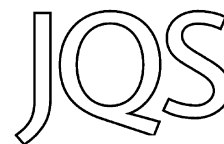


# Testate amoebae as a proxy for reconstructing Holocene water table dynamics in southern Patagonian peat bogs



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**ABSTRACT:** Testate amoebae are abundant and diverse in *Sphagnum* peat bogs and have been used extensively as indicators of past water table depths. Although these unicellular protists are widely dispersed with globally similar hydrological preferences, regional variations in communities demand region-specific transfer functions. Here we present the first transfer function for southern Patagonian bogs, based on 154 surface samples obtained from transects in five bogs sampled in 2012 and 2013. Significant variance was explained by pH, electrical conductivity and, in particular, water table depth. Transfer functions for water table were constructed using weighted averaging and evaluated by cross-validation and independent test sets. The optimal transfer function has predictive ability, but relatively high prediction errors given the wide range in sampled water tables. The use of independent test sets, as well as cross-validation, allows a more rigorous assessment of model performance than most previous studies. For a subset of locations we compare surface and subsurface samples to demonstrate significant differences in community composition, possibly due to vertical zonation. Our results provide the first quantification of hydrological optima and tolerances for several rare species, which may include Southern Hemisphere endemics and pave the way for palaeohydrological reconstructions in southern Patagonian bogs.

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**KEYWORDS:** peat; testate amoeba; Tierra del Fuego; transfer function; water table.

## Introduction

Peatland palaeoecology has been widely used to reconstruct environmental and climate change at centennial to millennial timescales (Barber, 1981; Barber *et al.*, 1994; Mauquoy *et al.*, 2002; Yu *et al.*, 2003; De Vleeschouwer *et al.*, 2009). In ombrotrophic bog ecosystems, water table fluctuations, vegetation assemblages and microfaunal dynamics are closely linked to changes in precipitation and temperature regime (Aaby, 1976; Schouten *et al.*, 1992; Charman *et al.*, 2004; Mauquoy and Yeloff, 2007). Sub-fossil shells ('tests') of the protist group testate amoebae are a widely used proxy in studies of palaeoecohydrological conditions in peatlands, often combined with analysis of plant macrofossils, humification and/or stable isotopes (e.g. Charman *et al.*, 1999; De Vleeschouwer *et al.*, 2009; Loisel *et al.*, 2009; Moschen *et al.*, 2009; Daley *et al.*, 2010). The community composition of these organisms in bogs is directly linked to the wetness of the bog surface (Warner and Charman, 1994; Mitchell *et al.*, 2013). As testate amoeba tests are also frequently well preserved in peat, identification of past assemblages is relatively straightforward. Testate amoebae are characterized by high reproduction rates (in the order of 10–27 generations per annum, Charman, 2001) and can therefore register rapid responses to variations in seasonal mire surface wetness, although the final resolution of the temporal signal reflects the accumulation rate of the peat.

For over two decades, testate amoeba transfer functions, which rely on the concept of uniformitarianism, have been

used to quantify the depth to the water table from palaeoecological assemblages (Warner and Charman, 1994; Charman *et al.*, 2007; Payne and Mitchell, 2007; Booth, 2008; Swindles *et al.*, 2009; Amesbury *et al.*, 2013; Lamarre *et al.*, 2013). In these studies, a modern amoeba training set is sampled from surface peat with corresponding measurements of environmental variables. Once amoeba assemblages have been determined, a series of ordinations is usually performed to test environmental controls. In most studies, water table depth (WTD) appears to be the variable accounting for the major part of the variations in assemblages; therefore, models are created to determine each taxon's optimum WTD along with its tolerance to this variable (Warner and Charman, 1994; Lamarre *et al.*, 2013). The performance of the water table reconstructions is generally verified by cross-validation or, preferentially, an independent test set (Telford and Birks, 2005; Payne *et al.*, 2012).

Although most testate amoeba species are cosmopolitan (i.e. not restricted to specific regions), there are regional variations in community composition and some taxa are limited to certain regions (Heger *et al.*, 2011; Turner *et al.*, 2013). The applicability of each transfer function is therefore spatially limited. While several transfer functions have been developed covering many European and North American peatland regions (Payne *et al.*, 2006; Charman *et al.*, 2007; Booth, 2008; Lamentowicz *et al.*, 2008; Payne *et al.*, 2008), there are few datasets for other areas, including all the Southern Hemisphere, with the exception of New Zealand (Charman, 1997). Because of the presence of testate amoeba species that may be exclusive to the Southern Hemisphere or South America, the creation of a testate

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amoeba transfer function for this region is an essential prerequisite for WTD reconstructions. For example, *Alocodera cockayni*, *Certesella certesi*, *Certesella martiali* and *Trigonopyxis microstoma* may be limited to the global areas that belonged to Gondwanaland (Foissner, 2009). The apparent absence of these species from regions that once constituted Laurasia may be explained by a combination of zonal wind patterns and regions without suitable habitats (Smith and Wilkinson, 2007; Smith *et al.*, 2008).

In this study, we present the first testate amoeba transfer function for southern South America. The development of this transfer function is a first step towards the reconstruction of a series of late Holocene peatland water table records across southern Patagonia (including Tierra del Fuego; 53–55°S) as part of the PATAGON research project. Despite an increased level of research undertaken in Tierra del Fuego over the last decade (Pendall *et al.*, 2001; Mauquoy *et al.*, 2004; Borromei *et al.*, 2010; Loisel and Yu, 2013), knowledge on Patagonian peatland palaeoecohydrology remains limited. Nevertheless, these peat bogs may hold important records of past environmental change, as they are located at the latitude of the southern Westerly wind belt. The latitudinal position and intensity of the southern Westerlies are linked to regional precipitation patterns (Tonello *et al.*, 2009; Lamy *et al.*, 2010; Garreaud *et al.*, 2013). Westerly belt dynamics may therefore cause substantial fluctuations in decadal-scale peatland water table dynamics in southern Patagonia. At the Holocene timescale, hypotheses for the linkages between the Westerly circulation dynamics and precipitation patterns across southern Patagonia have been postulated by Daley *et al.* (2012).

The aim of this study is to (i) examine the composition and biogeography of testate amoebae in southern Patagonia; (ii) quantify the relationship between amoeba assemblages and key environmental variables; (iii) construct and test a transfer function model to infer peat bog WTDs from palaeoassemblages and (iv) assess the ecology of taxa that are abundant in fossil samples but infrequent in training sets.

## Study region

The raised bogs included in the study are located in Chile and Argentina along a transect spanning 53–55°S and 67–72°W (Table 1, Fig. 1). The mean annual temperature in Punta Arenas (1990–2009), located at the western limit of the transect, is 6.1 °C (December–February: 10.3 °C; June–August: 1.7 °C). Ushuaia, near the eastern limit of the transect, has a mean annual temperature (1981–2002) of 6.2 °C (DJF: 9.6 °C; JJA: 2.4 °C). Annual precipitation is 433 mm (DJF: 114 mm; JJA: 103 mm) in Punta Arenas and 492 mm in Ushuaia (DJF: 143 mm; JJA: 119 mm) (IAEA/WMO, 2013). All sites are *Sphagnum magellanicum*-dominated ombrotrophic bogs and although some are affected by peat mining, samples were only taken from intact sections beyond the zone of water

table modification. Patagonian peatlands record low levels of nitrogen deposition (below 0.1–0.2 g N m<sup>-2</sup> a<sup>-1</sup>; Fritz *et al.*, 2012) and have preserved their pristine character with little human influence (Kleinebecker *et al.*, 2008). The peat bog vegetation of this region is characterized by a very low diversity of *Sphagnum* species with dominance by *Sphagnum magellanicum* from low lawns up to the highest hummocks and extreme microtopography as reflected by the measured WTD, which exceed 100 cm in Tierra Australis (TiA) bog. All sites have sections dominated by wet hollows, where *Sphagnum falcatulum* and *Sphagnum fimbriatum* occur in pools.

