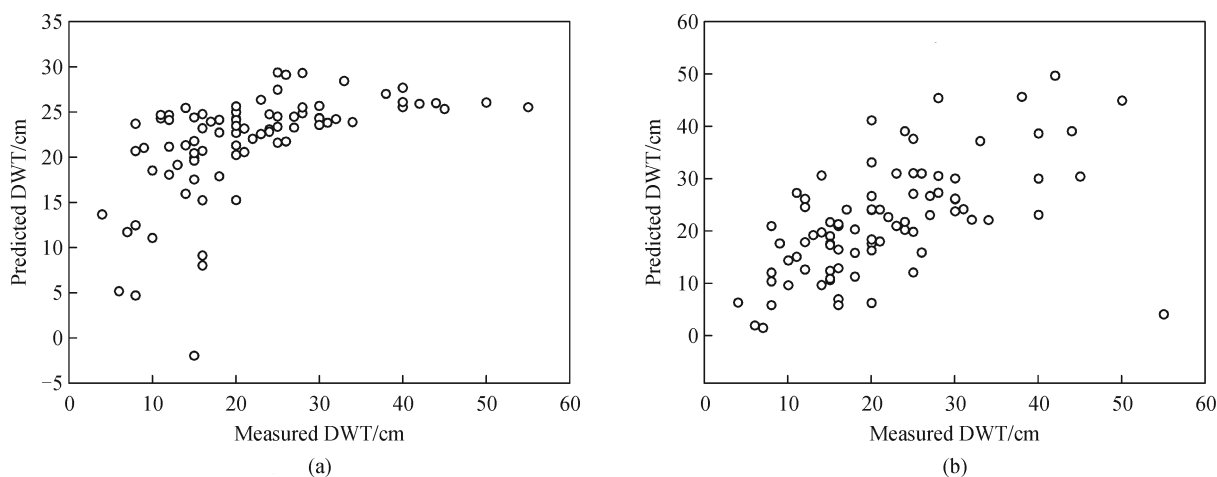
2009 data, transfer functions were developed (Table 2). While the RMSEP is only c.1cm poorer than at least one published model the  $R^2$  values are very low indeed. Plots of observed against predicted DWT values show this poor

relationship (Fig. 2). With weighted averaging the model predicts similar DWT values for all samples with DWT greater than c.25 cm, while with maximum likelihood there is a very general relationship between observed and

**Table 2** Transfer function performance of model structures tested in this study showing root mean squared error of prediction (RMSEP),  $R^2$  and maximum bias (MaxBias) assessed by boot-strap ('boot') or jack-knife ('jack') cross-validation for DWT

Model	RMSEP <sub>jack</sub>	RMSEP <sub>boot</sub>	$R^2_{jack}$	$R^2_{boot}$	MaxBias <sub>jack</sub>	MaxBias <sub>boot</sub>
Weighted averaging (tolerance downweighting, inverse regression)*	10.5	10.7	0.08	0.10	30.4	31.1
Maximum likelihood	11.4	12.5	0.14	0.33	24.7	28.4

Note: \* WA-PLS does not improve on the performance of simple WA



**Fig. 2** Performance of testate amoeba-hydrology transfer functions developed using (a) weighted averaging and (b) maximum likelihood. See Table 2 for details

