JOURNAL OF QUATERNARY SCIENCE (2008) **23**(1) 43–57

Published online 9 August 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/jqs.1114



# Testate amoebae as proxies for mean annual water-table depth in *Sphagnum*-dominated peatlands of North America

**ROBERT K. BOOTH***
Department of Earth and Environmental Science, Lehigh University, Bethlehem, Pennsylvania, USA

Booth, R. K. 2007. Testate amoebae as proxies for mean annual water-table depth in *Sphagnum*-dominated peatlands of North America. *J. Quaternary Sci.*, **Vol 23** pp. 43–57. ISSN 0267-8179.

Received 6 September 2006; Revised 22 December 2006; Accepted 8 January 2007

ABSTRACT: Peatland-inhabiting testate amoebae are sensitive indicators of substrate-moisture conditions and have increasingly been used in palaeohydrological studies. However, to improve accuracy of testate-amoeba-based hydrological inferences, baseline ecological data on rare taxa, a larger geographic network of calibration sites, and incorporation of long-term estimates of water-table depth are needed. Species–environment relationships at 369 sites from 31 peatlands in eastern North America were investigated. Long-term estimates of water-table depth were obtained using the method of polyvinyl (PVC) tape-discolouration. Transfer functions were developed using a variety of models, and validated through jackknifing techniques and with an independent dataset where water-table depths were directly measured throughout the growing season. Results indicate that mean annual water-table depth can be inferred from testate amoeba assemblages with a mean error of 6 to 8 cm, although there is a slight systematic bias. All transfer function models performed similarly and produced similar reconstructions on a fossil sequence. In a preliminary effort towards development of a comprehensive North American calibration dataset, data from this study were combined with previous studies in Michigan and the Rocky Mountains ($n = 650$). This combined dataset had slightly larger mean errors of prediction (8–9 cm) but includes data for several rare taxa. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: testate amoebae; Rhizopoda; peatlands; hydrology; palaeoclimate.

JQS Journal of Quaternary Science

## Introduction

Testate amoebae are a polyphyletic group of protozoa that produce decay-resistant and morphologically distinct shells, or tests. Within oligotrophic peatlands (i.e. nutrient-poor) the distribution of taxa is primarily controlled by substrate moisture, making them sensitive proxies for past and ongoing hydrological change (Charman, 2001). In the past decade, transfer functions to infer water-table depth from testate amoeba assemblages have been developed and validated in various regions of the world (e.g. Charman, 1997; Charman and Warner, 1997; Woodland *et al.*, 1998; Bobrov *et al.*, 1999; Mitchell *et al.*, 1999; Booth, 2002; Lamentowicz and Mitchell, 2005; Charman *et al.*, 2007; Payne *et al.*, 2006). These transfer functions have been used to address a wide range of environmental and palaeoenvironmental issues. For example, they have played an important role in understanding temporal relationships between vegetation and hydrology within raised bogs (McMullen *et al.*, 2004), delimiting spatial and temporal patterns of past centennial and sub-centennial scale drought events (Booth *et al.*, 2006), monitoring and informing bog restoration efforts (Buttler *et al.*, 1996; Davis and Wilkinson, 2004), and assessing the responses of terrestrial vegetation to past climate variability and change (Wilmhurst *et al.*, 2002; Booth and Jackson, 2003; Booth *et al.*, 2004).

Although the utility of testate amoebae as environmental indicators has been clearly demonstrated, improving the precision and accuracy of transfer functions should increase the applicability of testate amoebae in addressing a wider range of environmental issues. Several problems with current calibration datasets need to be addressed to improve accuracy, precision, and confidence. A primary concern has been that most calibration studies have not collected environmental and community data at the same temporal scale. For example, most training sets have been based on one-time measurements of water-table depth (i.e. water-table depth measured only at time of sampling) even though modern collections of testate amoeba assemblages integrate the last several years of accumulation. Therefore, comparison of species–environment relationships among studies carried out at different times of year in different regions has been problematic. Also, reconstructions can only be made along relative moisture gradients. The inclusion of annually averaged water-table depth measurements can provide more meaningful reconstructions (Woodland *et al.*, 1998), although long-term hydrological data are rarely

* Correspondence to: R. K. Booth, Department of Earth and Environmental Science, Lehigh University, 31 Williams Drive, Bethlehem, PA 18015, USA.
E-mail: robert.booth@lehigh.edu

available at sufficient spatial and temporal density and usually are prohibitably expensive to obtain.

The recent development and validation of a method of estimating water-table depth using polyvinyl chloride (PVC) tape may provide an inexpensive means of obtaining more comparable and meaningful estimates of water-table depth for calibration studies (Belyea, 1999; Booth *et al.*, 2005). The PVC-tape discolouration method is based on the observation that PVC-tape changes colour when exposed to reducing conditions (Belyea, 1999). Validation of the PVC-tape discolouration method in peatlands of northern Wisconsin indicates that the highest point of PVC-tape discolouration on tape-lined stakes inserted into peatlands is a good approximation of the mid-summer or mean position of the water-table depth during the growing season (Booth *et al.*, 2005). Other studies also have indicated strong relationships between peatland water-table depth and the height of PVC-tape discolouration (Bragazza, 1996; Belyea, 1999; Navrátilova and Hájek, 2005). However, precise relationships with water tables are not completely clear, with the position of discolouration ranging from the mean height of the water table, to the depth that experiences inundation 70% of the time, to a few centimetres below the highest water levels (Bragazza, 1996; Belyea, 1999; Booth *et al.*, 2005). The method may be problematic at fen sites with strongly fluctuating water tables (Schnitchen *et al.*, 2006).

Another problem with many calibration datasets has been the relatively small geographic range of sampling. Even though many taxa are cosmopolitan in distribution and occupy similar ecological niches in different regions (e.g. Booth and Zygmunt, 2005), application of transfer functions developed from assemblages in one region to fossil datasets from another region has sometimes been problematic (Charman *et al.*, 2006). Limited geographic sampling also may explain the lack of good modern analogues for some fossil assemblages (Charman, 2001). Lack of good modern analogues appears to be primarily due to poor representation of several taxa in modern calibration datasets (e.g. *Difflugia pulex*), but in some cases may be caused by taphonomic biases in highly decomposed peat (Wilmshurst *et al.*, 2003). Larger sampling networks and collection of long-term environmental data should improve our understanding of testate amoeba ecology and biogeography, leading to more accurate and precise environmental and palaeoenvironmental inferences.

In this study, I attempt to address some of these problems by investigating relationships among testate amoeba assemblages and hydrology from an array of 31 peatlands in eastern North American, extending from the mid-continent to the east coast at mid-latitudes. I use the PVC-tape discolouration method to provide long-term estimates of water-table depth at the sampling locations, and develop transfer functions for water-table depth. These transfer functions are validated using jackknifing (leave-one-out) techniques (Birks, 1998). To test the assumption that the transfer functions, which are based on PVC-discolouration estimates of water-table depth, predict mean annual water-table depths, I apply the transfer functions to an independent dataset where mean annual water-table depths were directly measured. Water-table depths also were reconstructed for a sequence of fossil assemblages, allowing comparison of reconstructions based on different transfer function models. Finally, I attempt to combine this newly developed dataset with data from previous studies in Michigan and the Rocky Mountains, in a preliminary effort toward the development of a comprehensive North American calibration dataset.

## Methods

### Field methods

The sampled peatlands included raised bogs and kettle peatlands in continental and eastern North America (Fig. 1). All peatlands were *Sphagnum*-dominated, and characteristics of the sites are shown in Table 1. Samples of testate amoeba communities were collected from within each peatland in an effort to capture the range of hydrologic variability (e.g. hummocks, hollows, pools). Methods of sampling were modelled after previous studies (Booth, 2001, 2002). At each sampling site, ~10 $cm^3$ of *Sphagnum* moss was collected. The samples consisted of the upper 3–5 cm of *Sphagnum* after the upper 1–2 centimetres of moss were removed, which typically corresponded to the brown area of the stem directly below the green portion. Vertical variation in assemblage composition occurs along the *Sphagnum* stem, and samples collected from this portion of the stem contain higher taxonomic diversity than

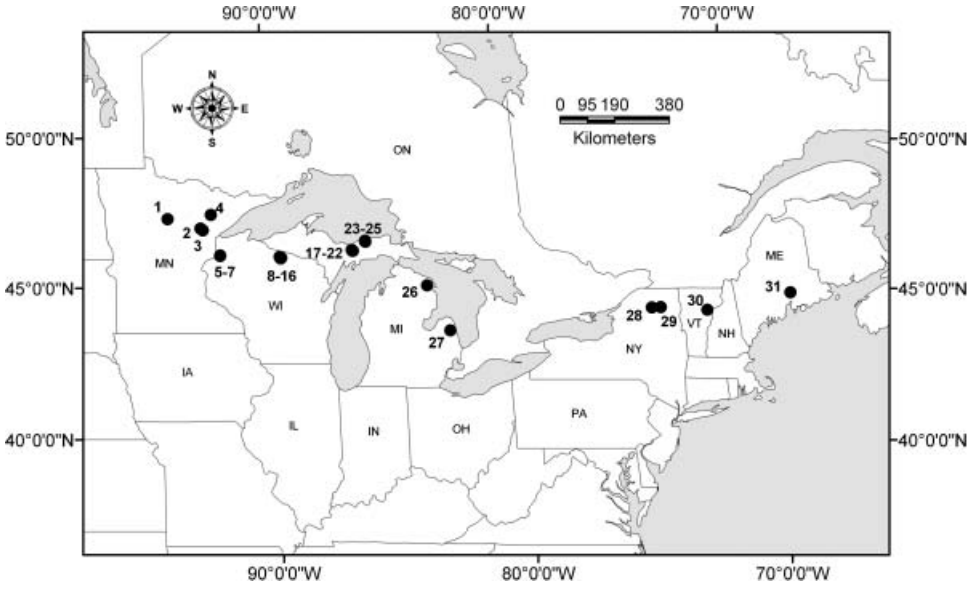


**Figure 1** Location of the 31 sampled peatlands in mid-continental and eastern North America. Site names and characteristics are shown in Table 1