## Methods

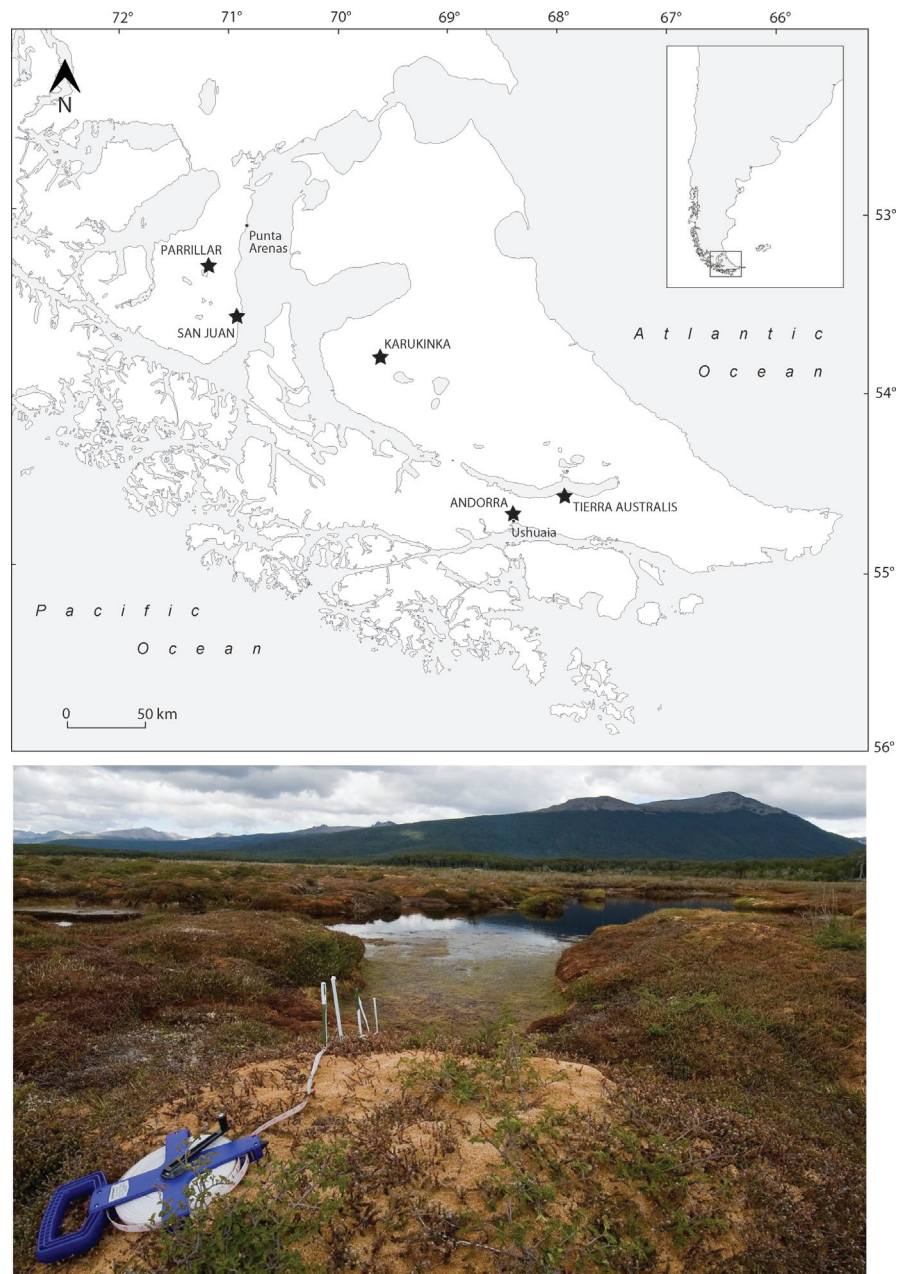
### General approach

With the more extensive use of transfer functions over the last decade, attention has increasingly focused on the true performance of these models. The performance of a transfer function may be highly dependent on the number of samples in the training set, the spatial distribution of these samples and their distribution along the environmental gradients included in the study. Many peat bogs are characterized by a surface microtopography of hummocks, hollows, lawns and pools, representing a within-site water table range that is often as high as the variability present between sites in the same region. Because of this high internal variability in environmental variables and the spatial distribution of bogs over the study area, testate amoeba training sets are generally sampled from a relatively low number of peat bogs, but with a high number of samples per site. Recent studies have shown that the clustered nature of resulting training sets may cause transfer function model performance to be too optimistic, as shown by standard cross-validation methods (Payne *et al.*, 2012). To correctly assess the uncertainty in the results, it is desirable to position modern samples evenly along the water table gradient (Telford and Birks, 2011). In this study, we included a variety of recently developed tests by cross-validation and using independent test sets, to evaluate the performance of transfer function models more robustly.

Another recurrent issue in studies of testate amoeba palaeoecology is the poor understanding of the ecology of some taxa that are present in fossil samples but less abundant or absent in surface samples. For example, *Diffflugia pulex* is frequently found in fossil samples constituting >60% of the total assemblage (e.g. Hendon *et al.*, 2001; Schoning *et al.*, 2005; van Bellen *et al.*, 2011; Lamarre *et al.*, 2012; Roland *et al.*, 2014), yet many studies lack these taxa because of their infrequent presence in the training set (Payne *et al.*, 2006; Booth, 2008; Bobrov *et al.*, 2013). Absence or rarity of these taxa in training sets is likely to cause inaccurate reconstructions where these taxa are abundant in fossil samples. This discrepancy may be explained by a preference

**Table 1.** Surface sample dataset: sites and characteristics.

Site	Location		2012 WTDs (cm)		2013 WTDs (cm)		2012 + 2013 WTDs (cm)
	Latitude (°S)	Longitude (°W)	Mean ± SD (n)	Range	Mean ± SD (n)	Range	Mean ± SD (n)
PAR	53.379	71.266	25 ± 24 (24)	0–81	37 ± 22 (10)	0–63	32 ± 21 (34)
SJB	53.651	70.966	–	–	44 ± 22 (35)	4–88	–
KAR	53.860	69.576	41 ± 27 (25)	0–86	39 ± 26 (10)	0–68	40 ± 26 (35)
AND	54.754	68.334	32 ± 24 (25)	0–92	–	–	–
TiA	54.616	67.771	43 ± 30 (25)	3–104	–	–	–
All sites	–	–	38 ± 26 (99)	0–104	40 ± 24 (55)	0–88	38 ± 25 (154)



**Figure 1.** Location of the studied peat bogs (black stars) sampled across southern Patagonia (above) and photograph showing an extreme microtopographical gradient and a testate amoeba sampling transect in Tierra Australis bog (below). This figure is available in colour online at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).

of such taxa for deeper levels than usually sampled, or a loss of suitable habitat in current peatlands, for instance due to pervasive air pollution. Previous research has shown that some species can live at considerably greater depths than the top 5 cm of the peat profile, which is generally sampled for training sets. Heal (1962) showed that numbers of live *Cryptodiffugia oviformis* increased downcore and were still increasing at 12 cm depth. Many studies have shown vertical zonation in testate amoebae structured by gradients of key variables, including moisture, light and availability of materials for xenosome test construction (Heal, 1962; Booth, 2002; Mitchell and Gilbert, 2004).