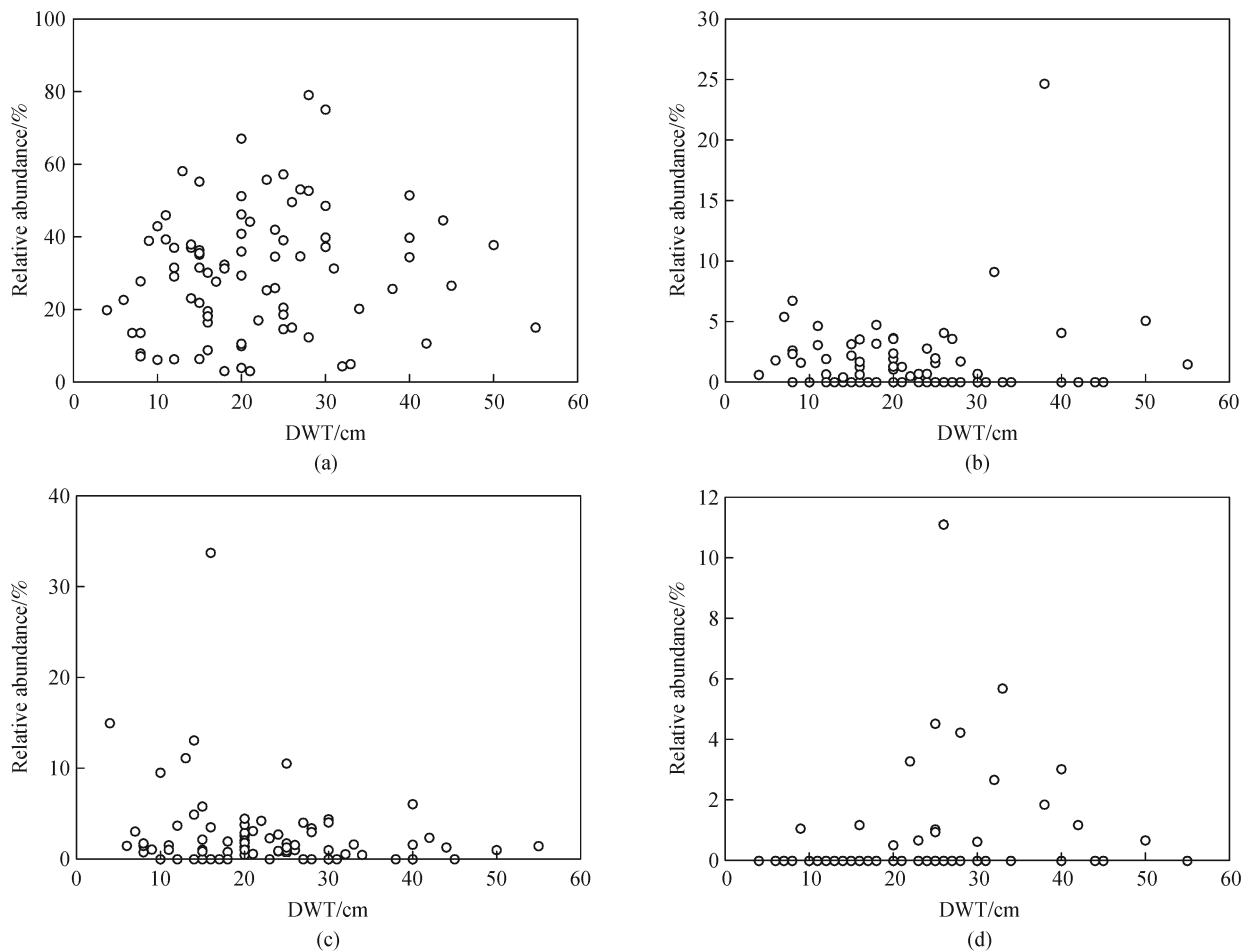
predicted values but a large amount of scatter. Many previous transfer functions have improved model performance by selective exclusion of rare taxa or samples with high residuals (a practice which may or may not be ecologically justified, Payne et al., 2006). While it would doubtlessly be possible to improve the model performance using these approaches, the lack of any very strong initial relationship between measured and predicted values makes this hard to justify. It is clearly not possible to build a transfer function model which could provide reliable palaeohydrological reconstructions on the basis of these data. Our attempt to identify indicator species of different sections of the hydrological gradient using the IndVal method failed to identify any taxa which are statistically significant indicators of any wetness class at  $P = 0.001$ .

