

Ecology of testate amoebae and their potential use as palaeohydrologic indicators from peatland in Sanjiang Plain, Northeast China

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Abstract Testate amoebae are sensitive indicators of substrate moisture in peatlands. Over the last decades, they have been studied to reconstruct hydrological changes since the Holocene. However, these studies have been geographically restricted to North America and Europe. We conducted the first investigation of testate amoebae on the largest continental fresh water wetland in the Sanjiang Plain, China. The objectives of this study were to provide baseline data on the ecology of testate amoebae in the peatlands of Northeast China and to assess the potential of using them as environmental indicators in this ecosystem. We examined modern testate amoeba assemblages and species-environmental relationships at 46 microsites within 5 waterlogged depressions. The environmental parameters measured included: depth to water table, pH, and loss on ignition. The results showed that the dominant species were *Trinema complanatum* type, *Euglypha rotunda* type, *Euglypha strigosa* type, and *Centropyxis cassis* type. Redundancy analysis demonstrates that water table depth has the most important effect on testate amoeba assemblages, explaining 16.7% ($p = 0.002$) of the total variance. pH was not a statistically significant factor for testate amoeba assemblages. Weighted averaging and weighted averaging partial least squares models were used to build transfer functions for depth to water table. The best performing transfer function was generated by the weighted averaging partial least squares model with an r^2_{LOSO} of 0.62 and $\text{RMSEP}_{\text{LOSO}}$ of 6.96 cm. Results indicate that testate amoebae in waterlogged depression peatland have the potential to be used as indicators for

hydrological changes and for palaeohydrologic reconstructions in the Sanjiang Plain.

Keywords palaeoecology, peatland, testate amoebae, transfer function, Sanjiang Wetland, Northeast China

1 Introduction

Testate amoebae are a ubiquitous group of shelled eukaryotic micro-organisms, living in aquatic or moist habitats in estuaries, lakes, rivers, wetlands, soils, litter, and moss (Ogden and Hedley, 1980; Meisterfeld, 2002a, b). They are small, abundant, and diverse (Mitchell et al., 2008), with proteinaceous, calcareous, or siliceous test (shell) (Ogden and Hedley, 1980). They are sensitive to a wide variety of environmental factors and have a rapid reproduction rate, which make them ideal ecological indicators for hydrology, nutritional status, and pollution levels (Charman and Warner, 1992; Booth, 2008; Roe et al., 2010; Payne et al., 2012a; Patterson et al., 2012, 2013). In addition, testate amoebae play a vital role in nutrient cycling (Wilkinson, 2008; Wilkinson and Mitchell, 2010). Because of their decay-resistant and morphologically distinct shells, testate amoebae are used in a variety of ecological and palaeoecological studies in a variety of ecosystems across the globe (Warner, 1988). Previous studies have demonstrated a strong relationship between testate amoebae and substrate moisture or depth to water table and pH, which have successfully been used to reconstruct hydrological changes from fossil assemblages data (Beyens and Chardez, 1987; Lamentowicz and Mitchell, 2005; Li, 2009; Swindles et al., 2009; Booth, 2010; Charman et al., 2012; Qin et al., 2012; Mitchell et al., 2013; Turner et al., 2013).

deep and a small layer of peat underlie this community. The second ring is formed by a mixture of *Carex meyeriana*, *Carex lasiocarpa*, *Glyceria spiculosa*, *Sphagnum* sp., and *Drepanocladus* sp. The underlying peat is mixed with moderately to highly decomposed *Sphagnum* and herbaceous peat with depths less than 70 cm. *Carex pseudo-curaica*, *Sphagnum*, and *Drepanocladus* dominate the plant community in the center of these depressions. This community forms a floating mat of peat, approximately 50 cm thick, which overlays a pocket of water and watery peat about 1–2 m deep. Usually these upland areas are partly surrounded by secondary deciduous-coniferous forest (*Betula platyphylla*, *Populus* sp., and *Larix* spp.).