### Field and laboratory methods

Surface samples were collected during two field campaigns in the austral summers of January 2012 and 2013 (Table 1). In 2012, the Chilean bogs Parrillar (PAR) and Karukinka (KAR) were sampled as well as the Argentinian sites of Andorra (AND) and Tierra Australis (TiA). Additional samples

were taken from PAR, KAR and San Juan Bog (SJB) in Chile in 2013. No additional material was collected from AND or TiA in 2013. To ensure the entire water table gradient was covered, five transects were used in each peat bog from pool (submerged *Sphagnum*) to hummock with five samples taken at equal intervals along each transect. *Sphagnum* capitula were removed, retaining the upper sections of the stems (1–5 cm depth, cf. Booth *et al.*, 2010) before storage in airtight plastic bags. For the 2012 samples, pH and electrical conductivity (EC) were measured in the field adjacent to each sample location but only for the locations with standing water; the pH and EC of the remaining samples were measured in the laboratory after soaking the samples in distilled water (Stanek, 1973). All 2013 samples' pH and EC were measured in the field from the water table. All measurements were performed using a Hanna 98129 pH/EC/TDS meter. WTD was measured relative to the peat bog surface with a metal tape. Where the water table was below the peat surface small pits were excavated and the water table was left to stabilize for 30–60 min before measurements were made.

The deepest water tables were measured after removal of the peat using a 5-cm-diameter Russian peat sampler. High, positive values indicate deep water table levels, whereas water table levels at or above the surface are assigned a value of 0 cm. Surface samples of 5–10 cm<sup>3</sup> were sampled next to each measurement point; these were weighed and dried at 105 °C to calculate moisture contents, quantified as percentages.

To investigate the differences between surface and subsurface amoeba assemblages, possibly representing a mixture of various communities over time, we analysed an additional 28 subsurface samples from 5 to 15 cm depth. Subsurface samples were taken in 2013 from PAR, SJB and KAR concurrently with surface samples. Live individuals were not differentiated from empty tests so these samples represent both live individuals of deeper-living species and the communities that lived on the surface in the recent past.

### Quantification of assemblages

Tests were extracted from the peat samples following the methods of Hendon and Charman (1997) and Booth *et al.* (2010), using 355- and 15- $\mu$ m sieves and distilled water. To identify taxa, the key developed by Charman *et al.* (2000) was primarily used, but adaptations by Booth (2008) were followed as well. Taxa possibly limited to the Southern Hemisphere, including taxa of *Certesella*, *Alocodera*, *Apo-dera* and *Schwabia*, were identified using photographs in Vucetich (1978), Smith *et al.* (2008) and Jung (1942) (Fig. 2). Tests were identified and counted using a transmitted light microscope at  $\times 400$  magnification with a count total of at least 150 specimens, which produced an optimal training set, including appropriate representation of rare taxa (Payne and Mitchell, 2008). Taxa were expressed as a percentage of the total count. In many published transfer functions, rare taxa have been excluded to improve model performance (Booth, 2008; Lamentowicz *et al.*, 2008; Amesbury *et al.*, 2013). We excluded taxa found in fewer than five samples, irrespective of their relative abundance within the assemblages. Among taxa excluded were *Centropyxis cassis* type, *Euglypha rotunda* type and *Hyalosphenia subflava*. Some taxa were grouped, including the genera *Corythion* and *Trinema*, although *Trinema lineare* was identified separately by its smaller test (Charman *et al.*, 2000). *Euglypha strigosa* type and *Euglypha compressa* type were grouped as *E. strigosa* type, and *Nebela collaris* and *Nebela bohémica* as *N. collaris-bohémica* type. One surface sample, from a hummock in PAR bog sampled in 2012, had to be omitted from the dataset because poor preservation prevented the identification of 150 specimens.

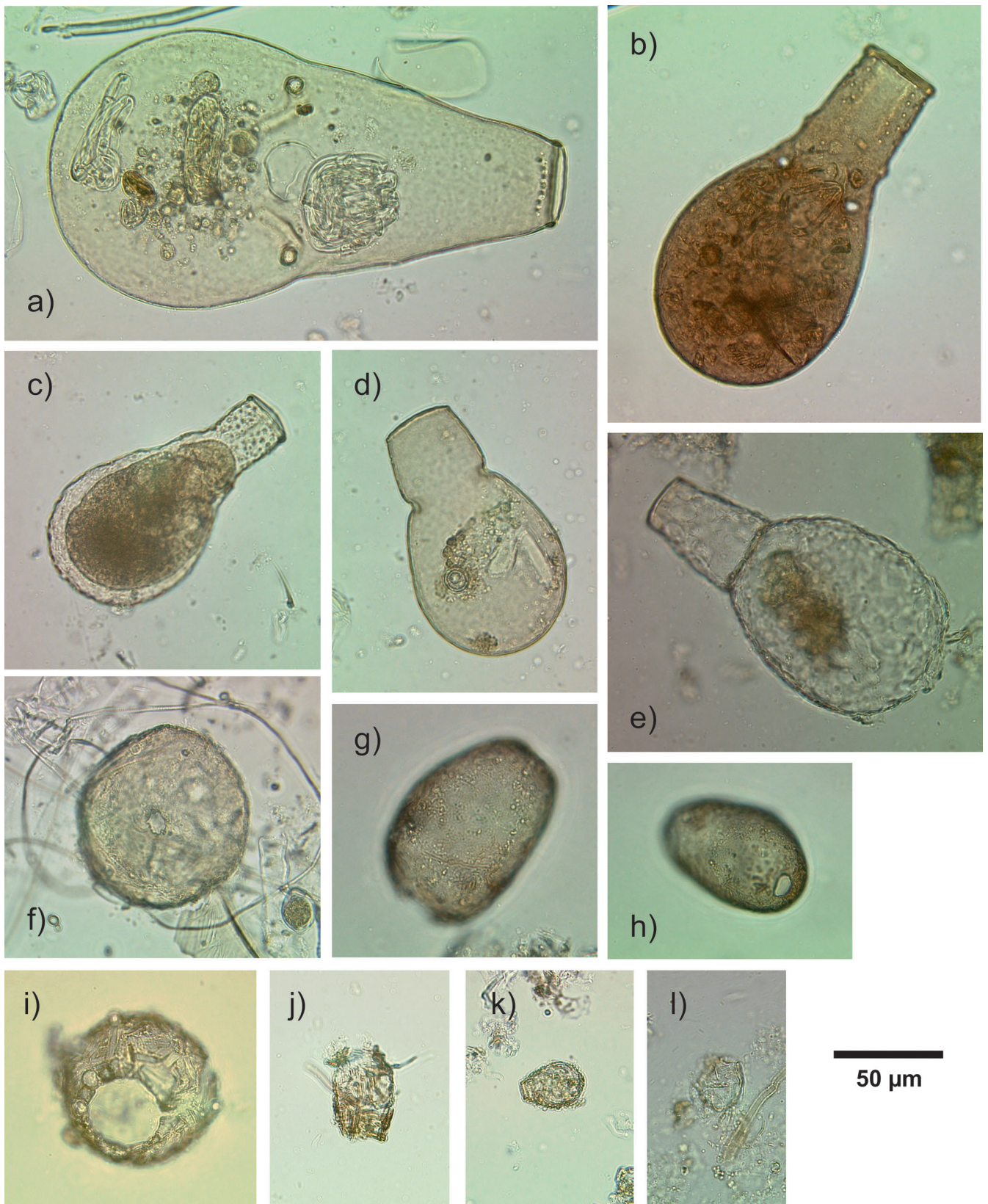
### Statistical analyses and transfer function development