The causes of these problems can be understood if we look at the relative abundances of selected species along the hydrological gradient. Figure 3 shows the abundances of four taxa: *Assulina muscorum*, *Centropyxis aculeata*, *Heleopera sphagni* and *Trigonopyxis arcuata* in the 2009 data. These four taxa are morphologically distinctive, relatively abundant and have ecologies which are well established (both qualitatively and quantitatively) from previous studies (e.g. Charman et al., 2000). *A. muscorum* is cosmopolitan but most abundant in drier niches; *C. aculeata* is typical of very wet conditions, *H. sphagni*

of relatively wet conditions and *Tr. arcuata* of drier conditions in hummocks. However the abundances of none of these taxa show very strong changes along the long hydrological gradient in this study. There is little indication of distinct optima for three of these taxa, while for *Tr. arcuata* there is a weak preference for intermediate conditions, inconsistent with indications from previous studies. If such abundant taxa which usually display strong hydrological preferences show little changes along the hydrological gradient it is little wonder that a transfer function cannot be successfully developed. The high abundance of *A. muscorum*, *Trinema-Corythion* type and *C. cassis* type in 2009 may implied a drier phase in this summer, although we are lacking such meteorological data.

## 4 Discussion

The most surprising ecological result of this study is how weak the hydrological control of testate amoeba communities appears in this site. Previous studies using essentially the same methodology have shown DWT to explain a much larger significant portion of variance; in one recent study (Payne et al., 2010) DWT explained almost 40% of variance. The small proportion of variance explained by



**Fig. 3** Relative abundance of four testate amoeba taxa along the hydrological gradient. (a) *Assulina muscorum*; (b) *Centropyxis aculeate* type; (c) *Heleopera sphagni*; (d) *Trigonopyxis arcula*

DWT here (only 1.9% in the full data) shows that although there is some hydrological control this is weaker than expected, and weaker than any study we are aware of using a similar methodology. Consequently, we are unable to either produce a transfer function or identify indicator species of different degrees of wetness.

In terms of the other environmental variables measured: temperature control has not been previously tested as an environmental control on amoeba communities in a quantitative study of this nature and has been relatively under-researched in general (Smith and Coupe, 2002). Previous research suggests that testate amoeba communities may respond to extreme events (Beyens et al., 2009) but are insensitive to even large-scale natural gradients (Mitchell et al., 2004). Given the large diurnal, seasonal and annual variability in temperature and small gradient it is unsurprising that we are unable to identify a significant relationship here. It is perhaps more surprising that pH does not emerge as a significant control. Several previous

studies have suggested that pH is an important control (e.g. Lamentowicz and Mitchell, 2005), and may be particularly important in fens although not all studies have found pH to explain variance independent of DWT (e.g. Payne et al., 2010).

The surprisingly weak hydrological control could be explained by either some methodological issue in this study or simply that other environmental variables are more important than hydrology to amoebae in this site. On analysis of the 2009 data we initially suspected that the weak relationship with hydrology might be related to the quick measurement of the DWT (only 30 min) which might have been insufficient time for the water table to equilibrate. However, when repeated with much longer time (6 to 12 h) in 2010 results were substantially the same.

A further methodological complication might be taxonomic error or inconsistency (Payne et al. 2011). As the first author was relatively new to testate amoeba analysis when the first samples were analyzed we opted to

harmonise all taxonomy to a very conservative level (Appendix 1). We do not believe that taxonomic resolution or error is likely to be a major cause of weak species-environment relationships as weak relationships are found even when considering each component data set (produced in a short-space of time by the same analyst) alone and modeling work has shown that relatively little ecological information is lost by even extremely conservative grouping.

We have no other hypotheses for potential methodological causes of the poor relationship with hydrology. Therefore, we conclude that hydrology appears to be less important to amoebae in this region than in more studied areas of the world.