DOI: 10.1002/jqs

**Table 1** Site characteristics for each peatland, arranged from west to east, including the location of the peatland, number of testate amoeba samples (after removal of outliers), the range of measured depths to water table (DTW) in 2004 and 2005, highest points of PVC-tape discolouration, the range of pH and the range of conductivity. The conductivity and pH data are from squeezed *Sphagnum* (S) and from water collected from the level of the water table (W). Site numbers refer to Fig. 1. For the peatlands in the region of Trout Lake (8–16) the range of average water-table depths are shown, based on monthly measurements during 2004

| Site number and name | Location | Elevation (m) | *n* | Range of DTW (2004) (cm) | Range of DTW (2005) (cm) | Range of highest PVC-tape discolouration (cm) | Range of pH | Range of conductivity |
|---|---|---|---|---|---|---|---|---|
| (1) Hole Bog, MN | 47° 18.000 N<br>−94° 14.942 W | 399 | 17 | −3 to 52 | −8 to 45 | 0 to 52 | (S) 3.56 to 3.87<br>(W) 3.83 to 3.96 | (S) 74 to 184.4<br>(W) 64.4 to 183.5 |
| (2) Toivola Bog B, MN | 46° 59.167 N<br>−92° 53.305 W | 384 | 7 | 0 to 62 | −7 to 52 | 0 to 44 | (S) 3.55 to 4.02<br>(W) 3.88 to 4.02 | (S) 85.2 to 197<br>(W) 70.1 to 86.1 |
| (3) Toivola Bog A, MN | 46° 55.609 N<br>−92° 47.702 W | 401 | 13 | 14 to 65 | −1 to 54 | 3 to 57 | (S) 3.4 to 3.82<br>(W) 3.73 to 3.91 | (S) 130.6 to 230<br>(W) 77.2 to 105 |
| (4) Ely Bog, MN | 47° 26.766 N<br>−92° 26.156 W | 423 | 12 | 12 to 72 | −2 to 52 | 0 to 55.5 | (S) 3.43 to 4.07<br>(W) 4.03 to 4.29 | (S) 102.7 to 226<br>(W) 43.6 to 69.8 |
| (5) Hornet Peatland, WI | 46° 4.593 N<br>−92° 7.212 W | 289 | 15 | −8 to 37 | −7 to 40 | −1.5 to 40 | (S) 3.61 to 4.18<br>(W) 3.92 to 4.27 | (S) 46.3 to 193.7<br>(W) 46.3 to 85.3 |
| (6) Small Sarracennia Peatland, WI | 46° 4.555 N<br>−92° 6.902 W | 291 | 10 | −9 to 23 | −11 to 26 | −6 to 23.5 | (S) 3.79 to 4.41<br>(W) 4.05 to 4.41 | (S) 22 to 83.5<br>(W) 22 to 64.6 |
| (7) Spruce Peatland, WI | 46° 5.678 N<br>−92° 6.723 W | 287 | 11 | −4 to 64 | −5 to 65 | −2.5 to 60.5 | (S) 3.42 to 4.08<br>(W) 3.88 to 4.05 | (S) 52.9 to 253<br>(W) 52.9 to 100.5 |
| (8) Trout Peatland H, WI | 46° 2.849 N<br>−89° 39.053 W | 499 | 8 | 9 to 48.7 | N/A | N/A | (S) 3.63 to 4.41<br>(W) 3.57 to 4.25 | (S) 42 to 138<br>(W) 41.1 to 61.6 |
| (9) Trout Peatland I, WI | 46° 2.806 N<br>−89° 38.903 W | 500 | 10 | 8.2 to 34.7 | N/A | N/A | (S) 3.5 to 3.89<br>(W) 3.9 to 4.20 | (S) 117.8 to 192<br>(W) 62.8 to 107.8 |
| (10) Trout Peatland G, WI | 46° 2.265 N<br>−89° 38.146 W | 497 | 8 | −0.2 to 49.2 | N/A | NA | (S) 3.38 to 3.87<br>(W) 3.7 to 4.06 | (S) 110 to 209<br>(W) 75.2 to 113 |
| (11) Trout Peatland F, WI | 46° 1.325 N<br>−89° 37.956 W | 504 | 8 | 13 to 37.5 | N/A | N/A | (S) 3.48 to 3.85<br>(W) 3.71 to 3.87 | (S) 146 to 222<br>(W) 70.5 to 129.4 |
| (12) Trout Peatland E, WI | 46° 0.287 N<br>−89° 37.644 W | 500 | 10 | 9.8 to 52.7 | N/A | N/A | (S) 3.59 to 3.74<br>(W) 3.87 to 4.08 | (S) 120.6 to 223<br>(W) 60.3 to 133 |
| (13) Trout Peatland D, WI | 45° 59.712 N<br>−89° 36.828 W | 506 | 15 | 2.5 to 44 | N/A | N/A | (S) 3.47 to 4.10<br>(W) 3.52 to 4.36 | (S) 109.1 to 305<br>(W) 24.4 to 262 |
| (14) Trout Peatland C, WI | 46° 0.459 N<br>−89° 36.304 W | 506 | 13 | 6.8 to 56.5 | N/A | N/A | (S) 3.45 to 3.67<br>(W) 3.73 to 4.43 | (S) 146.6 to 262<br>(W) 58.4 to 135.2 |
| (15) Trout Peatland A, WI | 46° 0.214 N<br>−89° 35.624 W | 514 | 11 | 4.2 to 39.2 | N/A | N/A | (S) 3.53 to 4.43<br>(W) 3.89 to 4.36 | (S) 125.3 to 232<br>(W) 69.2 to 180.1 |
| (16) Trout Peatland B, WI | 46° 1.155 N<br>−89° 35.335 W | 505 | 10 | −1.8 to 57.5 | N/A | N/A | (S) 3.44 to 4.07<br>(W) 3.68 to 4.69 | (S) 58.6 to 253<br>(W) 35.1 to 100 |
| (17) Grimes Peatland, MI | 46° 16.537 N<br>−86° 38.985 W | 258 | 10 | −8 to 33 | 40 to 75 | 4.4 to 33.0 | (S) 3.66 to 4.06<br>(W) 3.95 to 4.50 | (S) 46 to 143.3<br>(W) 46 to 88.3 |
| (18) Island Camp Peatland, MI | 46° 16.295 N<br>−86° 38.842 W | 261 | 10 | −8 to 31 | 70 to 88 | 3.5 to 38.5 | (S) 3.69 to 4.23<br>(W) 3.75 to 4.23 | (S) 35 to 126.7<br>(W) 35 to 80.8 |
| (19) Upper Twin Peatland, MI | 46° 16.546 N<br>−86° 38.848 W | 264 | 8 | 4 to 32 | 70 to 86 | 8 to 37 | (S) 3.74 to 4.03<br>(W) 3.76 to 4.20 | (S) 67.3 to 149<br>(W) 40.4 to 69.4 |
| (20) Small Red Pine Peatland, MI | 46° 14.866 N<br>−86° 37.929 W | 253 | 4 | 7 to 39 | 75 to 82 | 3 to 32 | (S) 3.83 to 3.95<br>(W) 3.68 to 3.94 | (S) 37.1 to 82.4<br>(W) 73.4 to 90.7 |
| (21) Big Red Pine Peatland, MI | 46° 15.194 N | | 9 | −2 to 54 | 13 to 99 | 1 to 52.5 | (S) 3.72 to 4.09 | (S) 46.4 to 105.4 |

(*Continues*)


DOI: 10.1002/jqs

**Table 1** (Continued)

| Site number and name | Location | Elevation (m) | *n* | Range of DTW (2004) (cm) | Range of DTW (2005) (cm) | Range of highest PVC-tape discolouration (cm) | Range of pH | Range of conductivity |
|---|---|---|---|---|---|---|---|---|
| | −86° 37.366 W | 254 | | | | | (W) 3.85 to 4.40 | (W) 33.7 to 74.1 |
| (22) Kentucky Trail Peatland, MI | 46° 14.807 N | | 5 | 26 to 50 | 77 to 90 | 28.5 to 54 | (S) 3.62 to 3.92 | (S) 84.9 to 196.2 |
| | −86° 36.046 W | 253 | | | | | (W) 3.73 to 3.78 | (W) 80.2 to 96.3 |
| (23) South Rhody Peatland, MI | 46° 33.871 N | | 18 | −34 to 22 | 0 to 37 | −5.5 to 22 | (S) 3.77 to 4.53 | (S) 15.56 to 156 |
| | −86° 4.481 W | 289 | | | | | (W) 4.11 to 4.53 | (W) 15.56 to 44.8 |
| (24) North Rhody Peatland, MI | 46° 34.965 N | | 12 | −29 to 7 | 0 to 30 | −7.5 to 6.5 | (S) 3.97 to 4.51 | (S) 14.01 to 77.3 |
| | −86° 4.374 W | 293 | | | | | (W) 4.17 to 4.51 | (W) 14.01 to 36.3 |
| (25) Adams Trail Peatland, MI | 46° 32.844 N | | 11 | −2 to 25 | −10 to 35 | −4 to 31 | (S) 3.75 to 4.08 | (S) 37.5 to 200 |
| | −86° 3.577 W | 286 | | | | | (W) 3.94 to 4.19 | (W) 37.5 to 115.3 |
| (26) Herron Peatland, MI | 45° 6.126 N | | 6 | 1 to 47 | 24 to 72 | 2 to 54.5 | (S) 3.59 to 3.75 | (S) 120.4 to 237 |
| | −83° 38.358 W | 213 | | | | | (W) 3.84 to 3.94 | (W) 70.4 to 94.1 |
| (27) Minden Bog, MI | 43° 36.584 N | | 24 | 13 to 70 | 23 to 70 | 2.5 to 65 | (S) 3.32 to 3.72 | (S) 136.5 to 293 |
| | −82° 50.147 W | 243 | | | | | (W) 3.27 to 3.64 | (W) 109 to 192 |
| (28) Spring Pond Bog, NY | 44° 22.083 N | | 26 | −3 to 54 | 3 to 58 | −1.5 to 54 | (S) 3.42 to 3.97 | (S) 39.8 to 195 |
| | −74° 29.958 W | 476 | | | | | (W) 3.67 to 3.99 | (W) 36 to 76.5 |
| (29) Bloomingdale Bog, NY | 44° 22.970 N | | 18 | 13 to 61 | 18 to 77 | 11 to 55.5 | (S) 3.48 to 3.78 | (S) 118.9 to 218 |
| | −74° 8.344 W | 479 | | | | | (W) 3.69 to 4.10 | (W) 53.8 to 95.2 |
| (30) Peacham Bog, VT | 44° 17.545 N | | 12 | −3 to 45 | −11 to 34 | 2 to 41 | (S) 3.51 to 3.91 | (S) 70 to 189.3 |
| | −72° 14.386 W | 468 | | | | | (W) 3.66 to 3.91 | (W) 62.8 to 74.5 |
| (31) Orono Bog, ME | 44° 52.286 N | | 18 | N/A | N/A | 8 to 36 | N/A | N/A |
| | −68° 43.630 W | 40 | | | | | | |

upper portions of the stem (Mitchell and Gilbert, 2004) and typically have been used for palaeohydrological calibration datasets (e.g. Charman and Warner, 1997; Charman, 1997; Woodland *et al.*, 1998; Booth, 2002). However, samples in this study were generally collected from a few centimetres higher on the *Sphagnum* stem than a previous study in Michigan, where some samples extended as deep as 12 cm in loose-growing *Sphagnum* (Booth, 2002).

Within 30 cm of each sample location, I measured pH, conductivity and depth to the water table. Care was taken to make these measurements from the same topographic setting as the testate amoeba sample. Conductivity and pH measurements were made on water squeezed from *Sphagnum* and on water collected from the depth of the water table. Water-table depths were measured relative to the substrate surface, with high values corresponding to deep water tables (i.e. dry sites) and negative values indicating standing water. I also lined 1 m long wooden stakes with PVC-tape and inserted them at each sampling point in summer 2004, except at Orono Bog where stakes were installed in summer 2005. Fifteen centimetres of each stake were left above the surface of the peatland. Stakes were retrieved approximately 1 yr later. The highest point of PVC-tape discolouration was measured on each stake. Ten stakes were destroyed by animals, broken in attempts to recover them, or lost. These sites were excluded from the dataset. It was not possible to return to Orono Bog in summer 2006 to retrieve PVC-lined stakes, so in the present analysis only measured water-table depths were used for those samples. Most sampling locations within Orono Bog were from locations that felt like they were floating, where water-table depths would not be expected to vary much during the course of the year.

At nine peatlands in northern Wisconsin (Trout Lake peatlands A to I, sites 8–16) I monitored seasonal changes in water-table depth at 97 testate amoeba sampling localities. Additional characteristics of these peatlands can be found in Kratz and Medland (1989) and Booth *et al.* (2005). Water-table depths were measured at approximately monthly time steps throughout the growing season of 2004 (April–October). These sites also were used in a validation study of PVC-tape inferred water-table depth (Booth *et al.*, 2005). Mean annual water-table depths were calculated from these data and used to test the accuracy of the transfer functions developed from the other North American samples.