The availability of testate amoeba and corresponding environmental data from two different growing seasons allowed us to test transfer functions based on the entire 2012 + 2013 dataset, the 2012 dataset only and the 2012 dataset plus the same sites re-sampled in 2013 (excluding SJB which was only sampled in 2013). We used the 2013 data as an independent test set to determine the performance of the transfer function based on the samples from 2012. The separate 2012 and 2013 datasets contained 99 and 55 samples, respectively, resulting in a 2012 + 2013 dataset totalling 154 samples with 32 different taxa. After exclusion and grouping of taxa discussed above the final 2012 training set contained 28 taxa.

Ordination was used to evaluate the data structure, environmental controls, and differences between sites and years. Taxon assemblages were explored in unconstrained ordinations (detrended correspondence analysis; DCA) and relationships with the measured environmental variables were evaluated using canonical correspondence analysis (CCA). CCA on 2012, 2013 and 2012 + 2013 datasets was performed with a downweighting of rare species. Environmental variables were selected by forward selection and a Monte Carlo permutation test (999 iterations) was used to test the significance of the environmental variables. The individual influence of environmental variables as well as site and year of sampling on amoeba assemblages were quantified by variation partitioning using partial CCA. A separate ordination of 2013 samples was performed to account for the differences in pH and EC measurement methods. Ordination was carried out using CANOCO for Windows 4.55 (Ter Braak and Smilauer, 1997–2006).

Transfer functions were constructed and tested using the Rioja 0.8–3 package (Juggins, 2012) in R 3.0.2 (R Core Team, 2009). We tested the models most frequently used in peatland palaeoecology: weighted averaging (WA), weighted averaging with tolerance downweighting (WA-Tol), weighted averaging-partial least-squares (WA-PLS) and ordinary partial least-squares (PLS) (Ter Braak and Juggins, 1993). The rationale behind weighted averaging regression is that the taxa occupy different niches in environmental space, with niches defined by a centre (optimum) and breadth (tolerance) (Juggins and Birks, 2012). The optima are obtained by a weighted averaging of the environmental values of the sites where different taxa are found. This reasoning is inverted to obtain reconstructions from past taxa assemblages: the weighted averaging of the species optima found in a sample then results in an estimate of the environmental variable. In contrast, PLS uses components to summarize variability within the taxon assemblages, extracted using both biological and environmental data. More detailed information on model characteristics can be found in Ter Braak and Juggins (1993) and Juggins and Birks (2012). Each model's performance was estimated based on four criteria: root mean square error of prediction (RMSEP),  $R^2$ , average bias and maximum bias from four methods of cross-validation and independent test sets where available. We implemented the two standard cross-validation approaches: bootstrapping and leave-one-out (LOO or jack-knifing). However, Payne *et al.* (2012) have shown that the within-site clustering of samples leads these methods to produce unrealistically optimistic performance statistics. To account for this we also applied leave-one-site-out (LOSO) cross-validation, which tests model performance when applied only to samples from different sites. Because of our attempt to evenly sample the WTD gradient by sampling along pool-hummock transects the dataset also includes another form of spatial structure; it is possible that model performance will be better when samples from the same transect remain in the training set thereby giving unrealistic performance estimates. As a first attempt to assess the impact of this sampling structure, which has been used in other recent studies (Amesbury *et al.*, 2013), we also apply a novel 'leave-one-transect-out' (LOTO) cross-validation in which each sampling transect is successively excluded. Note that as LOSO and LOTO are implemented separately we do not directly test the full impact of the transect-within-site structure, which may mean that real performance is weaker than either test. To test the potential impact of uneven sampling along the water table gradient we also apply the segment-wise RMSEP approach advocated by Telford and Birks (2011) to a sub-set of models.





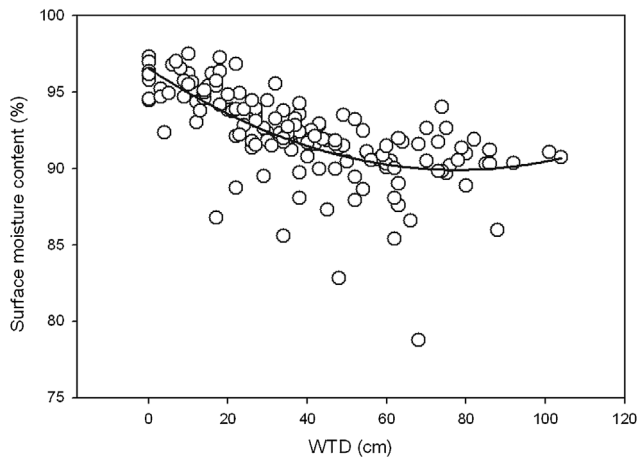
**Figure 2.** Typical testate amoebae encountered in this study displayed at equal scales: a) *Certesella australis* Vucetich; b) *Certesella martiali* Certes; c) *Certesella certesi* Penard; d) *Alocodera cockaynei* Wailies; e) *Apodera vas* Certes; f) *Trigonopyxis microstoma* Hoogenraad & De Groot; g) and h) *Schwabia* cf. *regularis* Jung; i) *Diffflugia globulosa* Dujardin type, previously described by Booth (2002); j) *Diffflugia pristis* Penard 1902 type; k) *Diffflugia pulex* Penard 1902; l) *Pseudodiffflugia fulva* Penard 1901 type. This figure is available in colour online at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).

## Results

### Training set

The complete dataset, including samples from both 2012 and 2013, shows a mean WTD of 38 cm ( $\sigma = 25$  cm;  $n = 154$ ), a

moisture content of  $92.0 \pm 4.2\%$ , a pH of  $4.1 \pm 0.6$  and an EC of  $93 \pm 67 \mu\text{S cm}^{-1}$  (Table 1). Regression analysis showed that the relationship between WTD and moisture content is significant and best represented by a quadratic function (2012 + 2013 dataset,  $R^2 = 0.5$  and  $P < 0.001$ ; Fig. 3). Mean



**Figure 3.** Curvilinear relationship between WTD and surface moisture content for the 2012+2013 dataset, showing a weaker correlation for the drier sites (moisture content =  $96.54024 + 0.001098 \times \text{WTD}^2 - 0.170755 \times \text{WTD}$ ;  $R^2 = 0.50$  and  $P < 0.001$ ).

taxonomic richness was 10.5 taxa per sample ( $\sigma = 3.8$ ,  $n = 154$ ), which is generally lower than numbers encountered in other studies (Booth, 2002; Payne *et al.*, 2006). Taxonomic richness is negatively correlated with WTD in linear regression, i.e. richness is significantly lower for dry microenvironments for both the complete datasets and the sites individually ( $-0.75 < r < -0.33$  and all  $P < 0.05$ ), with the exception of PAR bog. The most common amoebae in these datasets are *Assulina muscorum* (31.9/36.0% of total identified amoebae for the 2012 and 2012+2013 datasets, respectively), *Heleopera sphagni* (21.1/17.6%) and *E. strigosa* type (7.9/8.5%).

