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Comparing regional and supra-regional transfer functions for palaeohydrological reconstruction from Holocene peatlands

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Abstract

Testate amoebae-based transfer functions are commonly used in peatland palaeoclimate studies. These models have been developed in several regions of the world and are sometimes used for palaeohydrological reconstruction from fossil data in locations where no transfer functions exist. Limitations of this approach may include missing modern analogues and problems associated with site-specific or regional factors in testate amoebae ecology and biogeography. This study presents new testate amoebae-hydrology transfer functions based on data from six peatlands in Northern England. Transfer functions were generated for water table depth and moisture content using weighted averaging tolerance downweighted regression with inverse deshrinking and model performance was assessed using leave-one-out (jacknifing) cross-validation. To examine the robustness of applying transfer functions extra-regionally, we performed a number of spatially independent cross-validation (SICV) tests using contemporary testate amoebae and environmental data from established Northern Ireland and European transfer functions. Inferred water table depths were consistent with observed values in terms of position along the hydrological gradient, however magnitudes varied. We then applied the three independent transfer functions to fossil data from a peatland in Northern England to compare the reconstructions. The results show that the direction of the reconstructions is consistent in terms of wet/dry shifts. However, variation in the magnitudes of the reconstructed water tables is apparent. This probably reflects the sampling regime, including temporal/seasonal effects, and differences in testate amoebae ecology between regions and individual sites.

Keywords: Peatlands, testate amoebae, transfer function, palaeoclimate, Holocene, cross-validation

1. Introduction

The utilisation of transfer functions for quantitative palaeohydrological reconstruction from peatlands is now commonplace in several regions of the world (e.g. Booth, 2008; Charman, 1997; Lamentowicz et al., 2009; Lamentowicz et al., 2010; Langdon and Barber, 2005; Mitchell et al., 2001; Payne et al., 2006; Swindles et al., 2010). The hydrology of ombrotrophic ('rain-fed') peatlands is strongly linked to effective precipitation (precipitation – evapotranspiration), especially during the deficit period (Charman, 2007). Thus, inferences from peatland proxy-based palaeohydrological reconstructions contribute significantly to understanding Holocene hydroclimatic change. Although there are a number of peat-based hydrological proxies, such as plant macrofossils and peat humification, testate amoebae have become increasingly favoured by many researchers over the last two decades as transfer functions can be used for quantitative reconstruction of palaeo-water tables (e.g. Charman et al., 2007; Charman and Warner, 1992; Lamentowicz et al., 2010; Payne and Mitchell, 2007; Swindles et al., 2010; Wilmshurst et al., 2003; Woodland et al., 1998).

Testate amoebae are a polyphyletic group of single-celled organisms that form a shell, or test. They are abundant on bog surfaces, especially living within the water film of bryophytes, and on death their empty tests are preserved in accumulating peat (Charman, 2001; Smith et al., 2008; Swindles and Roe, 2007; Warner, 1990). Testate amoebae form a significant part of the microbial ecosystem in peatland soils, enhancing nutrient cycles by consuming bacteria, protozoa, micro-algae, fungi and micrometazoa (Coûteaux and Darbyshire, 1998; Foissner, 1999; Lamentowicz and Mitchell, 2005; Mitchell et al., 2008a; Mitchell et al., 2000a). Community composition of testate amoebae is influenced by a number of biotic and abiotic factors and they respond

rapidly to environmental change (Buttler et al., 1996; Mitchell et al., 2000a; Tolonen, 1986). The dominant control on testate amoebae in peatlands has repeatedly been shown to be bog surface wetness (water table depth, moisture content) particularly in ombrotrophic systems where pH and nutrient status has a limited range (Bobrov et al., 1999; Booth, 2001; Charman, 2001; Lamentowicz and Mitchell, 2005; Payne et al., 2006; Swindles et al., 2009; Woodland et al., 1998). Although the position of testate amoebae in the microbial network is complex, their sensitivity to hydrology makes them ideal biological indicator organisms in both contemporary and palaeoecological studies (Turner and Swindles, 2012).

The body of work on the ecology and biogeography of testate amoebae is extensive. Testate amoebae are often considered to be largely (with a number of exceptions) cosmopolitan in their distribution in *Sphagnum*-dominated peatlands, particularly in the Northern Hemisphere (Mitchell et al., 2000a; Smith et al., 2008). Testate amoebae-based hydrological transfer functions have been developed in many parts of the world; Alaska (Markel et al., 2010; Payne et al., 2006), Canada (Charman and Warner, 1997; Warner and Charman, 1994), Europe (Charman et al., 2007), Greece (Payne and Mitchell, 2007), New Zealand (Charman, 1997; Wilmshurst et al., 2003), North America (Booth, 2008; Booth and Zygmunt, 2005), Northern Ireland (Swindles et al., 2009), Switzerland (Mitchell et al., 1999), Poland (Lamentowicz et al., 2008; Lamentowicz and Mitchell, 2005), and the United Kingdom (Woodland et al., 1998). Due to spatial autocorrelation (Borcard et al., 1992; Lennon, 2000), transfer functions require validation against data sets that are completely independent of the training set (Belyea, 2007; Birks et al., 2010). However, very few models are rigorously tested using

spatially independent data and it has been suggested that they may generate reconstructions of unrealistic accuracy (Payne et al., 2011; Telford and Birks, 2005).