Our study sites consisted of five peatlands in two National Natural Reserves, four in Honghe National Natural Reserve (included in the “List of Ramsar wetlands of international importance”), and one in Dongfanghong National Natural Reserve (Fig. 1). Five waterlogged depression peatlands were selected based on their similarities in vegetation type. These selected peatlands are dish-like in shape and nearly equal in size, about 30,000 m², with the exception of Dongfanghong National Natural Reserve, which is only about 10,000 m². All of these sites are well protected and maintain their natural status. The hydrological regimes of these depression peatlands are mainly driven by rainfall. Groundwater flows from the wetland itself will also effluence these wetlands by seasonal flooding. Precipitation (snow and rain) significantly affects the distribution and succession of the flora in this type of peatland (Yang and Lv, 1996).

2.2 Field sampling

A series of transects were chosen to reflect the full range of hydrological and microtopographic environments at each site. Three to four samples were taken along each transect. For each sample, five replicates of approximately 5 cm × 5 cm × 5 cm of moss were designated within a 1 m² area. Thirteen transects were established and a total of 46 samples collected within one week in late September and early October. Depth to water table (DWT) was measured relative to the substrate surface at the end of sampling (after ca. 4 hr equilibration), with standing water recorded as negative values. A sample of water was collected in each hole left by sampling and measured for pH using a pH meter during each field sampling. Samples were stored at 4°C for further analysis.

2.3 Laboratory work

Each sample was divided in two subsamples. One was dried at 80°C and incinerated at 550°C for eight hours to calculate loss on ignition (LOI) (cf. Payne, 2011). Testate amoebae were isolated from moss following a combined

procedure according to Hendon and Charman (1997) and Lamentowicz et al. (2008). We mixed the other subsample with ca. 200 mL of deionized water. The samples were stirred for two hours to disaggregate them and to release the testate amoeba shells from the mosses. They were then sequentially washed through sieves with meshes of 300 μm and 15 μm. The fraction between the two sieves was centrifuged at 3,000 r/min for five minutes. Testate amoebae were identified and counted in drops of the concentrate mixed with glycerol at a magnification of ×200 or ×400 using a light microscope (Nikon 80i). We identified and counted at least 150 individual shells (Payne and Mitchell, 2009) for each sample. Taxonomy follows Ogden and Hedley (1980), Charman et al. (2000) and Meisterfeld (2002a, b).

2.4 Data analysis

To reduce the influence of rare taxa on the analysis, species that occurred in less than 10% of the samples were eliminated from the data set (Booth et al., 2008). Among these were *Centropyxis ecornis*, *Diffugia acuminata*, *Heleopera sylvatica*, *Hyalosphenia minuta*, *Nebela carinata*, *Physochila griseola*, *Placocista spinosa*, and *Trigonopyxis arcuata*.

The relationships between testate amoeba assemblages and environmental variables were explored using CANOCO 4.5 (Ter Braak and Šmilauer, 2002). Species data were square-root transformed prior to analysis. Detrended correspondence analysis (DCA) was used to determine the gradient length of the data set. Because the length of gradient was < 2 standard deviations, redundancy analysis (RDA) was used to test the strength of correlations between testate amoeba assemblages and environmental variables (Birks, 1995). The significance of three environmental parameters (DWT, pH, LOI) was tested by Monte Carlo permutation tests (499 random permutations).

Transfer functions for the environmental variables of community composition were developed. Weighted averaging (WA) and weighted averaging partial least squares (WAPLS) models were used. Model performance was assessed using the root mean squared error of prediction (RMSEP), the maximum bias (Max. bias), and correlation coefficients (R^2) with leave-one-site-out cross validation (Birks, 1998). The leave-one-site-out cross-validation was carried out because our samples had a clustered distribution with several samples per site (Payne et al., 2012b). Segment wise RMSEP was also applied to evaluate the bias of uneven sampling of wetness gradient (Telford and Birks, 2011). Water table depth was divided into ten equal segments and the RMSEP was calculated by weighting each segment equally. Transfer function analyses were carried out with the R software (v3.0.1) using the rioja library package (Juggins, 2012).