### Ordinations: DCA and CCA

Separate DCA of the 2012 and 2012+2013 dataset showed gradient lengths of  $3.80\sigma$  and  $3.88\sigma$ , respectively. Such gradient lengths generally justify the use of a unimodal response model (Ter Braak and Prentice, 1988). Both ordination plots (Fig. 4a for the 2012+2013 dataset) show a separation of known xerophilous taxa (*A. muscorum*, *Corythion-Trinema* type) towards the high values of axis 1 and

hydrophilous taxa at the low end (*Diffugia globulosa* and *Amphitrema wrightianum*). Testate amoeba assemblages have reasonable overlap between sites, suggesting that local site has a limited influence on amoeba community (Fig. 4b).

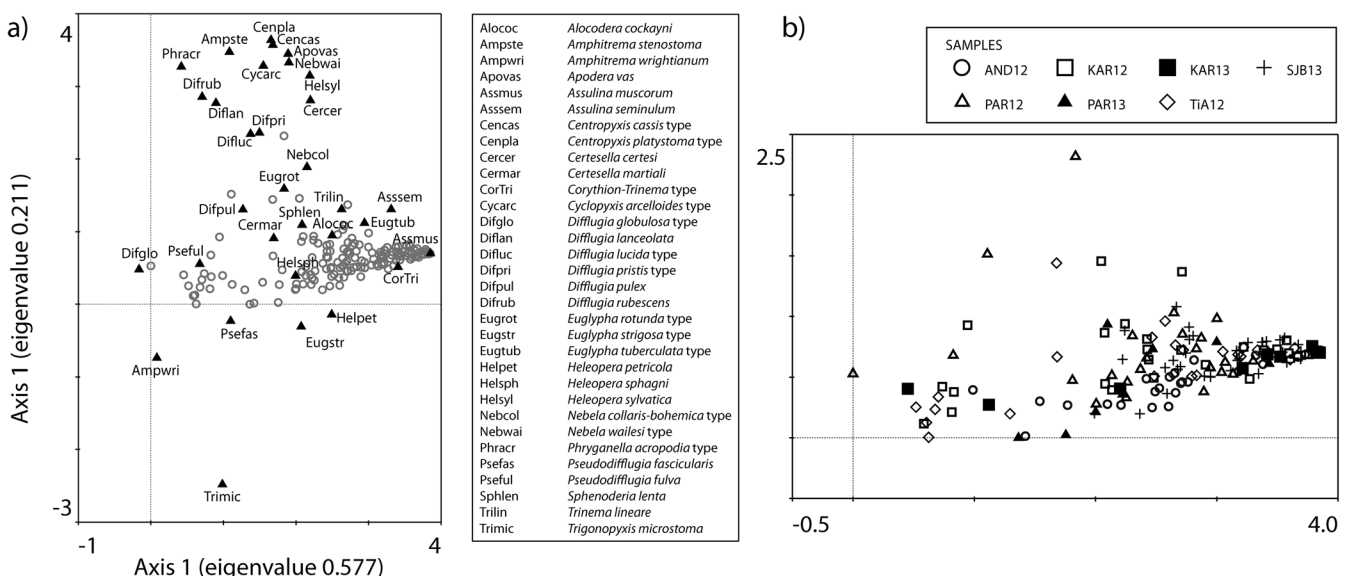
Relationships between assemblages and environmental variables were further explored by CCA, which showed that canonical axis 1 is clearly associated with WTD and explains 16.6 and 15.0% of the variability in amoeba assemblages for the 2012 and 2012+2013 datasets, respectively (Table 2; Fig. 5). Moisture content explained less variance than WTD and was not selected in forward selection ( $P = 0.097$ ) and therefore it was not included in variation partitioning (discussed below). CCA of the 2013 samples showed a significant, but minor, influence of pH and EC on testate amoeba communities (Table 2). The significant relationship with WTD supports the development of a transfer function.

### Variation partitioning

A series of variation partitioning analyses was performed on the 2012+2013 dataset to isolate effects of significant variables. In each analysis, WTD is consistently the most important environmental variable but pH and EC are significant controls on amoeba community composition as well, and both sites and years explain a significant part of the variability ( $P < 0.05$ ; Fig. 6). WTD+EC and pH, WTD and site as well as WTD and year together explain more of the variability in amoeba assemblages than the sum of their individual effects, explaining the negative values in Fig. 6.

### Transfer function models

Transfer function models were developed for WTD and tested using the four types of cross-validation outlined above. Of the different methods, the two-component WA-PLS model shows best performance, with a coefficient of determination of  $0.64 < R^2 < 0.72$  depending on the cross-validation method and RMSEP between 13.5 and 15.5 cm (Table 3). Maximum bias and average bias of the WA-PLS model are generally lower than those of the WA-Tol and PLS models, both in cross-validation and using the independent test set. Performance of the WA-PLS model with LOO cross-validation is reasonable with  $R^2$  of 0.72 and an RMSEP of 13.51 cm (Fig. 7a). Unsurprisingly, performance with the more



**Figure 4.** DCA of the 2012+2013 dataset: (a) biplot of species and samples suggesting a water table gradient along axis 1 with the high values corresponding to drier conditions; (b) ordination of samples grouped per site and sampling year, showing a good degree of overlap.



**Table 2.** Results of CCA, including WTD, pH and EC of both datasets.

Dataset	No. of observations	No. of taxa	Eigenvalue axis 1	Variance explained axis 1 (%)	Variance explained all canonical axes (%)
2012	99	28	0.402	16.6 ( $P=0.001$ )	24.0
2013	55	24	0.335	22.5 ( $P=0.001$ )	28.7
2012 + 2013	154	32	0.354	15.0 ( $P=0.001$ )	20.1

conservative LOSO cross-validation is weaker, suggesting some site-specific amoeba assemblage–WTD relationships, although the three models are not equally sensitive to this effect as WA-Tol out-performs PLS and WA-PLS using this method. Results further suggest little impact of the sampling structure by transects on model performance with LOTO results comparable with LOO for  $R^2$  and RMSEP. Segment-wise analyses of the RMSEP of the three models shows that values are similar for the WA-PLS and PLS models (Fig. 8). The RMSEPs of all three models remain below 15 cm for the wetter 70% of the WTD gradient, only to increase up to 30 cm for the driest samples. WA-Tol RMSEP is notably higher at the dry end of the gradient. Mean RMSEP of the 2012 WA-PLS model (13.79 cm) is close to the standard RMSEP (13.51 cm).

Results of the model performance tests using independent test sets show that model performance is considerably weaker than generally suggested by cross-validation, including the more conservative LOSO. However, breaking the test set down into those sites which were included in the training set but re-sampled in 2013 (KAR and PAR) and the site which was only sampled in 2013 (SJB) reveals a more nuanced picture (Table 3; Fig. 7b). Using the test set that includes KAR and PAR samples, i.e. the sites that are included in the training set, RMSEP is worse than suggested by cross-validation, but  $R^2$  is better. In this case,  $R^2$  is probably a more useful performance measure as the bias in predictions is of less palaeoecological interest than the performance in reconstructing relative trends. Using the independent site SJB, performance is consistently weaker than suggested by cross-validation results.