Dajiuhu peatland lies in a karst area with limestone bedrock. Although detailed chemical measurements were not undertaken, it is probable that base cation levels in the peat waters are high. It is conceivable that such geochemical gradients are particularly important to testate amoebae in this site. Recently, some ecological studies on testate amoebae in minerotrophic peatlands have indicated that caution should be exercised in the use of testate amoebae as hydrological indicators (Lamentowicz et al., 2010; Payne, 2011). Environmental variables other than wetness may be more important in fens than in bogs. Plant communities of the Dajiuhu Peatland are particularly complex with mixed stands of *Carex* sp., *Sanquisorba officinalis*, *Juncus* sp., and *Gentiana pseudo-aquatica* and may also be an important determinant of testate amoeba community.

Testate amoeba-based palaeoecology relies on the assumption that the present ecology of amoeba taxa can be extrapolated into the past. Currently the general consistency of amoeba hydrological preferences between multiple studies suggests that such relationships are stable in space (Charman et al., 2000; Booth and Zygmunt, 2005; Payne et al., 2008), and therefore likely to be also stable in time. However such studies are restricted to quite a limited region of the globe with the greatest concentrations in central and western Europe and North America. Further studies are needed to establish consistent hydrological preferences beyond these areas. This is certain the case in China, where testate amoebae have been relatively little studied but may prove to be valuable for a variety of applications in palaeoecology and biomonitoring. Future study of testate amoeba ecology is likely to provide considerable new scientific insights.

**Acknowledgements** This work was supported by the National Basic Research Program of China (No. 2011CB808800), the National Natural Science Foundation of China (Grant Nos. 40930210, 40921062, 40872202, 41072261 and 30800097), the Opening Research Fund of Key Laboratory of Wetland Ecology and Environment (No. WELF-2009-B-003), the Special Fund for Basic Scientific Research of Central Colleges, China University of Geosciences (Wuhan) (Nos. CUG090103 and G1323521132), and the 111 Project (No. B08030). Professor Robert K. Booth and Professor Edward A.D. Mitchell helped with species identifications, Professor Booth also helped with

initial statistical analysis and constructive comments on the research. We thank Jiantao Xue, Ze Zhang, Huan Yang and Zhiqi Zhang for their assistance of field works. Qianfan Zhang and Rong Zhu helped dealing with the processing of samples in the laboratory.

Author contributions: YQ designed and led the study, conducted all laboratory and microscopic analysis and wrote the paper. RJP conducted all statistical analysis and wrote the paper with YQ. YG, XH and HW helped with the field work.

## References

- An Z, Stephen C, John E, Wu X, Wang S, Liu X, Li X, Zhou W (2000). Asynchronous Holocene optimum of the East Asian Monsoon. *Quat Sci Rev*, 19(8): 743–762
- Beuens L, Ledeganck P, Graae B J, Nijs I (2009). Are soil biota buffered against climatic extremes? An experimental test on testate amoebae in arctic tundra (Qeqertarsuaq, West Greenland). *Polar Biol*, 32(3): 453–462
- Birks H J B (1995). Quantitative palaeoecological reconstructions. In: Maddy D, Brew S, eds. *Statistical Modelling of Quaternary Science Data*. Quaternary Research Association, Cambridge, UK
- Bobrov A A (2001). Findings of tropical group testate amoebae (Protozoa: Testacea) at the Far East (Sikhote Alin Reserves). *Biol Bull*, 28: 475–482
- Bobrov A A, Charman D J, Warner B G (1999). Ecology of testate amoebae (Protozoa: Rhizopoda) on peatlands in western Russia with special attention to niche separation in closely related taxa. *Protist*, 150(2): 125–136
- Bobrov A A, Mazei Y (2004). Morphological variability of testate amoebae (Rhizopoda: Testacealobosea: Testaceafillosea) in natural populations. *Acta Protozool*, 43: 133–146
- Booth R K (2001). Ecology of testate amoebae (protozoa) in two lake superior coastal wetlands: implications for palaeoecology and environmental monitoring. *Wetlands*, 21(4): 564–576
- Booth R K (2007). Testate amoebae as proxies of mean annual water table depth in *Sphagnum*-dominated peatlands of North America. *J Quaternary Sci*, 23(1): 43–57
- Booth R K (2011). Testing the climate sensitivity of peat-based paleoclimate reconstructions in mid-continental North America. *Quat Sci Rev*, 29(5–6): 720–731
- Booth R K, Lamentowicz M, Charman D J (2010). Preparation and analysis of testate amoebae in peatland palaeoenvironmental studies. *Mires and Peat*, 7: 1–7
- Booth R K, Zygmunt J R (2005). Biogeography and comparative ecology of testate amoebae inhabiting *Sphagnum*-dominated peatlands in the Great Lakes and Rocky Mountain regions of North America. *Divers Distrib*, 11(6): 577–590
- Charman D J (1997). Modelling hydrological relationships of testate amoebae (Protozoa: Rhizopoda) on New Zealand peatlands. *J R Soc N Z*, 27(4): 465–483
- Charman D J (2007). Summer water deficit variability controls on peatland water-table changes: implications for Holocene palaeoclimate reconstructions. *Holocene*, 17(2): 217–227
- Charman D J, Barber K E, Blaauw M, Langdon P G, Mauquoy D, Daley T J, Hughes P D M, Karofeld E (2009). Climate drivers for peatland