## Laboratory methods

Standard methods were used to isolate testate amoebae from the collections of *Sphagnum* moss (Hendon and Charman, 1997). Samples were boiled in distilled water for approximately 10 min and sieved through nested screens with openings of 355 μm and 15 μm. The material between 355 and 15 μm was collected, stained with Safranine dye, and stored in glycerol. Testate amoebae were identified and tallied at 400× magnification, normally until 150 specimens were encountered although in some samples this total could not be achieved. The relative abundance of each taxon was calculated as a percentage of the count total. Samples with count totals less than 100 were eliminated from the dataset, because percentages are likely to be unreliable. Taxonomy generally follows Charman *et al.* (2000), except as detailed in Table 2. *Habrotrocha angusticollis*, a rotifer commonly found and preserved in association with testate amoebae, was included in the analysis and count total.

## Analytical methods

Outliers and rare taxa can have a large influence on ordination techniques and transfer function development (McCune and

**Table 2** Taxonomic sources and notes for taxa encountered in this study where identification differed from Charman *et al.* (2000)

| Taxon | Taxonomy and notes |
|---|---|
| *Amphitrema wrightianum* Archer | Includes *A. stenostoma* Nüsslin. |
| *Arcella crenulata* Deflandre | Ogden and Hedley (1980). |
| *Centropyxis ecornis* Ehrenberg type | Separated from *C. cassis* type by an invaginated aperture that is only slightly off-centre. Tests circular or sub-circular in outline. Similar to description in Ogden and Hedley (1980), and a representative photomicrograph can be found in Lamentowicz and Mitchell (2005). |
| *Corythion-Trinema* type | May include *T. lineare*. |
| *Cyclopyxis arcelloides* (Penard) Deflandre type | Small (generally <70 μm in diameter), spheroidal or sub-spheroidal tests with aperture diameter greater than 0.75 the diameter of the test. Aperture rim smooth, and not armored with quartz grains. Similar to definition used by Booth (2002). |
| *Difflugia globulosa* Dujardin type | Large (generally >70 μm in diameter), spheroidal or sub-spheroidal tests with aperture diameter usually greater than 0.75 of the test. The rim of the aperture is usually armored with quartz grains, and large quartz grains are often abundant on surface of test. Aperture rim sometimes slightly raised above the surface of the test. Similar to definition used by Booth (2002). |
| *Euglypha strigosa* (Ehrenberg) Leidy type | May include *E. compressa* and *E. ciliata*. |
| *Habrotrocha angusticollis* Murray | As illustrated by Warner and Chengalath (1988). |
| *Phryganella acropodia* (Hertwig & Lesser) type | Intermediate sized (generally 40–80 μm in diameter), spheroidal or sub-spheroidal tests with aperture diameter usually less than 0.75 of the test. A few quartz grains are sometimes incorporated into the test, although more typically fungal hyphae and spores are included. The aperture is not armoured with quartz grains. Similar to definition used by Booth (2002). |
| *Pontigulasia* spp. | Ogden and Hedley (1980). |
| *Tracheuglypha dentata* Moniez | Ogden and Hedley (1980). |
| *Trigonopyxis minuta* Schönborn and Peschke | Bobrov *et al.* (1999). |
| Unknown type | Small (20–40 μm in length) pyriform test with terminal, collared aperture at the end of a short neck. Test material is clear and tests are not agglutinate. Very tidy appearance and morphologically invariable. May be referable to *Hyalosphenia minuta*. |



DOI: 10.1002/jqs

Grace, 2002). To reduce the effect of rare taxa, those taxa present in less than five samples, including *Arcella gibbosa*, *Difflugia rubescens*, *D. pulex*, *D. pristis*, *Nebela barbata*, *N. galeata*, *Pontigulasia* sp., *Sphenoderia lenta* and *Tracheleuglypha dentata* were excluded from the analysis. To reduce the effect of anomalous samples, 12 samples with unusual assemblages that were co-dominated by species characteristic of opposite sides of the hydrological gradient were removed from the dataset. Details of these samples, and further justification for their removal, are discussed in the results and discussion section. After removing these 12 outliers, as well as samples with inadequate count totals and samples where PVC-inferred water-table depths were not available, the resulting dataset contained 369 samples, including 93 samples from the Trout Lake peatlands.

To describe patterns of variation in testate amoeba assemblages and compare these patterns to measured environmental gradients, I used non-metric multidimensional scaling (NMDS) ordination (Kruskal, 1964; McCune and Grace, 2002). Species data were square root transformed prior to ordination analyses. NMDS does not make assumptions regarding underlying species distributions along compositional gradients and therefore has advantages over other ordination techniques (McCune and Grace, 2002; Clarke, 1993). I used Sorenson's distance measure in the analyses, along with the automated search feature of PC-ORD (McCune and Mefford, 1999) to identify the best solution and dimensionality. The automated search feature performed 40 runs with real data, each with a random starting configuration and consisting of solutions for one through six dimensions. Fifty runs with randomised data were then performed and statistics on the final stress at each dimensionality were accumulated. The best solution for each dimensionality was identified by comparing final stress values.

Transfer functions were developed using a variety of commonly used models, including weighted averaging (WA), weighted averaging with tolerance downweighting (WA-Tol), weighted average partial least square (WA-PLS), and weighted modern analogue technique (WMAT). I also developed a transfer function based on an ordination approach in which fossil samples were projected into the NMDS ordination of the calibration dataset (NMDS prediction) (McCune and Grace, 2002). In NMDS prediction, samples were positioned in the ordination space based on their similarity to the calibration samples, using Sorenson's distance metric (McCune and Grace, 2002). The various methods differ in their underlying assumptions, with WA, WA-Tol and WA-PLS based on a unimodal species response model, and WMAT and NMDS prediction lacking explicit species response models (Birks, 1998; McCune and Grace, 2002). The software package PC-ORD was used for the NMDS prediction technique (McCune and Mefford, 1999) and the software package C2 was used to develop and validate the other transfer function models and apply them to fossil data (Juggins, 2003). Jackknifing methods (leave-one-out) were used to assess model performance by comparing the root mean square error of prediction (RMSEP), coefficient of determination ($r^2$), average bias (Ave-bias), and an estimate of the largest systematic mean error along the environmental gradient (Max-bias). The models were also tested on the independent dataset from the Trout Lake peatlands, where water-table depths during the 2004 growing season were measured.

## Results and Discussion

### Relationships among testate amoebae and environmental conditions

A two-dimensional NMDS ordination of the entire dataset ($n = 369$, final stress = 19.8), including the samples from the Trout Lake peatlands, represented 81% of the variability in the testate amoeba data (Fig. 2). Correlations with environmental data indicate a strong relationship between assemblage composition and substrate moisture conditions, particularly

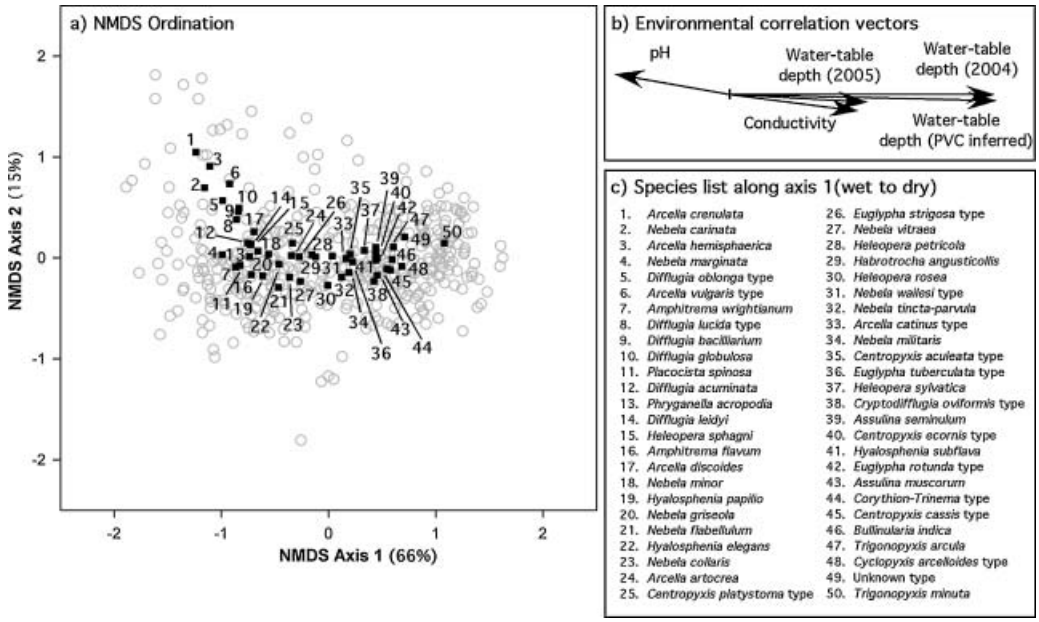


**Figure 2** (a) Results of the two-dimensional NMDS ordination of all testate amoeba samples ($n = 369$). The solution represents 81% of the variability in the data, with axes 1 and 2 representing 66% and 15% respectively. Samples are denoted with grey circles and the position of taxa is shown with black squares. Taxa are numbered according to their position along axis 1. (b) Environmental vectors indicating the direction and strength of environmental correlations ($r$), with the length of vectors indicating higher correlations. Only correlations greater than 0.3 are shown. Table 2 lists the maximum correlation of each variable with the ordination structure. (c) List of taxa with numbers corresponding to those in (a), arranged along the axis 1 gradient from wet to dry (water-table depth)



DOI: 10.1002/jqs

**Table 3** Correlations ($r^2$) between NMDS axis-1 scores and measured environmental variables for the complete dataset ($n = 369$), after rotating the ordination to maximise the correlation of axis 1 with each environmental variable

| Variable | $r^2$ |
|---|---|
| Conductivity (S) | 0.32 |
| Conductivity (W) | 0.21 |
| pH (S) | 0.37 |
| pH (W) | 0.21 |
| Depth to water table (measured 2004) | 0.68 |
| Depth to water table (measured 2005) | 0.29 |
| Depth to water table inferred from PVC-tape discolouration | 0.68 |

PVC-tape inferred water-table depth and water-table depth measured in 2004 (Fig. 2(b), Table 3). Secondary environmental controls on assemblage composition included pH and conductivity, consistent with patterns observed in previous studies (e.g. Charman and Warner, 1992; Booth, 2002) (Fig. 2(b), Table 3). Water-table depths measured in summer 2005 were much less correlated with assemblage composition than those measured in 2004 or inferred from PVC-tape discolouration (Fig. 2(b), Table 3). This lower correlation was probably the result of extremely dry conditions at some sites during the 2005 sampling, particularly in Upper Michigan. Several sites in central Upper Michigan, particularly Kentucky Trail, Upper Twin, Island Camp, Grimes, Big Red Pine and Small Red Pine (sites 17–22) had extremely deep water tables (often exceeding 70 cm) in 2005. These deep water tables were often very difficult to measure, and were probably unrepresentative of the average conditions experienced during much of the preceding year.

The pattern of taxon distribution along the water-table depth gradient (Fig. 2(c)) was similar to other studies in North America and elsewhere (e.g. Warner, 1987; Warner and Charman, 1994; Woodland *et al.*, 1998; Booth and Zygmunt, 2005; Charman *et al.*, 2007). Taxa common to drier sites are positioned on the right side of the NMDS ordination and include *Trigonopyxis minuta*, *T. arcula*, *Cyclopyxis arcelloides* type, *Bullinularia indica* and an unknown type (Fig. 2). *T. minuta* was almost exclusively found in very dry sites, supporting the contention of Bobrov *et al.* (1999) that differentiation of this taxon from the rest of the *T. arcula* group provides important ecological information. Taxa common to wetter hollows and pools are positioned on the left side of the NMDS ordination and include *Nebela carinata*, *N. marginata*, *Amphitrema wrightianum* and several species of *Arcella* and *Difflugia* (Fig. 2).