3 Results

3.1 Species diversity and community

After making a number of taxonomic groupings to ensure consistency (Table 1), 39 testate amoeba taxa or ‘type’ from 16 genera were identified for all samples (Fig. 2(a)). Testate amoeba communities were predominantly composed of generalist taxa, which are abundant in European and North American wetlands, with the most abundant being *Trinema complanatum* type (17.5% of all tests), *Euglypha rotunda* type (15.0%), *Centropyxis cassis* type (14.3%) and *Euglypha strigosa* type (11.9%). Other species found (greater than 1% in number) included *Cyclopyxis arcelloides* type (8.9%), *Tracheleuglypha dentata* (7.1%), *Trinema lineare* (5.3%), *Euglypha tuberculata* (2.9%), *Assulina muscorum* (2.6%), *Centropyxis aculeata* type (1.6%), and *Arcella discoides* type (1.4%).

Some common species found in wetlands in Europe and North America, including *Amphitrema wrightianum*, were absent in our study. Also, only one specimen of *Trignopyxis arcuata* was found. Five species of the genus *Nebela* account for only 0.4% of all the species, far less than in the neighboring region of Mount Changbai (Li, 2009).

3.2 Species-environment relationship

Because the maximum gradient of length of DCA is 1.91, RDA was used to investigate the relationships between environmental parameters and testate amoeba assemblages. RDA axes one (Eigenvalue = 0.180) and two (Eigenvalue = 0.050) together explain 23.0% of the variance in the species data and 83.4% of the species-environment relationship. The measured environmental variables collectively explained 25.3% of testate amoeba community variation (Figs. 2(a) and 2(b)). The highest variation in the testate amoeba data was explained by DWT, accounting for 16.7%, while pH and LOI explained 5.0% and 4.6% respectively. Monte-Carlo permutation tests showed that DWT ($p = 0.002$) were the dominant controls of testate amoeba species distributions. Other environmental variables did not show a significant relationship at the 5% alpha level (Table 2).

Taxa from genus *Arcella* and species with spined forms (esp. *Centropyxis aculeata* type) appear at the wetter end of

the gradient. *Diffflugia* species generally occur at intermediate to wet locations, while *Euglypha* species are generally indicators of intermediate to drier conditions. Taxa of the genera *Assulina*, *Trinema*, and *Corythion* are found in drier locations (Fig. 2).

3.3 Development of transfer functions

The significant taxa-hydrological result for DWT indicated in the RDA suggests that a transfer function can be developed for DWT based on the waterlogged depression peatlands data set. The performance of WA and WAPLS models for water table depth are shown in Table 3. The two-component WAPLS model performed the best ($RMSEP_{LOSO} = 6.96$ cm, $r^2_{LOSO} = 0.62$) (Table 3). Many previous studies of testate amoebae have improved the performance of transfer function by removing outliers (e.g., Payne et al., 2006; Charman and Blundell, 2007). In this study, however, we confine ourselves to discussing the potential of using testate amoebae as environmental and palaeoenvironmental indicators in water-logged depressions. All data were used in our preliminary transfer function. Segment-wise RMSEP shown in Fig. 3 indicate that RMSEP values are biased by the uneven sampling of the water table gradient. For all models, RMSEP was considerably lower than the standard deviation of all water table measurements ($SD = 11.77$ cm, $n = 46$) suggesting that all models have predictive capacity (Payne et al., 2012b). The comparison of the observed and model estimated DWT is shown in Fig. 4.