- palaeoclimate records. *Quat Sci Rev*, 28(19–20): 1811–1819
- Charman D J, Brown A D, Hendon D, Karofeld E (2004). Testing the relationship between Holocene peatland palaeoclimate reconstructions and instrumental data at two European sites. *Quat Sci Rev*, 23(1–2): 137–143
- Charman D J, Hendon D, Woodland A A (2000). The identification of testate amoebae (Protozoa: Rhizopoda) from British oligotrophic peats. Quaternary Research Association Technical Guide Series, Cambridge, UK
- Clarke K R (1993). Non-parametric multivariate analysis of changes in community structure. *Aust J Ecol*, 18(1): 117–143
- Dufrêne M, Legendre P (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol Monogr*, 67: 345–366
- Hendon D, Charman D J (1997). The preparation of testate amoebae (Protozoa: Rhizopoda) samples from peat. *Holocene*, 7(2): 199–205
- Juggins S (2003). C2 user guide. Software for ecological and palaeoecological data analysis and visualisation. University of Newcastle, Newcastle upon Tyne, UK
- Lamentowicz M, Lamentowicz L, Knaap W O, Gabka M, Mitchell E A (2010). Contrasting species-environment relationships in communities of testate amoebae, bryophytes and vascular plants along the fen-bog gradient. *Microb Ecol*, 59(3): 499–510
- Lamentowicz M, Mitchell E A D (2005). The ecology of testate amoebae (Protists) in *Sphagnum* in relation to peatland ecology. *Microb Ecol*, 50: 48–63
- Li H K, Bu Z J, Wang S Z, An Z S, Zhao H Y, Ma Y Y, Chen X (2009). Environmental implications of the modern testate amoebae in the peatlands in Changbai Mountains. *Quaternary Science*, 29: 817–824 (in Chinese)
- Markel E, Booth R K, Qin Y (2010). Testate amoebae and  $\delta^{13}\text{C}$  of *Sphagnum* as surface-moisture proxies in Alaskan peatlands. *Holocene*, 20(3): 463–475
- Meisterfeld R (2002). Order Arcellinida Kent. In: Lee J J, Leedale G F, Bradbury P, eds. *An Illustrated Guide to the Protozoa*. Lawrence Kansas: Allen Press
- Mitchell E A, Bragazza L, Gerdol R (2004). Testate amoebae (Protista) communities in *Hylocomium splendens* (Hedw.) B.S.G. (Bryophyta): relationships with altitude, and moss elemental chemistry. *Protist*, 155(4): 423–436
- Mitchell E A, Buttler A J, Warner B G, Gobat J M (1999). Ecology of testate amoebae (Protozoa: Rhizopoda) in *Sphagnum* peatlands in the Jura mountains, Switzerland and France. *Ecoscience*, 6: 565–576
- Mitchell E A, Charman D J, Warner B G (2008). Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. *Biodivers Conserv*, 17(9): 2115–2137
- Mitchell E A, Gilbert D (2004). Vertical micro-distribution and response to nitrogen deposition of testate amoebae in *Sphagnum*. *J Eukaryot Microbiol*, 51(4): 480–490
- Ning Y Z, Shen Y F (1999). Community structure and its characteristics of soil protozoa in typical zones of China. *Journal of Northwest Normal University*, 35: 50–54
- Payne R (2011). Can testate amoeba-based palaeohydrology be extended to fens? *J Quaternary Sci*, 26(1): 15–27
- Payne R, Charman D J, Matthews S, Eastwood W (2008). Testate amoebae as palaeoclimate proxies in Sürmene Ağaçaş Yaylasi peatland (Northeast Turkey). *Wetlands*, 28(2): 311–323