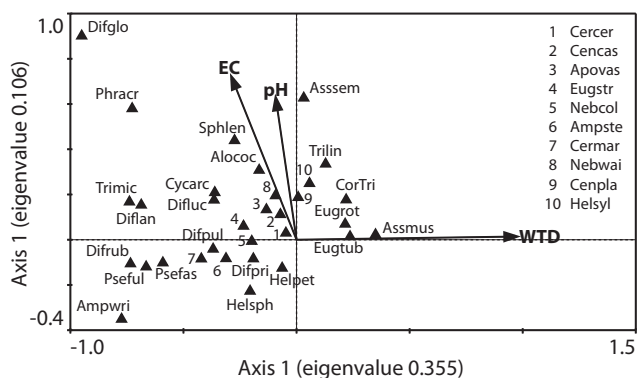
### Taxa optima and tolerances

The total range of weighted-average WTD optima is 49 cm; *A. muscorum* and *D. globulosa* are positioned at the extremes of the WTD range at 54 and 5 cm depth, respectively (Fig. 9). At the dry end *Corythion–Trinema* type (optimum of 51 cm) and *Euglypha tuberculata* type (49 cm) are present. Besides

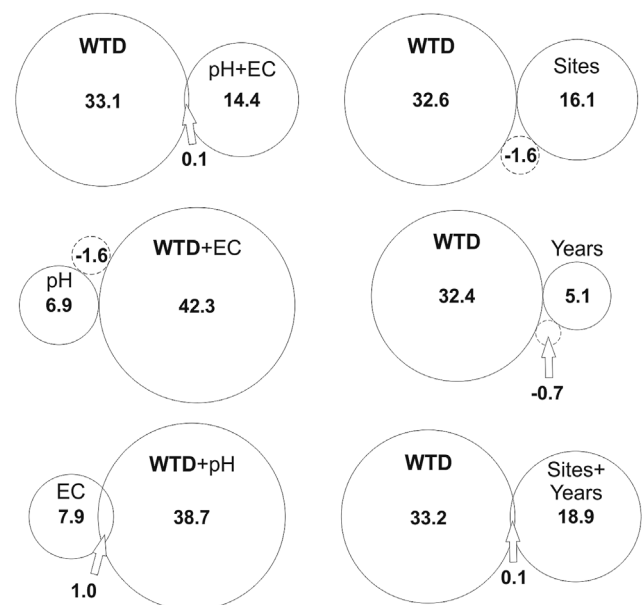
*D. globulosa*, the typical hydrophilous *A. wrightianum* (8 cm) is frequently observed in low lawns towards pool transitions. *D. pulex*, which is often absent in previously published training sets, was encountered frequently enough here to include in the transfer functions as 55 specimens were counted in 33 samples of the 2012 dataset. *D. pulex* shows an optimum at the moderately wet end of the gradient at 21 cm depth, although its tolerance is wide ( $\pm 22$  cm). The globally restricted taxa *C. certesi* (39 cm) and *A. cockaynei* (45 cm) have optima between the middle and dry end of the gradient, whereas *T. microstoma* is clearly associated with low lawns and hollows (15 cm).

### Subsurface sample characteristics

A CCA of the 28 subsurface samples and environmental variables measured in the corresponding surface samples shows that assemblages are primarily associated with WTD along axis 1 (eigenvalue = 0.400; variance explained by axis 1 = 20.6%;  $P=0.001$ ). These subsurface samples show a good overlap in assemblages with the surface samples when projected onto the surface sample ordination (Fig. 10a). Nevertheless, the surface samples hold significantly lower relative abundances of *D. pulex* (83% decrease compared with subsurface samples; Fig. 10b) and *H. subflava* (97% decrease). *H. subflava* is rare in our data and therefore not included in the transfer function. The surface samples also show significantly lower relative abundance of *Diffugia pristis* type and higher abundance of *E. strigosa* type (Fig. 10b). Figure 10b suggests that hydrophilous taxa may be



**Figure 5.** CCA biplot of the 2012 + 2013 dataset showing species and environmental variables. Axis 1 is associated with WTD. See Fig. 4 for species abbreviations.



**Figure 6.** Variation partitioning results for the 2012 + 2013 dataset showing the individual and shared effects of the significant variables, indicating WTD is the dominant variable explaining variability in amoeba assemblages. Details of the sites and samples are given in Table 1.

**Table 3.** 2012 WTD model performance as shown by cross-validation and independent test sets.

Performance measure	Cross-validation				Independent test sets		
	LOO	Bootstrap (1000)	LOSO	LOTO	All 2013 data	KAR and PAR bogs 2013*	SJB bog 2013 <sup>†</sup>
WA-Tol Model (inv. deshrinking)							
RMSEP	13.81	14.29	14.83	13.91	21.42	20.66	21.85
R <sup>2</sup>	0.71	0.71	0.66	0.70	0.57	0.80	0.39
Average bias	0.35	0.60	-0.29	0.23	12.97	12.58	13.19
Maximum bias	39.73	40.15	42.41	39.55	30.62	39.03	34.20
WA-PLS (two-component Model)							
RMSEP	13.51	14.01	15.48	13.69	18.23	16.65	19.07
R <sup>2</sup>	0.72	0.72	0.64	0.71	0.62	0.87	0.43
Average bias	-0.02	<0.01	-0.33	-0.09	10.39	12.70	9.07
Maximum bias	33.25	33.22	36.86	33.59	26.07	36.66	30.92
PLS (two-component Model)							
RMSEP	13.63	14.15	15.57	13.86	18.87	17.77	19.47
R <sup>2</sup>	0.72	0.72	0.63	0.71	0.62	0.84	0.45
Average bias	0.08	0.12	-0.29	-0.15	11.74	14.14	10.37
Maximum bias	33.47	33.69	37.52	34.11	27.19	39.31	31.33

\*Sites included in 2012 dataset re-visited in 2013. <sup>†</sup>Site sampled in 2013 but not included in 2012 dataset.

slightly more abundant in subsurface samples and xerophilous taxa relatively more abundant in surface samples, but no significant relationship was found between WTD optimum and surface/subsurface abundance for the 15 most common taxa. Although there are differences in species composition, the taxonomic richness of subsurface samples ( $x = 10.7$ ) is not significantly different from that of the corresponding surface samples ( $x = 9.0$ ; paired  $t$  test,  $P = 0.20$ ). An analysis of the variance components shows that most of the variance in species richness of the combined surface and subsurface sample datasets is present at the microtopographic location level (i.e. position along the transect; 42.8%), followed by site (37.1%), sample type (i.e. surface or subsurface; 4.4%) and transect (1.8%; all  $P < 0.05$ ). This implies that vertical micro-distribution is a relatively minor cause of variability in amoeba taxonomic richness compared with the influence of sample location, which is strongly linked to WTD.