4 Discussion

This is the first study conducted to analyze testate amoeba assemblages and their relationship with hydrological variables in the largest concentration of freshwater wetlands in China. Waterlogged depression peatlands were primarily selected due to their natural dry-wet gradient. We collected baseline data of testate amoeba ecology in this region. Consistent with similar studies from other geographic regions (e.g., Charman and Blundell, 2007; Booth et al., 2008; Turner et al., 2013), the results of RDA confirmed that the structure of the testate amoeba community is principally explained by DWT. These good, modern analogues demonstrate a strong qualitative

Table 1 Combinations and means of separation of some testate amoeba species differing from Charman et al. (2000)

Species name in Charman et al. (2000)	Species name used here	Important grouping criteria
<i>Corythion-Trinema</i> type	<i>Corythion dubium</i>	Test ovoid; anterior part resembling a spoon
	<i>Trinema complanatum</i> type	Test broad elliptical
<i>Euglypha ciliata</i>	<i>Euglypha strigosa</i> type	Test ovoid in lateral view; spines scattered over surface
<i>Euglypha compressa</i>		
<i>Euglypha strigosa</i>		

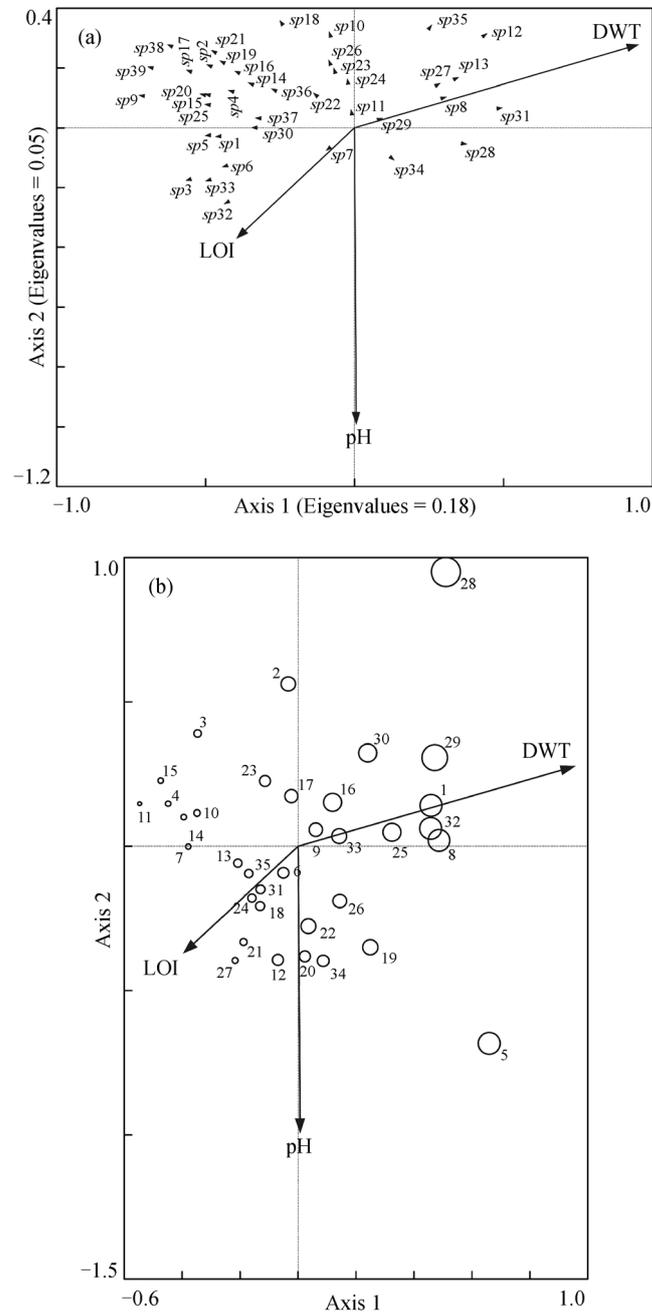
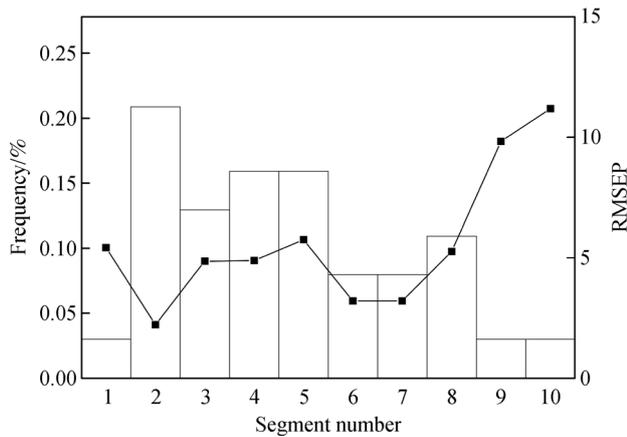