Interestingly, 10 of the 12 samples that were identified as outliers, and removed prior to analysis because of unusual testate amoeba assemblages, were from the peatlands in central Upper Michigan that experienced extremely low water-table depths in 2005. These assemblages were co-dominated by taxa that are generally characteristic of opposite sides of the moisture gradient. In particular, these samples contained abundant taxa characteristic of dry habitats, such as *Hyalosphenia subflava*, *Nebela militaris* and *Nebela tincta-parvula*, along with abundant taxa common to very wet habitats, including *Arcella discoides*, *Nebela griseola* and *Difflugia globulosa*. Although species with very wet and very dry optima occasionally occur in the same samples in modern environments, they are not usually co-dominant. Charman *et al.* (2007) recently described similar anomalous assemblages from a peatland in Germany, and attributed them to unusual characteristics of the peatland, and in particular the high bulk density of the peat at the site. Although I did not measure bulk density in the present study, qualitative observations do suggest higher bulk density of peat associated with these unusual samples. The large difference between measured water-table depths in 2004 and 2005 at these sites suggests that they may experience greater seasonal or interannual variability in water-table depth, leading to increased decomposition, higher bulk density and probably unusual assemblages of testate amoebae. Species with wet and dry optima may be active at different times of the year, and the collected samples integrate this seasonal variability. All 12 unusual assemblages also were from hollows or sites intermediate in topography, and these sites tend to experience greater fluctuations in surface-moisture throughout the growing season than hummocks. Although inclusion of the 12 anomalous samples in the dataset does not significantly change the NMDS ordination, and does not affect RMSEP of the transfer functions by more than 0.2 cm, I still eliminated them from further analysis because they may negatively impact the characterisation of water-table depth optima of a few taxa, particularly those that are otherwise only abundant in dry habitats. Clearly more research is needed on these unusual testate amoeba assemblages and their relationship to environmental conditions, as well as on the seasonal dynamics of testate amoeba populations, particularly at sites that experience a large range of moisture variability throughout the year.

The second axis of the NMDS solution represents much less variance than the first (15% versus 65%) and is not as straightforward to interpret in terms of an environmental gradient. Samples on the wet side of the gradient (the left of axis 1) show more variability along the second axis than samples on the dry side of the gradient (Fig. 2(a)), suggesting that wet habitats may be more variable in testate amoeba composition than dry habitats (Warner, 1987; Booth, 2002). This pattern may be related to increased variability in pH, conductivity and other environmental variables among wetter habitats.

Potential differences in the relative importance of factors controlling community composition on the two sides of the moisture gradient were investigated by performing separate NMDS analysis on samples from the two sides of axis 1 (i.e. 'wet' and 'dry' sides) (Fig. 2(a), (b)). Each analysis resulted in a three-dimensional NMDS solution (final stress equals 14.0 and 17.5, left and right respectively), and reveals that substrate moisture is the most important variable controlling assemblage composition within communities of both sides of the moisture gradient (Fig. 3). However, on the 'wet' side of the gradient, pH is a secondary control (Fig. 3(a); Table 4) whereas on the dry 'side' of the gradient, pH is much less important (Fig. 3(b); Table 4). Water-table depth and pH were also negatively correlated, and pH was much less variable in dry habitats, making interpretation complicated. However, previous studies have shown that in minerotrophic peatlands, water chemistry variables such as pH and conductivity may be more important than water-table depth in structuring testate amoeba communities (Booth, 2001; Opravilová and Hájek, 2006). However, these apparent differences may result from the length of the environmental gradients sampled, with longer pH gradients sampled in studies of minerotrophic peatlands and longer gradients of water-table depth sampled in studies of ombrotrophic peatlands.

## Improved modern analogues?

The present dataset provides improved characterisation of species–environment relationships from a wider region and



DOI: 10.1002/jqs

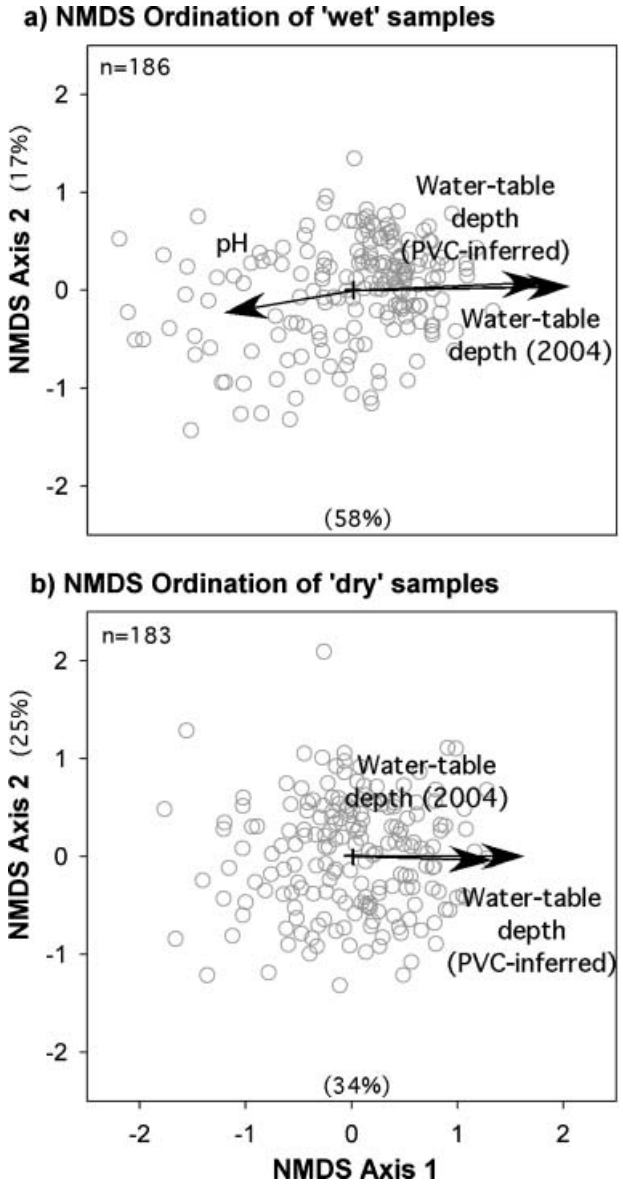


**Figure 3** First two axes of a three-dimensional NMDS ordination of testate amoeba samples from (a) the 'wet' side of the gradient, defined as samples on the left half of the NMDS ordination of all samples (as shown in Fig. 2(a)), and (b) the 'dry' side of the gradient, defined as samples on right half of the NMDS ordination of all samples (as shown in Fig. 2(a)). The direction and strength of environmental correlations ($r$) greater than 0.3 are shown, with longer vectors corresponding to higher correlations. Percentage variance represented by each axis is listed. The third axis of each solution is not shown, because it is not correlated with any measured environmental variable and represents a relatively small amount of variance (13% and 19% for (a) and (b), respectively). All environmental correlations are listed in Table 4

larger number of samples than previously studied in North America. However, one of the aims of this study was to obtain improved ecological information on taxa that have tended to be scarce in modern calibration datasets, particularly those that are often common in the fossil record, such as *Difflugia pulex*. The dataset does provide information on several taxa that were rare in previous calibration sets, such as *Nebela vitraea*, *N. wailesi* type and *Cryptodifflugia oviformis* type. However, only two samples contained *Difflugia pulex*, even though this taxon was encountered within several of the same peatlands during a previous study (Booth, 2002). It is unclear why the taxon was not found in the present study, but it may be related to ongoing hydrological change. Alternatively, the species may occur more frequently on lower portions of the *Sphagnum* stem, and may have been better sampled in the study of Booth (2002).

**Table 4** Correlations ($r^2$) between NMDS axis-1 scores and measured environmental variables for the separate NMDS analysis of 'wet' and 'dry' sides of the NMDS ordination shown in Fig. 2, after rotating the ordinations to maximise the correlation of axis 1 with each environmental variable. The corresponding ordination diagrams are shown in Fig. 3

| Variable | 'Wet' dataset | 'Dry' dataset |
|---|---|---|
| Conductivity (S) | 0.19 | 0.04 |
| Conductivity (W) | 0.08 | 0.05 |
| pH (S) | 0.33 | 0.07 |
| pH (W) | 0.10 | 0.01 |
| Depth to water table (measured 2004) | 0.50 | 0.47 |
| Depth to water table (measured 2005) | 0.18 | 0.13 |
| Depth to water table inferred from PVC-tape discolouration | 0.48 | 0.53 |

## Transfer function development and validation with independent dataset

The relatively low correlation between water-table depths measured in 2005 and the composition of testate amoeba assemblages strongly affects the performance of the transfer functions, as shown by comparisons of WA transfer functions developed using the different water-table depth datasets (Fig. 4). Transfer functions developed using the 2004 water-table depth measurements and the PVC-tape inferred water-table depth measurements perform substantially better than the model using 2005 measurements (Fig. 4). The poor performance of the transfer function developed using water-table depths measured in 2005 highlights the potential large effects of one-time measurements of water-table depth (Fig. 4). Because PVC-inferred water-table depths should correspond most closely to mean annual water-table depths (Booth *et al.*, 2005), and should be more comparable between samples collected at different times of year in different regions, I use this dataset to more fully develop and test the various transfer function models.

In addition to the more commonly used transfer function models, I explored the technique of NMDS prediction to infer water-table depths from testate amoeba assemblages. Although ordination methods often have been used in palaeoenvironmental reconstruction, applications typically apply the axis-1 scores from ordinations of fossil data as a semi-quantitative proxy for some variable that is presumably related to the dominant axis of variation. For example, detrended correspondence analysis (DCA) of plant macrofossil data has been used to generate semi-quantitative indices of past changes in peatland surface moisture (e.g. Barber *et al.*, 2000). NMDS prediction differs from this approach, because a calibration dataset is used to define the ordination space, and the fossil samples are passively positioned within this space without influencing the ordination (e.g. Fig. 5(a)). Therefore, the fossil samples are constrained by the calibration dataset, and the relationship between environmental variables and axis-1 scores of the calibration samples can be directly modelled (e.g. Fig. 5(b)), providing a means to transform the predicted axis-1 scores of fossil samples into quantitative estimates of the environmental variable.