- Payne R, Kishaba K, Blackford J, Mitchell E A (2006). The ecology of testate amoebae in southcentral Alaskan peatlands: building transfer function models for palaeoenvironmental inference. *Holocene*, 16: 403–414
- Payne R, Lamentowicz M, Mitchell E A D (2011). The perils of taxonomic inconsistency in quantitative palaeoecology: experiments with testate amoeba data. *Boreas*, 40(1): 15–27
- Payne R, Mitchell E A (2009). How many is enough? Determining optimal count totals for ecological and palaeoecological studies of testate amoebae. *J Paleolimnol*, 42(4): 483–495
- Payne R, Ryan P A, Nishri A, Gophen M (2010). Testate amoeba communities of the drained Hula wetland (Israel): implications for ecosystem development and conservation management. *Wetlands Ecol Manage*, 18(2): 177–189
- Payne R J, Mitchell E A D (2007). Ecology of testate amoebae from mires in the Central Rhodope Mountains, Greece and development of a transfer function for palaeohydrological reconstruction. *Protist*, 158(2): 159–171
- Penard E (1902). Faune Rhizopodique du Bassin du Léman. Genève: Henry Kundig
- Qin Y, Booth R K, Gu Y, Wang Y, Xie S (2009). Testate amoebae as indicators of 20th century environmental change in Lake Zhangdu, China. *Fundamental and Applied Limnology. Arch Hydrobiol*, 175: 29–38
- Qin Y, Gu Y, Xie S, Zhou X (2007). Recent environmental change in Swan Oxbow of the Yangtze River: evidence from testate amoebae records. *Geological Science and Technology Information*, 26: 37–42 (in Chinese)
- Qin Y, Xie S, Gu Y, Zhou X (2008a). *Pontigulasia pangulostoma* nov. spec., a new testate amoeba from the peat land of Shennongjia Mountains, China. *Acta Protozool*, 47: 155–160
- Qin Y, Xie S, Smith H G, Swindles G T, Gu Y (2011). Diversity, distribution and biogeography of testate amoebae in China: implications for ecological studies in Asia. *Eur J Protistol*, 47(1): 1–9
- Qin Y, Xie S, Swindles G T, Gu Y, Zhou X (2008b). *Pentagonia zhangduensis* nov. spec. (Lobosea, Arcellinida), a new freshwater species from China. *Eur J Protistol*, 44(4): 287–290
- Shen Y F (1983). Protozoa of the Teibetan Plateau. In: Jiang X Z, Shen Y F, Gong X J, eds. *Aquatic Invertebrates of the Teibetan Plateau*. Beijing: Science Press (in Chinese)
- Smith H, Coupe S (2002). Testate amoebae — past, present and future. *Eur J Protistol*, 37(4): 367–369
- Swindles G T, Charman D J, Roe H M, Sansum P A (2009). Environmental controls on peatland testate amoebae (Protozoa: Rhizopoda) in the North of Ireland: implications for Holocene palaeoclimate studies. *J Paleolimnol*, 42(1): 123–140
- Woodland W A, Charman D J, Sims P C (1998). Quantitative estimates of water tables and soil moisture in Holocene peatlands from testate amoebae. *Holocene*, 8(3): 261–273
- Yang J, Feng W, Miao W (2004). A taxonomic catalogue of freshwater and soil testacea in China with a discussion of their faunal similarity. *Acta Hydrobiologica Sinica*, 28: 426–433 (in Chinese)
- Yang J, Meisterfeld R, Zhang W J, Shen Y F (2005). *Diffflugia mulanensis* nov. spec., a freshwater testate amoeba from Lake Mulan,