Fig. 2 Biplot of redundancy analysis (RDA) on all data. (a) Species and environmental variables. Species codes: Sp1, *Arcella catinus* type; Sp2, *Arcella dentata*; Sp3, *Arcella discooides* type; Sp4, *Arcella gibbosa* type; Sp5, *Arcella hemisphaerica*; Sp6, *Arcella vulgaris*; Sp7, *Argynnia dentistoma*; Sp8, *Assulina muscorum*; Sp9, *Centropyxis aculeata* type; Sp10, *Centropyxis cassis* type; Sp11, *Centropyxis platystoma* type; Sp12, *Cyclopyxis arcelloides* type; Sp13, *Corythion dubium*; Sp14, *Corythion pulchellum*; Sp15, *Diffflugia bacilliarum*; Sp16, *Diffflugia bacillifera*; Sp17, *Diffflugia lucida* type; Sp18, *Diffflugia pristis* type; Sp19, *Diffflugia oblonga*; Sp20, *Diffflugia* sp.; Sp21, *Euglypha acanthophora*; Sp22, *Euglypha strigosa* type; Sp23, *Euglypha cristata*; Sp24, *Euglypha rotunda* type; Sp25, *Euglypha tuberculata*; Sp26, *Heleopera petricola* type; Sp27, *Heleopera rosea*; Sp28, *Nebela collaris*; Sp29, *Nebela penardiana*; Sp30, *Nebela carinata*; Sp31, *Nebela tubulosa*; Sp32, *Lesquereusia epistomium*; Sp33, *Lesquereusia spiralis*; Sp34, *Placocista glabra*; Sp35, *Trinema complanatum* type; Sp36, *Trinema lineare*; Sp37, *Cryptodiffflugia oviformis*; Sp38, *Sphenoderia lenta*; Sp39, *Tracheleuglypha dentata*. (b) RDA sampling plots and hydrological variables biplot. Larger circles represent the higher values of relative depth to water table.

Table 2 Results of redundancy analysis of testate amoeba data, showing percentage variance explained using various combinations of variables and co-variables and the *p*-value determined by Monte-Carlo permutations tests (499 permutations)

Explanatory variables	Co-variables	% variance explained	<i>p</i>
DWT, pH, LOI	n.a.	25.3	
DWT	pH, LOI	16.7	0.002
pH	DWT, LOI	5.0	0.100
LOI	DWT, pH	4.6	0.142

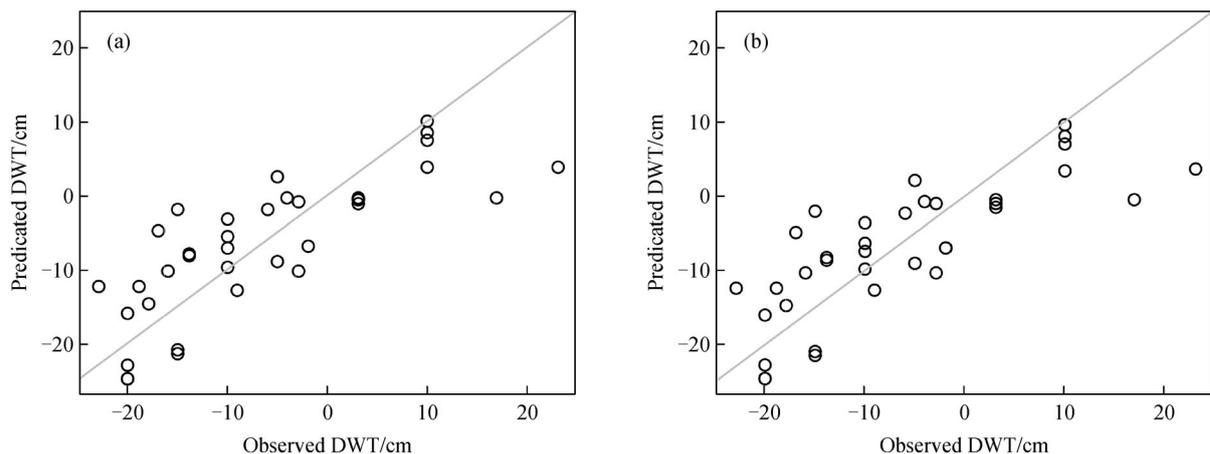
**Fig. 3** Segment-wise RMSEP for the highest performing WAPLS model. Sampling distribution of the dataset was evenly divided into 10 segments.