DOI: 10.1002/jqs

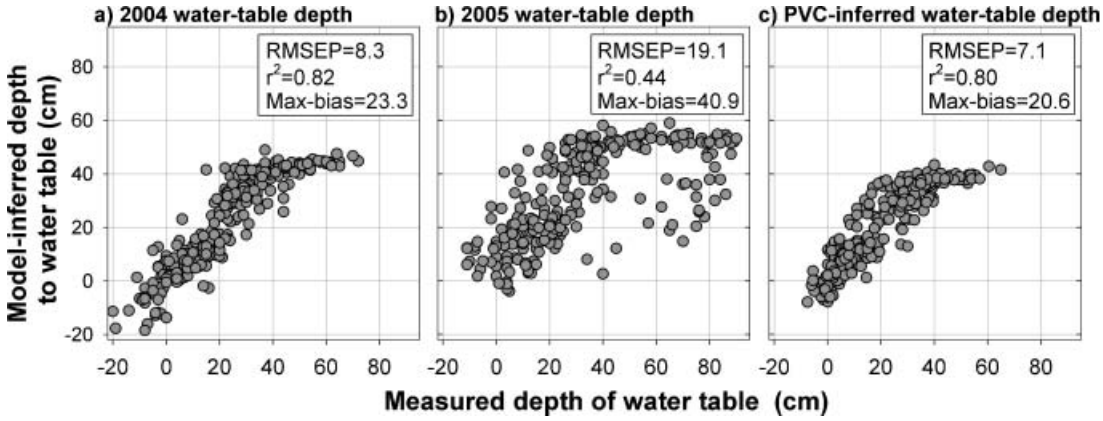


**Figure 4** Jackknifed (leave-one-out) validation and relevant statistics of transfer functions based on a weighted averaging model using the partial dataset ($n = 276$) (i.e. excluding Trout Lake peatlands) for (a) water-table depth data collected in summer 2004, (b) water-table depth data collected in summer 2005, and (c) water-table depth data inferred from PVC-tape discolouration

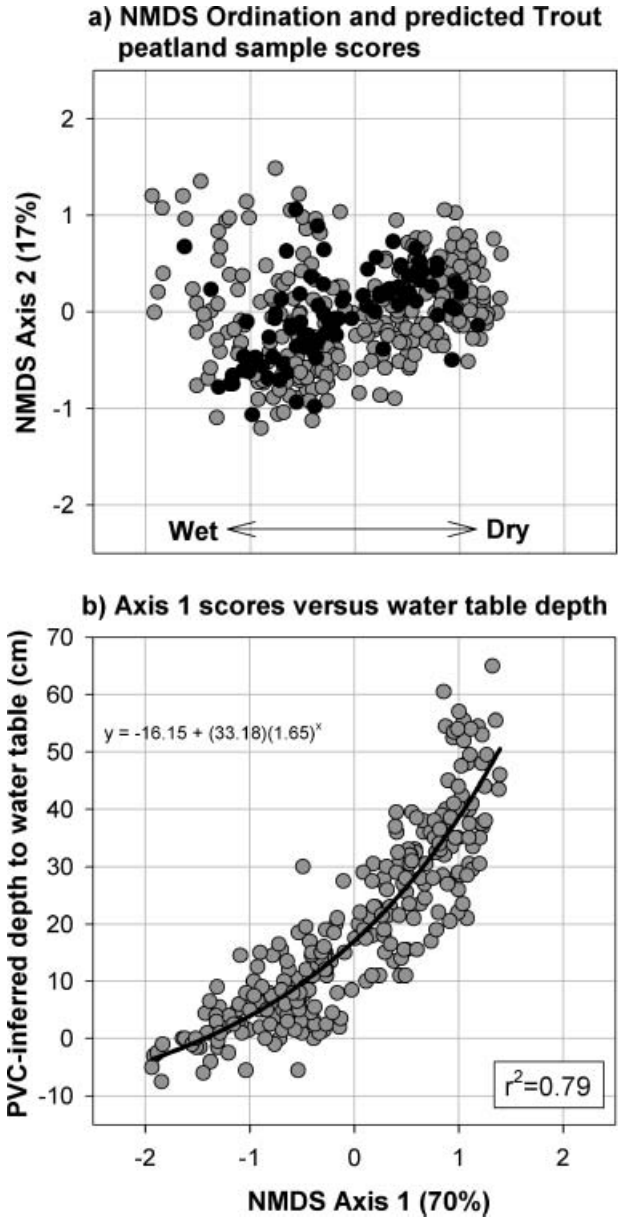


**Figure 5** (a) NMDS ordination of the partial dataset ($n = 276$) (i.e. excluding Trout Lake peatlands) with Trout Lake samples projected into the ordination using the technique of NMDS prediction. (b) Relationship between NMDS axis one scores for the modern calibration samples and the observed water-table depth (PVC-inferred), which can be used to infer the water-table depth of the samples from the Trout Lake peatlands from their projected axis-1 scores

A two-dimensional NMDS ordination of the calibration dataset, excluding the Trout Lake peatland samples ($n = 276$, final stress = 15.5), represented 87% of the variability in the data (Fig. 5(a)). This ordination is very similar to the NMDS ordination of the complete dataset (Fig. 2). The ordination was rotated to maximise the correlation of axis 1 with water-table depths (PVC inferred), and the relationship between axis 1 and water-table depth was modelled (Fig. 5(b)). Observed and predicted values were then compared (Fig. 6(i), (j)). Although it is not feasible to perform jackknifed validation of this method, apparent error statistics (e.g. RMSE, $r^2$) can be cautiously used to evaluate performance, although they are probably overly optimistic because the predicted data points are included in the model (Birks, 1998).

Transfer functions developed from the dataset, excluding the samples from the Trout Lake peatlands, performed similarly in cross-validation (Fig. 6). For the various models, RMSEP and RMSE ranged from 6.6 to 7.3 cm and $r^2$ values ranged from 0.80 to 0.83. Although the performance of all models is similar with respect to RMSEP and $r^2$, decreased performance occurs in all models on the dry side of the gradient, where the models all predict wetter conditions than observed (Fig. 6). This pattern is a common feature of weighted-average-based models in testate amoebae studies (e.g. Mitchell *et al.*, 1999; Booth, 2002; Lamentowicz and Mitchell, 2005; Payne *et al.*, 2006) and WA-based transfer functions derived from other organisms, because of the underestimation and overestimation of optima on the edges of the environmental gradient (Birks, 1998). Error plots and maximum bias estimates (Max-bias) indicate that this error is least with the WA-PLS model and most with the WA-Tol model (Fig. 6). The two models that do not have underlying species response models, WMAT and NMDS prediction, also underestimate the depth to water table on the dry side of the gradient, performing similarly to the WA and WA-Tol models (Fig. 6).

To obtain a more realistic validation of the transfer functions, I tested them on the samples from the Trout Lake peatlands. Because water-table depths were directly measured at these sites, as opposed to inferred from PVC-tape discolouration, this also provides a test of whether the transfer functions are accurately inferring mean annual water-table depth. The transfer functions shown in Fig. 6 were used to infer the water-table depth of the Trout Lake peatland samples using all five models. For the NMDS prediction method, the Trout Lake peatland samples were positioned within the NMDS ordination based on their similarity with calibration samples (Fig. 5(a)), and NMDS axis-1 scores were transformed to water-table depths using the modelled relationship (Fig. 5(b)). Cross-validation of the various models revealed somewhat higher



DOI: 10.1002/jqs

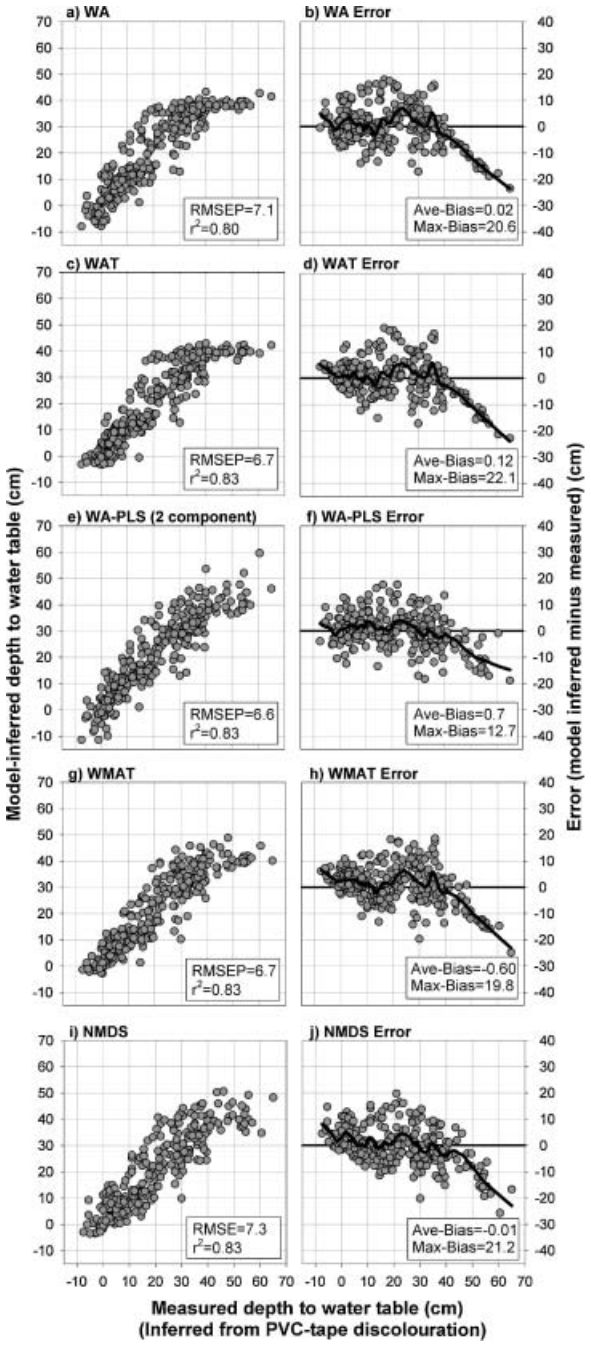


**Figure 6** Cross-validation of transfer functions, corresponding error plots, and relevant statistics using the partial dataset ($n = 276$) (i.e. excluding Trout Lake peatlands) and five different models, including weighted averaging (a, b), weighted averaging tolerance downweighted (c, d), weighted averaging partial least squares (e, f), weighted average modern analogue technique (g, h), and the NMDS prediction technique (i, j). Cross-validation of (a)–(h) were through jackknifing techniques, and prediction statistics are shown. Statistics for the NMDS prediction technique are apparent errors, and therefore are probably overly optimistic. A lowess smoothing of the error plots is shown with a black line

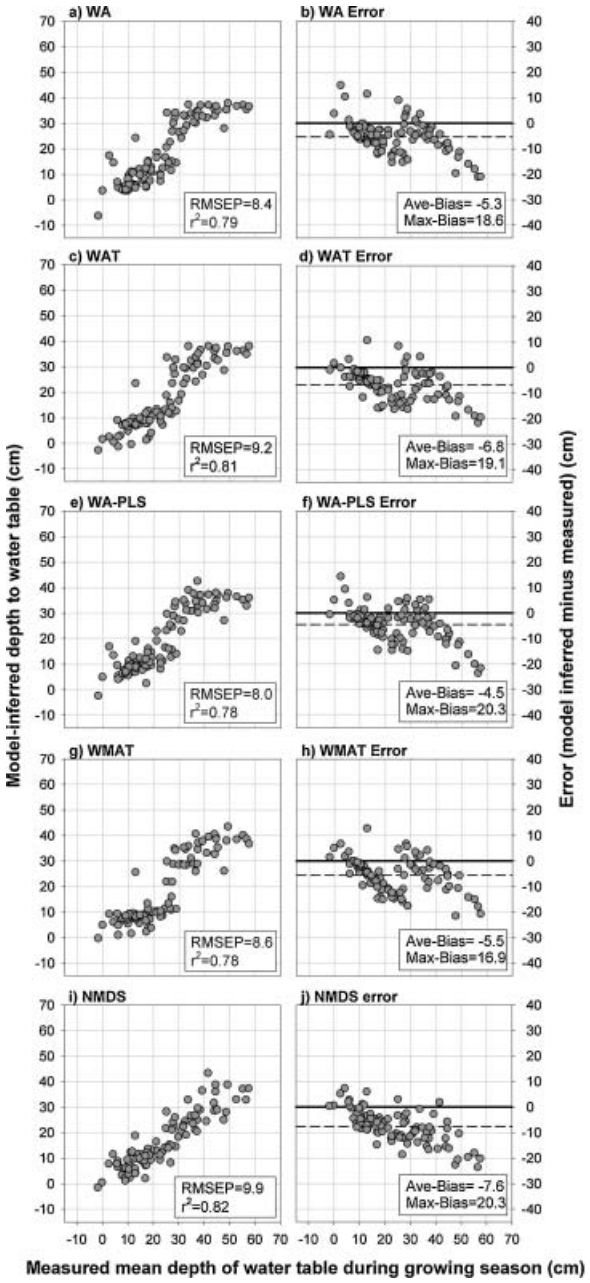


**Figure 7** Results of testing the transfer functions on the samples from the Trout Lake peatlands, where mean annual water-table depth was measured. Cross-validation of transfer functions, corresponding error plots, and relevant statistics are shown for five different models, including weighted averaging (a, b), weighted averaging tolerance downweighted (c, d), weighted averaging partial least squares (e, f), weighted average modern analogue technique (g, h), and the NMDS prediction technique (i, j)

RMSEP values than the jackknifing approach, ranging from 8.0 to 9.9 cm (Fig. 7). Values of $r^2$ were similar to those obtained via jackknifing approaches, ranging from 0.78 to 0.82. The performance of the transfer functions is again poorest on the dry end of the gradient, and in this case the WA-PLS model underestimates deep water tables as much as the other models (Fig. 7). However, the WA-PLS model still had the lowest RMSEP, predicting water table depth with a mean error of ±8.0 cm.