The 2012 WA-PLS transfer function was applied to the subsurface samples to verify if these samples may be good predictors of actual WTD. The inferred values from these assemblages were validated using the measured, actual WTD. The rationale behind this exercise is to evaluate if these subsurface samples are characterized by deep-dwelling live amoebae or if the assemblages are merely fossil, representing past surface assemblages. If the former were the case, one would expect the transfer function results of the subsurface samples to be biased to the wetter end of the gradient, which has been observed previously in other regions (Booth, 2002; Payne and Pates, 2009) and could be explained by the fact that these subsurface samples are located closer to the present-day water table. Alternatively, the latter hypothesis, with subsurface samples suggesting drier conditions than observed, may then point towards a succession effect and recent ecohydrological change. The model works reasonably well for these subsurface samples, although there is a structural bias towards the dry end of the gradient (Fig. 10c), supporting the succession/eco-hydrological change hypothesis.

## Discussion

### *Southern Patagonia testate amoeba ecology*

The taxa observed in these bogs, located along a longitudinal gradient in southern Patagonia, show WTD optima similar to

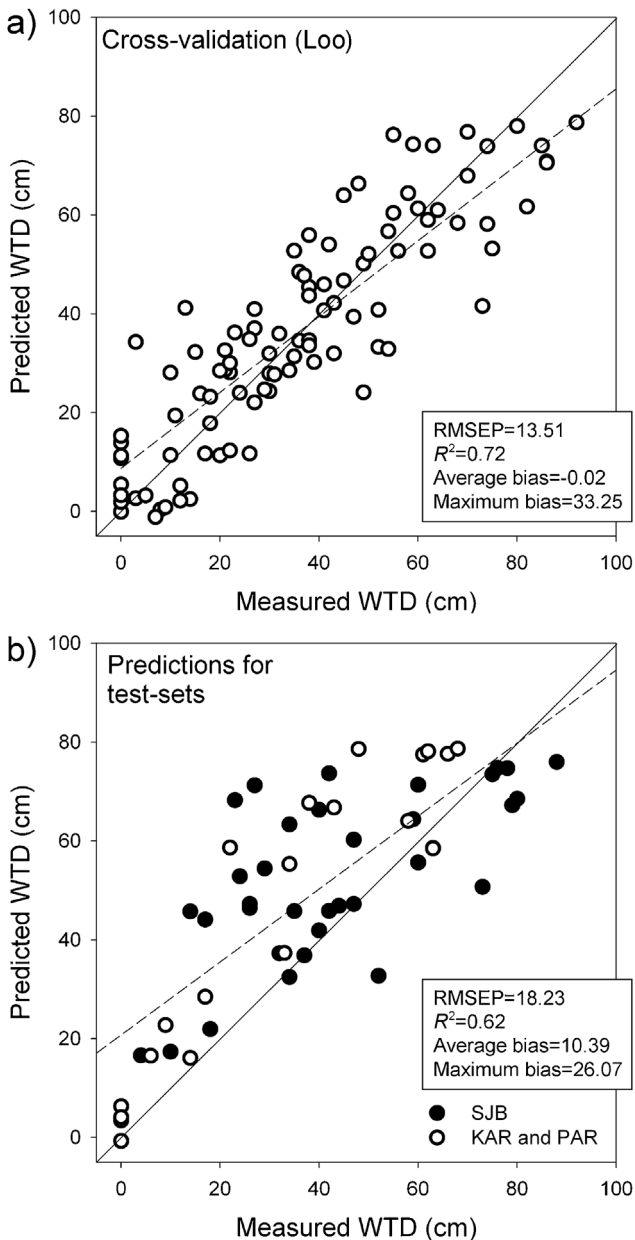
those from Northern Hemisphere bogs. *A. muscorum*, *E. tuberculata* type and *Corythion-Trinema* type are generally associated with the dry end of the sampled water table gradient in studies from North America, Europe and Asia (Booth, 2008; Amesbury *et al.*, 2013; Qin *et al.*, 2013), whereas *D. globulosa* and *A. wrightianum* are invariably found at the wetter end of the gradient. *C. certesi* is likely to be restricted to the Southern Hemisphere and occupies intermediate WTD positions in southern Patagonia, although Charman (1997) found a relatively wet optimum for *C. certesi* in New Zealand. Interestingly, *Archerella flavum*, formerly *Amphitrema flavum* (Gomaa *et al.*, 2013), was absent from the bogs in this study, yet it is very common in the Northern Hemisphere. In New Zealand, *A. flavum* has only been observed in a few samples and in low amounts and this taxon appears to be absent in China (Qin *et al.*, 2013), which suggests it may have a restricted global distribution. The low species richness of testate amoebae parallels the low plant species richness in these peatlands, which are largely dominated by a single *Sphagnum* species. This may imply that the higher species richness of northern peatlands is partially due to the greater diversity of niches provided by different *Sphagnum* species.

### *Transfer function performance*

LOO and bootstrap cross-validation (RMSEP of 13.51 and 14.01 cm) imply a rather poor model performance when compared with other testate amoeba/water table transfer functions, which generally show RMSEP in the order of 6–8 cm (Charman *et al.*, 2007; Booth, 2008; Amesbury *et al.*, 2013; Lamarre *et al.*, 2013). One possible explanation for this difference may be the wide range of WTD values measured in our study (Payne *et al.*, 2006; Lamentowicz *et al.*, 2010), combined with the relatively small number of taxa (32 taxa in a total of 154 samples of the 2012 + 2013 dataset). Measured WTD varied between 0 and 104 cm, which is a much wider range than those of <60 cm presented in many published training sets (Wilmshurst *et al.*, 2003; Charman *et al.*, 2007; Lamentowicz *et al.*, 2008; Lamarre *et al.*, 2013).

The availability of data on testate amoeba assemblages and environmental variables for individual bogs, transects and years allows a detailed assessment of transfer function



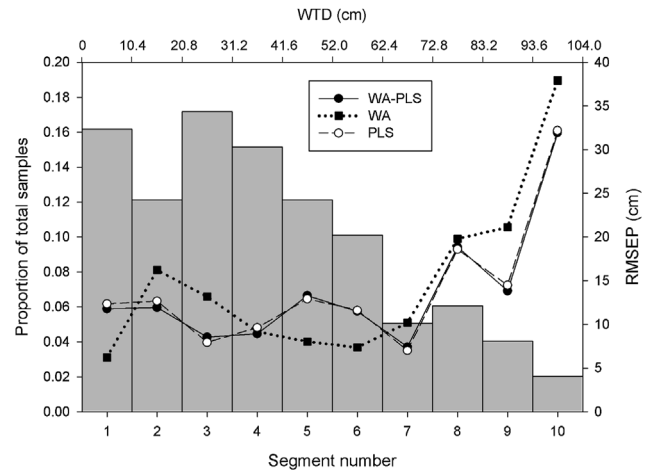


**Figure 7.** WA-PLS model performance as shown by correlation of measured and predicted WTDs: (a) by leave-one-out cross-validation and (b) from independent test sites sampled in 2013.

performance. Model performance was much worse using the independent test set and including all the sites than for the other cross-validation methods. Using the independent test set, the performance was worse still for SJB, absent from the 2012 training set, than for the sites that were sampled in both years. Although this result may partly be explained by the fact that, of all sites, SJB has the deepest mean water table and scarce wet hollows and pools (Table 1), it also demonstrates that caution is required when applying the transfer function to sites not covered by the training set.