correlation between DWT and testate amoeba assemblages. This suggests that testate amoeba-based transfer function for DWT could be used to quantitatively infer rapid changes in past moisture conditions and climate of the Sanjiang Plain.

Some differences in community composition were observed in comparison to similar studies in other regions. *Amphitrema* and *Archerella* species are very common in northern hemisphere peatland (e.g., Charman and Blun-

dell, 2007; Booth, 2008; Swindles et al., 2009; Bobrov et al., 2010); however, they have not yet been reported in Chinese peatland (Li, 2009; Qin et al., 2012, 2013b). The absence of *Amphitrema* species in our study may be due to the limited study samples or to the minerotrophic characters in Chinese peatland (Qin et al., 2012, 2013b). Only one specimen of *Trigonopyxis arcula* was identified in this work, while *T. arcula* accounts for 2.4% of individuals in the wetlands from Mount Changbai (Li, 2009). *T. arcula* was considered to be an indicator for a dryer environment, with optimal water-table values of 10–60 cm (Booth, 2001; Li et al., 2008). However, the samples we collected were located in a slightly wetter habitat, which could be the reason why few *T. arcula* were encountered in this work. Both taxon number and specimen totals in the genus *Nebela* were far less than for Li's (2009) work in Mount Changbai. The reason for this result could be the difference in wetland type. Peatlands in this study are *Carex*-dominated poor fens. Compared with waterlogged depression peatlands, peatlands at Mount Changbai have a deeper peat deposit and are *Sphagnum*-dominated ombrotrophic. Further study in these two regions is needed to provide a better understanding of testate amoeba composition in peatlands of Northeast China.

Relationships between measured environmental variables and testate amoeba communities, conducted by

**Fig. 4** Biplot of observed vs. predicted depth to water table (DWT). (a) Prediction resulting from the weighted averaging model. (b) Prediction resulting from the weighted averaging partial least squares model.