- China. *Eur J Protistol*, 41(4): 269–276
- Yang J, Shen Y F (2005). Morphology, biometry and distribution of *Diffugia biwae* Kawamura, 1918 (Protozoa: Rhizopoda). *Acta Protozool*, 44: 103–111
- Zhao Y, Hoelzer A, Yu Z (2007). Late Holocene natural and human-induced environmental change reconstructed from peat records in eastern Central China. *Radiocarbon*, 49: 789–798
- Zhu C, Ma C, Yu S, Tang L, Zhang W, Lu X (2009). A detailed pollen record of vegetation and climate change in Central China during the past 16000 years. *Boreas*, 38: 69–76

**Appendix 1** Combination of some testate amoebae species

Group	Species probably included	Type name used here
Large <i>Arcella</i> species (78–105 µm), aperture bordered by a shallow lip	<i>Arcella discoides</i> , <i>A. rotundata</i> , <i>A. rotundat</i> var. <i>aplanata</i> , <i>A. polypora</i> , <i>A. megastoma</i>	<i>A. discoides</i> type
Large <i>Arcella</i> (73–114 µm), pores around aperture are always clearly visible	<i>A. catinus</i> , <i>A. arenaria</i>	<i>A. catinus</i> type
All non-spined <i>Centropyxis</i> tests with aperture offset from center of test	<i>Centropyxis cassis</i> , <i>C. aerophila</i> , and <i>C. platystoma</i>	<i>C. cassis</i> type
All <i>Centropyxis</i> taxa tests with a variable number of spines	<i>C. aculeata</i> , <i>C. gibba</i> , <i>C. spinosa</i> and all subspecies of <i>C. aculeata</i>	<i>C. aculeata</i> type
All <i>Cyclopyxis</i> species	<i>Cyclopyxis arcelloides</i> , <i>Cy. kahli</i>	<i>Cy. kahli</i> type
Spined <i>Euglypha</i> tests	<i>Euglypha strigosa</i> , <i>E. compressa</i> , <i>E. ciliata</i>	<i>E. strigosa</i> type
Large pyriform <i>Nebela</i> species (150–200 µm) with and without keel	<i>Nebela galeata</i> , <i>N. penardiana</i>	<i>N. penardiana</i> type
Pyriform and other small <i>Diffugia</i>	<i>Diffugia pulex</i> , <i>D. pristis</i> and <i>D. rubescens</i>	<i>D. rubescens</i> type
Pyriform to narrow ovoid, large <i>Diffugia</i>	<i>D. oblonga</i> , <i>D. bacillifera</i>	<i>D. oblonga</i> type
Smaller flask-shaped <i>Nebela</i> , possibly including some <i>Hyalosphenia elegans</i> where plates not clear	<i>N. lageniformis</i> , <i>N. minor</i> , <i>N. barbata</i> , and <i>Hyalosphenia elegans</i>	<i>N. wailesi</i> type
All <i>Corythion</i> and <i>Trinema</i> taxa, exclude <i>T. lineare</i>	<i>Corythion dubium</i> , <i>Trinema enchelys</i> , <i>Tr. complanatum</i> and <i>Tr. penardi</i>	<i>Corythion-Trinema</i> type
Sub-ovoid or ovoid-globose <i>Diffugia</i> species	<i>D. urceolata</i> , <i>D. tuberculata</i> , <i>D. globulosa</i>	<i>D. globulosa</i> type
All <i>Plagiopyxis</i> species	<i>Plagiopyxis callida</i> and possibly other small <i>Plagiopyxis</i> species	<i>P. callida</i>