Testing the transfer functions with the samples from the Trout Lake peatlands also reveals that, on average, the models predict shallower water tables (i.e. wetter conditions) than the measured means during the growing season (Fig. 7). This average bias ranges from underestimation of 4.5 cm in the WA-PLS model to 7.6 cm in the NMDS prediction model (Fig. 7). The most likely cause of this bias is variability in the PVC-tape discolouration method, which although corresponded with mean water-table depth in validation studies in northern Wisconsin (Trout Lakes peatlands) (Booth *et al.*, 2005), may not always precisely record mean water-table depth. Some variability in the method may occur between regions, and among different peatland types. For example,



DOI: 10.1002/jqs

Bragazza (1996) found that PVC-tape discolouration corresponded to the horizon that experienced inundation 70% of the time, and Belyea (1999) found that the highest point of PVC-tape discolouration was correlated with the highest water levels, although she noted an average bias of several centimetres. Both of these studies indicate discolouration probably occurs somewhat above the mean position of the water table. This slight wet bias in PVC-tape-inferred estimates of mean water tables may explain the wet-bias in the transfer functions.

## Transfer function development for the complete eastern North America dataset

Transfer functions were developed from the complete eastern North American dataset ($n = 369$) using PVC-inferred estimates of water-table depth for all sites except the Trout Lake peatlands, where measured mean annual water-table depths were used. The use of measured water-table depths for the Trout Lake peatland sites should decrease the amount that predicted values are offset from mean water-table depths, but this cannot be quantified without another independent dataset. Cross-validation of the transfer functions based on the complete dataset resulted in RMSEP and RMSE ranging from 6.8 to 7.9 cm, and $r^2$ values ranging from 0.76 to 0.81 (Fig. 8). As with the transfer functions developed from the partial dataset, the WA-PLS model had the least amount of bias on the dry side of the gradient (Fig. 8).

Several recent testate amoeba calibration studies have demonstrated that removal of outliers can substantially improve the performance of transfer functions in cross-validation (Woodland *et al.*, 1998; Wilmhurst *et al.*, 2003; Charman *et al.*, 2007; Payne *et al.*, 2006). Typically samples with residuals higher than some cut-off value are eliminated from the analysis, and the transfer function is redeveloped without these samples. Recent studies have removed samples with residuals greater than 20% of the full range of the water-table depth gradient, with resulting reductions in RMSEP ranging from about 2 to 6 cm (Payne *et al.*, 2006; Charman *et al.*, 2007). By removing outliers with residuals greater than 20% of the full range (14.5 cm), the performance statistics of the transfer functions in this study were improved by about 1 cm, resulting in a mean error of ±5.9 cm and a maximum bias on the dry side of the gradient of 9.2 cm for the WA-PLS model (Table 5).

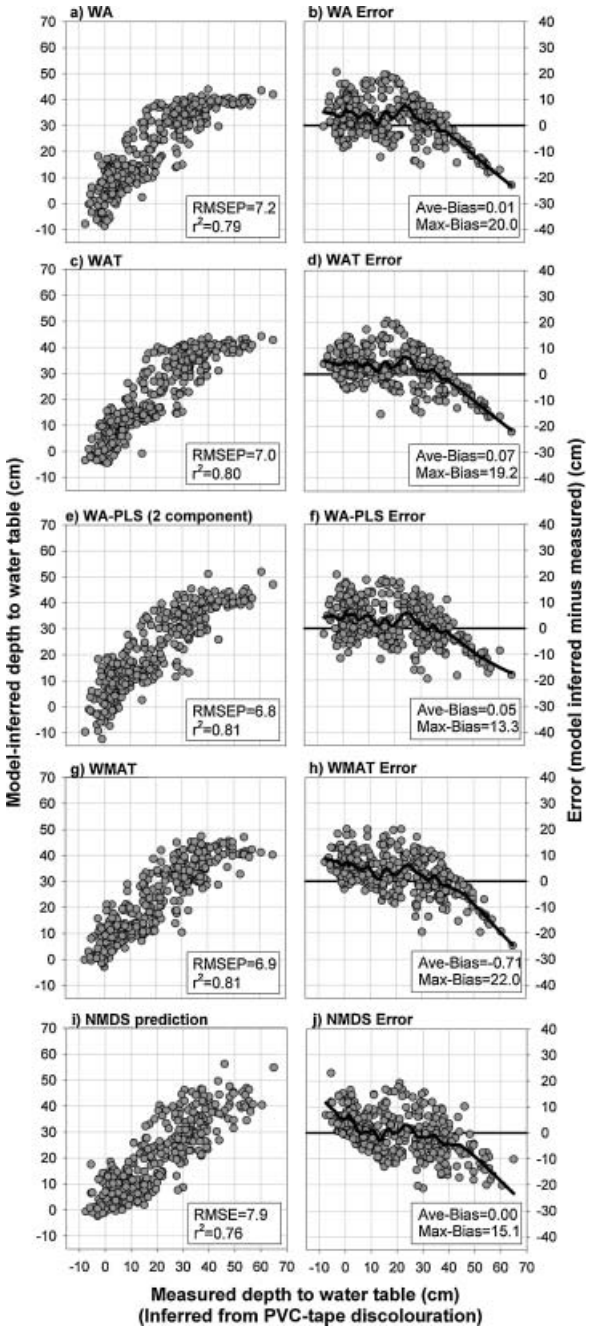


**Figure 8** Cross-validation of transfer functions using the entire eastern North America dataset and five different models, including weighted averaging (a, b), weighted averaging tolerance downweighted (c, d), weighted averaging partial least squares (e, f), weighted average modern analogue technique (g, h), and the NMDS prediction technique (i, j). Cross-validation of (a)–(h) were through jackknifing techniques, and prediction statistics are shown. Statistics for the NMDS prediction technique are apparent errors, and therefore are probably overly optimistic. A lowess smoothing of the error plots is shown with a black line

## Comparison of reconstructions on a fossil record

Water-table depth reconstructions at Minden Bog, an ombrotrophic peatland in Michigan, are similar using the different transfer function models (Fig. 9). Reconstructions also are similar to those produced by a regional calibration dataset, based only on samples from Michigan (Booth and Jackson, 2003). Although reconstructions based on the different models covary, differences in the absolute values of the reconstructions occur (Fig. 9). This pattern is particularly apparent in samples with deep water tables (i.e. dry conditions). WA-PLS, and to a lesser extent NMDS prediction, are able to predict deeper water tables than the other models, consistent with the results from cross-validation. The WMAT reconstruction seems unrealistic, with most reconstructed water tables either very wet or very dry, with few intermediate depths or gradual transitions (Fig. 9). Telford and Birks (2005) note that MAT models may have overly optimistic predictive statistics because of problems with spatial autocorrelation.

## Toward a calibration dataset for North America

Although one-time measurements of water-table depth make comparison between studies difficult, and clearly have the potential to introduce considerable error (Fig. 4), a



DOI: 10.1002/jqs

**Table 5** Performance of five different transfer function models for water-table depth using the complete eastern North America dataset ($n = 369$). Values after removal of samples with residuals larger than 20% of the gradient length (14.5) are shown in parentheses. Apparent error statistics are shown for NMDS prediction

| Model | $r^2$ | Average bias (cm) | Maximum bias (cm) | RMSEP (cm) |
|---|---|---|---|---|
| WA | 0.79 (0.83) | 0.02 (0.02) | 21.3 (16.0) | 7.4 (6.6) |
| WA-Tol | 0.82 (0.85) | 0.10 (0.10) | 20.4 (14.2) | 6.8 (6.1) |
| WA-PLS (two component) | 0.82 (0.85) | 0.07 (<0.00) | 12.7 (9.2) | 6.9 (5.9) |
| WMAT | 0.82 (0.85) | −0.41 (−0.60) | 22.1 (11.4) | 6.9 (5.9) |
| NMDS prediction | 0.76 (0.82) | <0.00 (<0.00) | 15.1 (10.0) | 7.9 (6.6) |

comprehensive calibration dataset for North American peatlands is desirable for widespread applicability. I attempted to combine the data from this study with several previous calibration studies in North America that have used similar methods and taxonomy, including datasets from Michigan and the Rocky Mountains (Booth and Zygmunt, 2005). Although the same research group performed all identifications, some taxonomic harmonisation, or lumping of similar taxa, was necessary because the level of taxonomic precision varied somewhat between the studies. The taxonomic groupings used in this analysis generally follow Booth and Zygmunt (2005), although a few taxa that were combined in that analysis are left separate in the present analysis (e.g. *Centropyxis arcelloides* type, *Phryganella acropodia* type). Although the combined North American dataset has reduced taxonomic precision for a few taxa (e.g. *Arcella catinus/artocrea* type, *A. vulgaris* type), it also contains species that were rare or absent in the present study, including *Difflugia pulex*. The Trout Lake samples were excluded from the initial analysis of this combined dataset so that they could be used to test the transfer functions, but the full dataset ($n = 650$) was used for jackknifed cross-validation.

A NMDS ordination of the combined North American (NA) dataset represents 79% of the variance in the data (final stress = 14.3) and reveals that even though water-table depths were based on both one-time measurements and PVC-discolouration in this combined dataset, a strong correlation between assemblage composition and water-table depth still occurs (Fig. 10). The ordination also reveals that testate amoeba assemblages from the Rocky Mountains are somewhat different in composition from those from eastern North America, a pattern consistent with a recent comparative analysis (Booth and Zygmunt, 2005). However, the strong correlations with water-table depth suggest that transfer functions can be developed from the combined dataset, although some error is expected because of the disparity in times of water-table depth measurements.