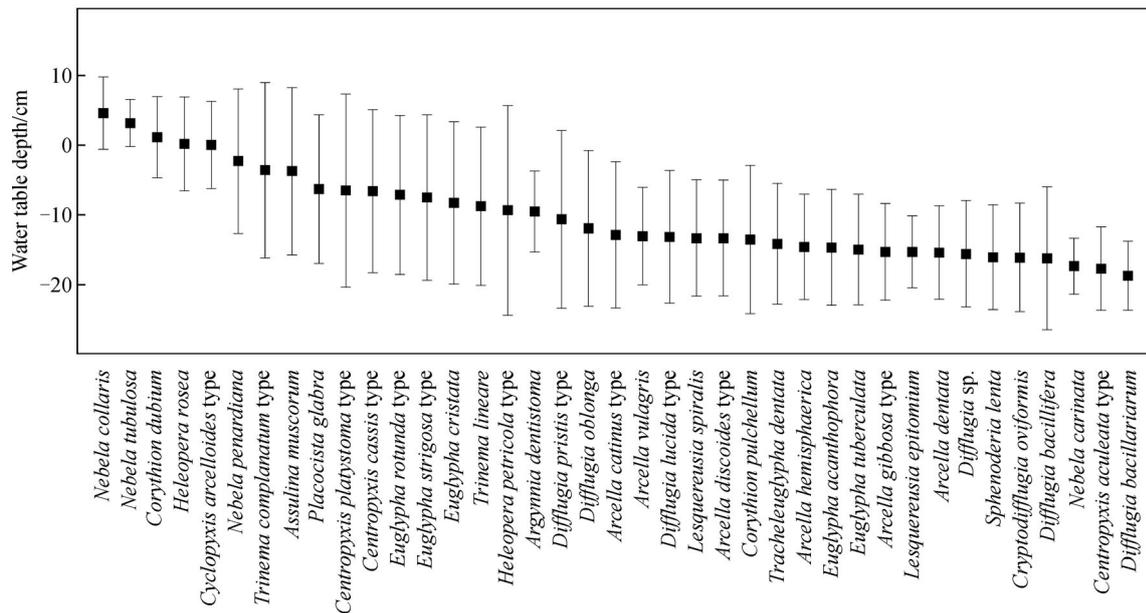


Fig. 5 Testate amoeba taxa optima and tolerances for water table depth based on weighted averaging.

Table 3 Transfer function performance of models for DWT

Model	r^2_{LOSO}	Max. bias LOSO	RMSEP LOSO
WA.inv	0.56	19.97	7.70
WA.cla	0.58	14.10	8.81
WA.inv.tol	0.55	20.42	7.85
WA.cla.tol	0.58	14.85	8.40
WAPLS Component 2	0.62	19.16	6.96

Abbreviations: WA = weighted averaging model; WAPLS = weighted averaging partial least squares model; RMSEP = root mean squared error of prediction; Max. bias = maximum bias.

redundancy analysis, have demonstrated a strong environmental control of community composition. As found in most studies of testate amoebae in North America (Payne et al., 2006; Booth et al., 2008; Amesbury et al., 2013), Europe (Charman and Blundell, 2007; Mitchell et al., 2013; Turner et al., 2013), and Asia (Li et al., 2009; Mazei and Chernyshov, 2011; Qin et al., 2013b), surface moisture conditions (expressed as depth to water table) have been identified as a primary control of community composition (Fig. 2). For example, on the dry side of the gradient, communities include *Cyclopyxis arcelloides*, *Assulina muscorum*, and *Centropyxis cassis* type. In more mesic sites, *Centropyxis aculeata* type and *Arcella discoides* are the characteristic taxa. Key taxa occur at similar relative positions along the hydrology gradient (Fig. 5), confirming the robustness of these organisms as indicators of hydrological changes. The results demonstrate the persis-

tent and comparable control of DWT on the distribution of testate amoebae taxa across Europe, North America, and Northeast China, and indicate that the substrate moisture conditions could be inferred from their assemblage. In most of these studies, pH was found to be the second most significant variable (Li, 2009; Mitchell et al., 2013). However, in contrast, it did not significantly affect community composition in the present study, as was also true for peatland in central China (Qin et al., 2013b). One possible reason could be due to a relatively short gradient of pH (Qin et al., 2013b). Another explanation could be that the pH was diluted by precipitation, since sampling was performed about two weeks after the last rain.

The marked changes in testate amoebae assemblages are interpreted as the result of past changes in wetland hydrology. The results of this study show that testate amoebae in waterlogged depressions are responsive to hydrology and the transfer function should allow accurate prediction of water tables. Assuming the modern assemblage provides a good analogue, past water table depth can be quantitatively inferred from fossil data sets (Fig. 4). Quantitative palaeohydrologic reconstructions have been calculated for many wetlands in North America (e.g., Booth, 2010) and Europe (e.g., Charman and Blundell, 2007; Charman et al., 2012). By applying the transfer function to the fossil testate amoebae data, it will be possible to quantitatively reconstruct the past moisture conditions in this system, although a study of the fossil testate amoebae has not yet been carried out.

This work provided information on the ecology of testate amoebae in a previously unexplored region and

peatland type in China. Although the fossil testate amoebae have not yet been assessed, they could provide detailed information on past changes in substrate moisture content in this system. However, additional data on testate amoebae ecology in waterlogged depression peatland is needed before it can be considered to be of widespread applicability.

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