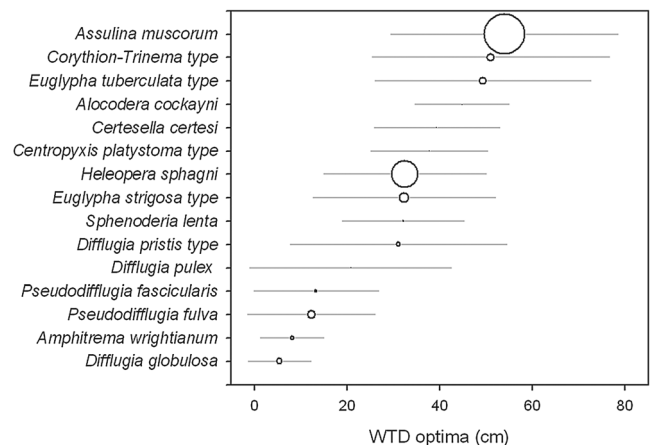
*An in-depth look at WTD and surface moisture*

In most palaeohydrological transfer functions, WTD has been selected as the variable to be reconstructed. However, WTD is merely an easily and robustly measurable proxy for the hydrological variables to which amoebae actually respond, such as water film thickness, and it might thus be expected

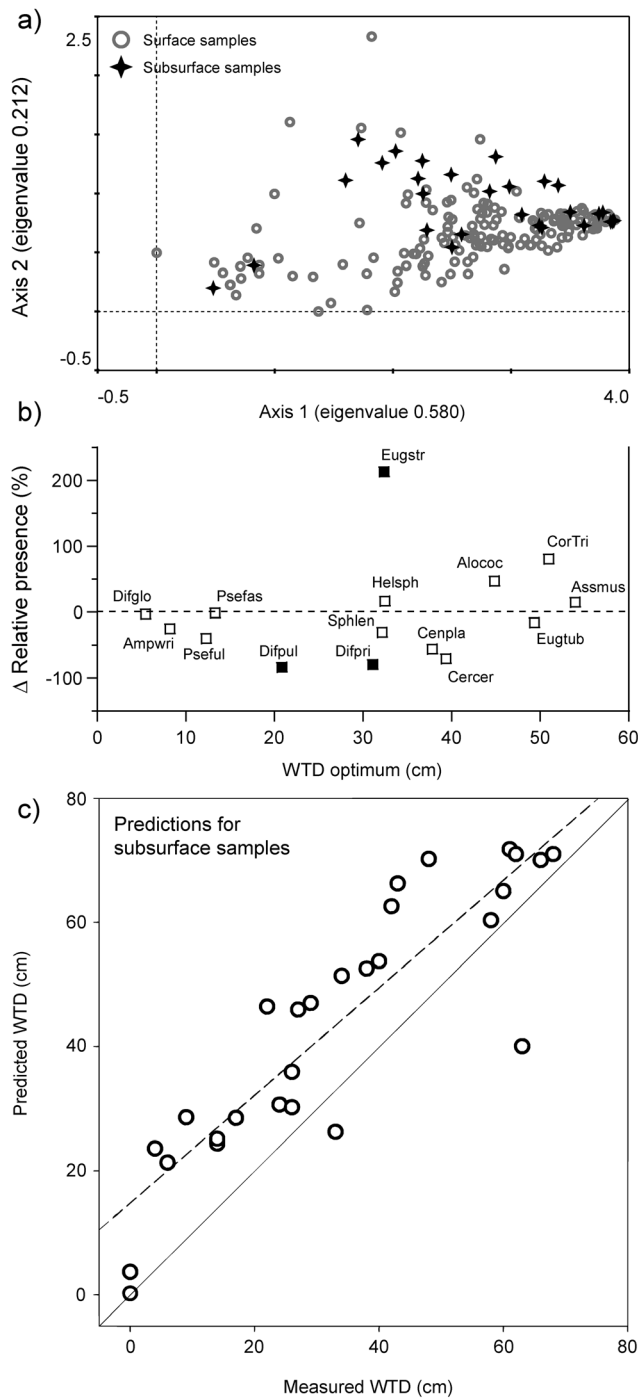


**Figure 8.** Segment-wise RMSEP values and corresponding proportion of samples along the water table gradient for the three tested models. Segment limits were calculated by dividing the sampled range in water tables by the number of segments (10); WTD increases with segment number.

that surface moisture content would be a better proxy. Nevertheless, this study and several previous studies showed that moisture content is a less useful variable when aiming to explain assemblage compositions that integrate communities living over a substantial period (Charman *et al.*, 2007; Swindles *et al.*, 2009; Turner *et al.*, 2013). The most likely explanation for this is that surface moisture content is subjected to short-term (i.e. daily; Adema *et al.*, 2006) fluctuations that are difficult to capture in one-off sampling methods (Sullivan and Booth, 2011). The meteorology of this region, in particular the high wind speeds, may have a strong influence on surface moisture conditions while having less effect on the water table position. Discrepancies between water table position and surface moisture content have been discussed regarding testate amoeba ecology by Charman *et al.* (2007) who associated *D. pulex* and *Pseudodiffugia fulva* with relatively high water tables but low surface moisture using high-resolution soil moisture analyses. Corroborating these findings, Sullivan and Booth (2011) associated *D. pulex* with surface conditions showing high ‘short-term environmental variability’.



**Figure 9.** Testate amoeba optima and tolerances of the 15 most common taxa based on weighted averaging calculated on the 2012 dataset. Marker size represents the relative abundance of each taxon in the dataset.



**Figure 10.** Comparison of surface and subsurface samples: (a) 28 subsurface samples plotted within the DCA ordination of the surface sample dataset; (b) relationship between surface–subsurface difference in taxon abundance and WTD optimum for the 15 most common taxa. Negative values indicate a lower abundance, and positive values a higher abundance in the surface sample. Filled markers show taxa which have a significantly different abundance between surface and subsurface samples ( $P < 0.05$ ). See Fig. 4 for species abbreviations. (c) Correlation between measured WTD from 28 surface samples and predicted WTD from adjacent subsurface samples, showing a general bias to drier predictions than measured.

## Conclusions

The ombrotrophic bogs of southern Patagonia hold relatively species-poor assemblages of testate amoebae that are strongly linked with WTD, with secondary influences of EC and pH. All sites included in the study, located along a longitudinal transect, have comparable environmental conditions in terms

of WTD, as well as similar amoeba assemblages. The taxa most commonly found in the surface include *A. muscorum*, *H. sphagni* and *E. strigosa* type. Despite the low diversity of taxa observed, we have been able to quantify WTD optima and tolerances for a few of the rarer taxa, possibly restricted to the Southern Hemisphere or South America, including *C. certesi* (optimum at 39 cm), *C. cockaynei* (45 cm) and *T. microstoma* (15 cm), as well as for *D. pulex* (21 cm), which appears rare or absent in surface samples in other regions of the world. Our WA-PLS transfer function will allow the first quantitative WTD reconstructions from ombrotrophic bogs of southern Patagonia. The novel cross-validation and independent test set validation methods show that care must be taken when applying the transfer function to sites not included in the training set. Finally, our results highlight a few taxa, including *D. pulex* and *D. pristis* type, which have differing abundance in surface and subsurface samples and suggest the presence of vertical zonation.

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**Abbreviations.** CCA, canonical correspondence analysis; DCA, detrended correspondence analysis; EC, electrical conductivity; LOO, leave-one-out; LOSO, leave-one-site-out; LOTO, leave-one-transect-out; PLS, ordinary partial least-squares; RMSEP, root mean square error of prediction; WA, weighted averaging; WA-PLS, weighted averaging-partial least-squares; WA-Tol, weighted averaging with tolerance downweighting; WTD, water table depth

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