Transfer functions developed from the combined North American dataset (NA) perform surprisingly well, given that water-table depths were measured in different years in different regions (Table 6). WA-PLS and WMAT performed the best in cross-validation, although because of the previously mentioned problems with WMAT-based reconstructions and the like-

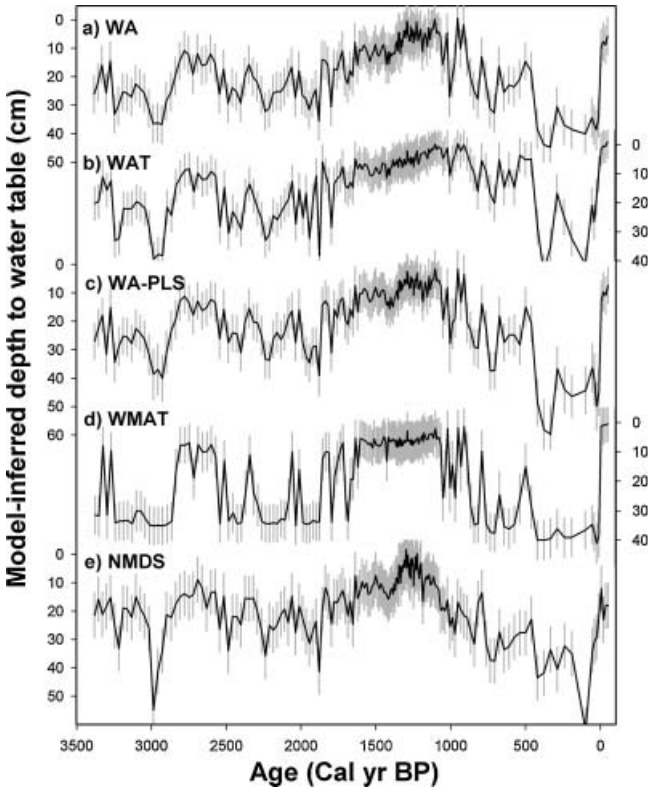


**Figure 9** Comparison of water-table depth reconstructions at Minden Bog, MI, based on five models, including (a) weighted averaging, (b) weighted averaging tolerance downweighted, (c) weighted averaging partial least squares, (d) weighted averaging modern analogue and (e) NMDS prediction. Errors bars show estimated prediction errors based on bootstrapping for (a)–(d). The error bars for (d) are standard errors

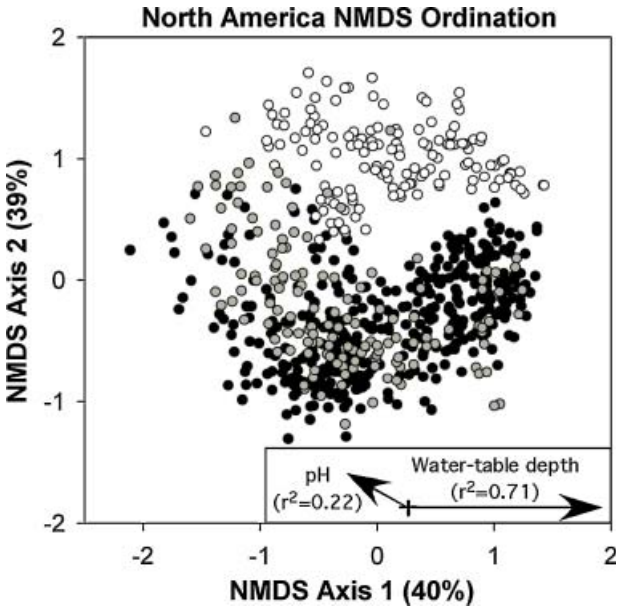


**Figure 10** (a) NMDS ordination of testate amoeba samples from this study (solid circles), a regional calibration dataset from Michigan (shaded circles) and a regional calibration dataset from the Rocky Mountains (open circles) ($n = 650$, final stress = 18.5). Percentage variance represented by each axis is shown. Inset shows direction and strength of environmental correlations, where the length of the vector is proportional to the strength of the correlation. Correlations with axis 1 are listed



DOI: 10.1002/jqs

**Table 6** Performance of five different transfer function models for water-table depth using the complete North America dataset, which includes samples from the present study as well as previous studies in Michigan and the Rocky Mountains ($n = 650$). Values after removal of samples with residuals larger than 20% of the gradient length (15.2 cm) are shown in parentheses. Apparent error statistics are shown for NMDS prediction

| Model | $r^2$ | Average bias (cm) | Maximum bias (cm) | RMSEP (cm) |
|---|---|---|---|---|
| WA | 0.74 (0.80) | 0.01 (0.01) | 21.2 (21.1) | 8.0 (6.9) |
| WA-Tol | 0.74 (0.81) | 0.05 (0.06) | 21.0 (20.4) | 7.9 (6.8) |
| WA-PLS (two component) | 0.76 (0.83) | 0.02 (0.02) | 16.2 (11.6) | 7.6 (6.2) |
| WMAT | 0.79 (0.84) | −0.50 (−0.51) | 19.2 (13.7) | 7.1 (6.0) |
| NMDS prediction | 0.72 (0.67) | −0.49 (<0.00) | 38.7 (16.3) | 8.3 (8.4) |

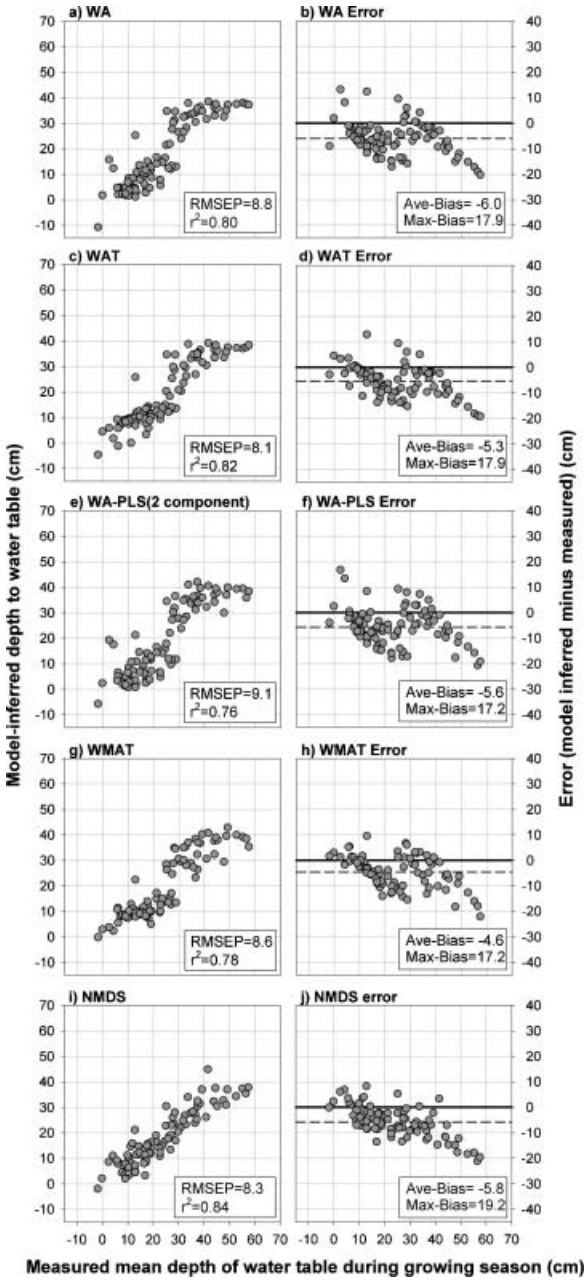


**Figure 11** Results of testing the transfer functions developed from the combined North American dataset on the samples from the Trout Lake peatlands, where mean annual water-table depth was measured. Cross-validation of transfer functions, corresponding error plots, and relevant statistics are shown for five different models, including weighted averaging (a, b), weighted averaging tolerance downweighted (c, d), weighted averaging partial least squares (e, f), weighted average modern analogue technique (g, h), and the NMDS prediction technique (i, j)

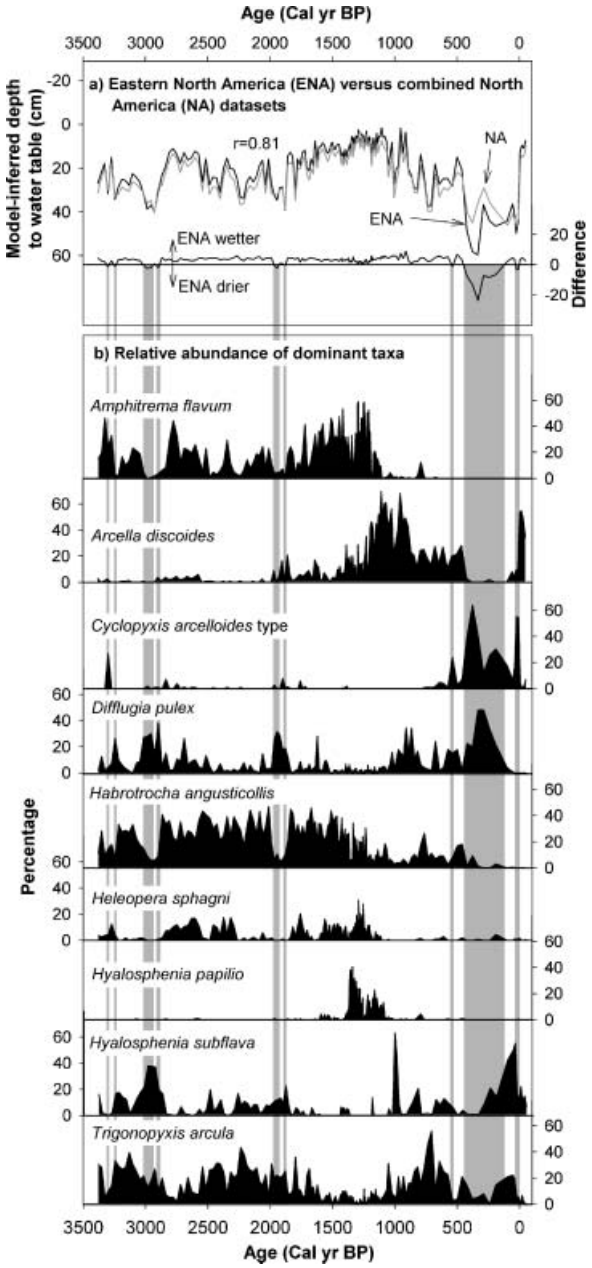


**Figure 12** (a) Comparison of reconstructions based on weighted averaging models from the dataset developed in this study (eastern North America, ENA) and the combined North American dataset (NA). (b) Dominant testate amoeba taxa (maximum abundance >30%) in the Minden Bog fossil record, plotted for comparison to the reconstructions based on the ENA and NA datasets. Grey bars denote times when the ENA dataset predicts drier conditions than the NA dataset



DOI: 10.1002/jqs

lihood of overly optimistic prediction statistics (Telford and Birks, 2005) the preferred model is WA-PLS. Water-table depth can be inferred with a mean error of 7.6 cm with this model, and removal of samples with residuals greater than 20% of the gradient length (15.2 cm) can decrease mean error by a little over a centimetre (Table 6). Testing the NA dataset on the samples from Trout Lake suggests slightly higher mean errors, and a wet-bias of about 4–6 centimetres is again present (Fig. 11). It is rather surprising that the NA dataset performs only slightly more poorly than the ENA dataset, given the error that one-time measurements of water-table depth can sometimes cause, and clearly would have caused if we had used the 2005 measured water tables for the ENA transfer functions (Fig. 4).

Comparison of fossil reconstructions at Minden Bog, based on the ENA and NA datasets, reveal that WA-PLS reconstructions using the two datasets are highly correlated ($r = 0.81$), although absolute values differ at times (Fig. 12). The eastern North America dataset tends to predict slightly wetter conditions than the NA dataset, probably because it is based on PVC-tape inferred water-table depths. However, the ENA dataset predicts drier conditions than the NA dataset at several discrete time intervals, and these appear to be associated with high abundance of *Difflugia pulex*, particularly when it is co-dominant with *Cyclopyxis arcelloides* type and/or *Hyalosphenia subflava*. *Difflugia pulex* is not present in the ENA dataset, and has been absent or rare in many calibration datasets from other regions (Woodland *et al.*, 1998). However, the taxon appears to be an indicator of moderately dry conditions (Charman *et al.*, 2000; Booth, 2002) and this has been recently confirmed in a European study (Charman *et al.*, 2007). *Cyclopyxis arcelloides* type and *Hyalosphenia subflava* have optimum abundance in dry conditions in both the ENA and NA datasets. In the Minden Bog reconstruction, lack of *D. pulex* in the ENA dataset places more emphasis on the other taxa, so when *D. pulex* is abundant and associated with dry taxa like *C. arcelloides* and *D. pulex*, it is likely that the ENA dataset overestimates depth to the water table.

## Conclusions

Testate amoebae can be used to reconstruct accurately depth to water table in *Sphagnum*-dominated peatlands of North America. The calibration dataset developed in this study from peatlands in eastern North America can be used to infer water-table depth with a mean error of about 6 to 8 cm. Combining this dataset with previous data from the Rocky Mountains and Michigan increases mean error only slightly and provides ecological information on more taxa, including taxa like *D. pulex* that are relatively common in some fossil records yet rare in many modern calibration datasets. Combined use of the two datasets, particularly on fossil records containing *D. pulex*, is recommended until better ecological data on taxa that are relatively uncommon in surface samples can be obtained. Water-table estimates based on these datasets are a good approximation of the mean height of the water table, although inferences are typically about 4–6 cm above the mean depth to water table (i.e. slightly wetter than the mean condition). The incorporation of PVC-tape-based estimates of water-table depth is an improvement over previous North American calibration datasets, because it provides comparable, inexpensive, estimates of water-table depth, closely approximating mean water-table depth. Use of the method should facilitate the development of comparable calibration datasets from different regions, allowing improved understanding of testate amoeba ecology and biogeography, and leading to wider applicability of these sensitive environmental indicators.

*Acknowledgements* Funding for this project came from collaborative research grants from the National Science Foundation's Ecology and Earth System History Programs and a cooperative agreement with United States Geological Survey—Great Lakes Science Center. I thank the Nature Conservancy, The Adirondack Park, The University of Maine, The Michigan Department of Natural Resources, and various other landowners for permission to sample sites. Jennifer Zygmunt performed the analysis of the samples from the Rocky Mountains. Amy Beaudet provided extensive field assistance, and Nick Zachar and Andy Fraas provided laboratory assistance. The project was improved by discussions with Sara Hotchkiss and Steve Jackson. The comments of Steve Jackson improved an early version of the manuscript. Reviews by Dan Charman and Edward Mitchell also improved the manuscript.

## References

Barber KE, Maddy D, Rose N, Stevenson AC, Stoneman R, Thompson R. 2000. Replicated proxy-climate signals over the last 2000 yr from two distant UK peat bogs: new evidence for regional paleoclimate teleconnections. *Quaternary Science Reviews* **19**: 481–487.

Belyea LR. 1999. A novel indicator of reducing conditions and water-table depth in mires. *Functional Ecology* **13**: 431–434.

Birks HJB. 1995. Quantitative paleoenvironmental reconstructions. In *Statistical Modelling of Quaternary Science Data. Technical Guide 5*, Maddy D, Brew JS (eds). Quaternary Research Association: Cambridge; 161–254.

Birks HJB. 1998. Numerical tools in palaeolimnology—Progress, potentialities, and problems. *Journal of Paleolimnology* **20**: 307–332.

Bobrov AA, Charman DJ, Warner BG. 1999. Ecology of testate amoebae (Protozoa: Rhizopoda) on peatlands in western Russia with special attention to niche separation in closely related taxa. *Protist* **150**: 125–136.

Booth RK. 2001. Ecology of testate amoebae in two Lake Superior coastal wetlands: implications for paleoecology and environmental monitoring. *Wetlands* **21**: 564–576.

Booth RK. 2002. Testate amoebae as paleoindicators of surface-moisture changes on Michigan peatlands: modern ecology and hydrological calibration. *Journal of Paleolimnology* **28**: 329–348.

Booth RK, Jackson ST. 2003. A high-resolution record of late Holocene moisture variability from a Michigan raised bog. *The Holocene* **13**: 865–878.

Booth RK, Zygmunt JR. 2005. Biogeography and comparative ecology of testate amoebae inhabiting Sphagnum-dominated peatlands in the Great Lakes and Rocky Mountain regions of North America. *Diversity and Distributions* **11**: 577–590.

Booth RK, Jackson ST, Gray CED. 2004. Paleoecology and high-resolution paleohydrology of a kettle peatland in Upper Michigan. *Quaternary Research* **61**: 1–13.

Booth RK, Hotchkiss SC, Wilcox DA. 2005. Discolouration of polyvinyl chloride (PVC) tape as a proxy for water-table depth in peatlands: validation and assessment of seasonal variability. *Functional Ecology* **19**: 1040–1047.

Booth RK, Notaro M, Jackson ST, Kutzbach JE. 2006. Widespread drought episodes in the western Great Lakes region during the past 2000 years: geographic extent and potential mechanisms. *Earth and Planetary Science Letters* **242**: 415–427.

Bragazza L. 1996. Delimitation of the aerobic peat layer in a Sphagnum mire on the southern Alps. *Oecologia Montana* **5**: 41–46.

Buttler A, Warner BG, Grosvernier P, Matthey Y. 1996. Vertical patterns of testate amoebae (Protozoa: Rhizopoda) and peat-forming vegetation on cutover bogs in the Jura, Switzerland. *New Phytologist* **134**: 371–382.

Charman DJ. 1997. Modelling hydrological relationships of testate amoebae (Protozoa: Rhizopoda) on New Zealand peatlands. *Journal of the Royal Society of New Zealand* **27**: 465–483.

Charman DJ. 2001. Biostratigraphic and palaeoenvironmental applications of testate amoebae. *Quaternary Science Reviews* **20**: 1753–1764.

Charman DJ, Warner BG. 1992. Relationship between testate amoebae (Protozoa: Rhizopoda) and microenvironmental parameters on a forested peatland in northeastern Ontario. *Canadian Journal of Zoology* **70**: 2427–2482.

Charman DJ, Warner BG. 1997. The ecology of testate amoebae (Protozoa: Rhizopoda) in oceanic peatlands in Newfoundland, Canada: modelling hydrological relationships for paleoenvironmental reconstruction. *Ecoscience* **4**: 555–562.

Charman DJ, Hendon D, Woodland WA. 2000. *The identification of testate amoebae (Protozoa: Rhizopoda) in peats*. Technical Guide No. 9, Quaternary Research Association, London; 147.

Charman DJ, Blundell A. and ACCROTELM members. 2007. A new European testate amoebae transfer function for paleohydrological reconstruction on ombrotrophic peatlands. *Journal of Quaternary Science* **22***:* 209–221.

Clarke , KR. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**: 117–143.

Davis SR, Wilkinson DM. 2004. The conservation management value of testate amoebae as 'restoration' indicators: speculations based on two damaged raised mires in northwest England. *The Holocene* **14**: 135–143.

Hendon D, Charman DJ. 1997. The preparation of testate amoebae (Protozoa: Rhizopoda) samples from peat. *The Holocene* **7**: 199–205.

Juggins S. 2003. In *C2 User guide. Software for ecological and palaeoecological data analysis and visualization*. University of Newcastle, Newcastle upon Tyne, UK; 69.

Kratz TK, Medland VL. 1989. Relationship of landscape position and groundwater input in northern Wisconsin kettle-hole peatlands. In *Freshwater Wetlands and Wildlife*, Sharitz RR, Gibbons JE (eds). DOE Symposium Series No. 61, USDOE Office of Scientific and Technical Information: Oak Ridge, TN; 1141–1151.

Kruskal JB. 1964. Nonmetric multidimensional scaling: a numerical method. *Psychometrika* **29**: 115–129.

Lamentowicz M, Mitchell EAD. 2005. The ecology of testate amoebae (Protists) in Sphagnum in north-western Poland in relation to peatland ecology. *Microbial Ecology* **50**: 48–63.

McCune B, Grace JB. 2002. Analysis of ecological communities. MjM Software, Gleneden Beach, OR; 300.

McCune B, Mefford MJ. 1999. PC-ORD. Multivariate analyses of ecological data. Version 4.34. MjM Software, Gleneden Beach, OR.

McMullen JA, Barber KE, Johnson B. 2004. A paleoecological perspective of vegetation succession on raised bog microforms. *Ecological Monographs* **74**: 45–77.

Mitchell EAD, Gilbert D. 2004. Vertical micro-distribution and response to nitrogen deposition of testate amoebae in Sphagnum. *Journal of Eukaryote Microbiology* **51**: 485–495.

Mitchell EAD, Buttler A, Warner BG, Gobat JM. 1999. Ecology of testate amoebae (Protozoa: Rhizopoda) in Sphagnum peatlands in the Jura mountains, Switzerland and France. *Ecoscience* **6**: 565–576.

Navrátilová J, Hájek M. 2005. Recording relative water table depth using PVC tape discolouration: Advantages and constraints in fens. *Applied Vegetation Science* **8**: 21–26.

Ogden CG, Hedley RH. 1980. *An Atlas of Freshwater Testate Amoebae*. British Museum of Natural History and Oxford University Press: London and Oxford, UK; 222 pp.

Opravilová V, Hájek M. 2006. The variation of testacean assemblages (Rhizopoda) along the complete base-richness gradient in fens: a case study from the western Carpathians. *Acta Protozoologica* **45**: 191–204.

Payne RJ, Kishaba K, Blackford JJ, Mitchell EAD. 2006. Ecology of testate amoebae (Protista) in south-central Alaska peatlands: building transfer-function models for paleoenvironmental studies. *The Holocene* **16**: 403–414.

Schnitchen C, Charman DJ, Magyari E, Braun M, Grigorszky I, Tóthmérész B, Molnár M, Szántó Z. 2006. Reconstructing hydrological variability from testate amoebae analysis in Carpathian peatlands. *Journal of Paleolimnology* **36**: 1–17.

Telford RJ, Birks HJB. 2005. The secret assumption of transfer functions: problems with spatial autocorrelation in evaluating model performance. *Quaternary Science Reviews* **24**: 2173–2179.

Warner BG. 1987. Abundance and diversity of testate amoebae (Rhizopoda, Testacea) in Sphagnum peatlands in southwestern Ontario, Canada. *Archiv für Protistenkunde* **133**: 173–189.

Warner BG, Charman DJ. 1994. Holocene changes on a peatland in northwestern Ontario interpreted from testate amoebae (Protozoa) analysis. *Boreas* **23**: 271–279.

Warner BG, Chengalath R. 1988. Holocene fossil *Habrotrocha angusticollis* (Bdelloidea: Rotifera) in North America. *Journal of Paleolimnology* **1**: 141–147.

Wilmhurst JM, McGlone MS, Charman DJ. 2002. Holocene vegetation and climatic change in southern New Zealand: linkages between forest composition and quantitative surface moisture reconstructions from an ombrogeneous bog. *Journal of Quaternary Science* **17**: 653–666.

Wilmhurst JM, Wiser SK, Charman DJ. 2003. Reconstructing Holocene water tables in New Zealand using testate amoebae: differential preservation of tests and implications for the use of transfer functions. *The Holocene* **13**: 61–72.

Woodland WA, Charman DJ, Sims PC. 1998. Quantitative estimates of water tables and soil moisture in Holocene peatlands from testate amoebae. *The Holocene* **8**: 261–273.




DOI: 10.1002/jqs