Whilst the environmental preferences of these organisms are generally consistent between regions, their sensitivity to microsite variations and localised idiosyncrasies (Mitchell et al., 2000b) suggest it would be unreasonable to make palaeoecological inferences for one region using contemporary data from another (Charman et al., 2000). Therefore, a comprehensive understanding of the local ecology of testate amoebae is ideally a prerequisite when taking a transfer function approach to palaeoenvironmental reconstruction in any given area. To date, information on testate amoebae species-environment relationships in Britain comprise the dataset of Woodland et al. (1998) (163 samples in 9 sites) and 18 samples from a single British site included in the European transfer function of Charman et al. (2007). A need for further contemporary data from Britain is pertinent when considering the plethora of peatland palaeohydrological reconstructions from the region (e.g. Barber and Langdon, 2007; Blundell and Barber, 2005; Charman et al., 2004; Charman et al., 1999; Daley and Barber, in press; Hendon and Charman, 2004; Langdon and Barber, 2005). However, producing single-site training sets for each individual investigation would be impractical, and therefore the complexity of applying training sets extra-regionally needs further consideration.

The aims of this study are to 1) examine environmental controls on contemporary testate amoebae communities in the Northern England region; 2) develop local transfer functions for quantitative palaeohydrological reconstruction based on fossil testate amoebae assemblages from the area; 3) to explore the issue of spatial scale and biogeography in transfer function development by comparing the results to recently

published regional (Swindles et al., 2009) and European (Charman et al., 2007) transfer functions; 4) to take a novel approach to model validation, using spatially independent data to rigorously test the potential of employing transfer functions extra-regionally.

2. Methods

2.1 Study Sites

Six sites located in North and West Yorkshire, United Kingdom (Fig.1) were selected for this study to encompass a range of peatland environments within a similar climatic regime. Although the majority of palaeohydrological studies have been carried out on ombrotrophic raised bogs, capturing taxa associated with other bog types may be critical to understanding bog development trajectories. We included a variety of bog types in site selection to 1) ensure the inclusion of as many modern analogues as possible; and 2) sample full environmental gradients as recommended by Payne et al. (2011). However, we acknowledge that this sampling strategy cannot account for the true complexity of the autogenic and allogenic processes operating within peatlands (cf. Swindles et al., 2012). The sites comprise Malham Tarn Moss, Swarthmoor Bog, Oxnop Moor, Fleet Moss, Thornton Mire, and Ilkley Moor (Table 1).

Malham Tarn Moss (TM) forms the major part of a mire complex located to the west of Malham Tarn, a shallow marl lake. The study site is an active ombrotrophic raised bog of around 40 ha formed over calcareous fen peat and lake sediments. Vegetation across the raised bog is dominated by heather *Calluna vulgaris* and hare's-tail cottongrass *Eriophorum vaginatum,* with localised abundances of cross-leaved heath *Erica tetralix,* deer grass *Scirpus cespitosus*, common cottongrass *Eriophorum angustifolium* and bog asphodel *Narthecium ossifragum.* Bryophytes include several Sphagna (including

Sphagnum capillifolium, S. papillosum, S. cuspidatum, S. magellanicum) and hypnoid species which are locally abundant. There are isolated Scots pine *Pinus sylvestris* growing on the surface toward the tarn, and fen carr *Salix* spp. dominates the bog edges. Grips (drains) were cut radially across *TM* from the late 19th Century (Cooper and Proctor, 1998), however the site was donated to the National Trust in 1947, and progressive restorative work from the 1970s onwards has seen most of the grips blocked to instigate water table recovery. The site is designated a Site of Special Scientific Interest (SSSI), has Special Area of Conservation (SCA) status and is listed as a wetland of international importance under the Ramsar Convention.

Swarth Moor (SM) is an ombrotrophic raised mire with a classic dome profile which runs into fen grassland communities to the north and south. The 34 ha site has SSSI status, though the edges of the bog have been cut for peat in the past, and a number of cross-cutting grips are evident on the bog surface which have in-filled naturally. Vegetation is characterised by *E. vaginatum,* purple moor grass *Molinia caerulea, E. tetralix* and *C. vulgaris,* with localised cranberry *Vaccinium oxycoccus*, bog rosemary *Andromeda polifolia,* white beak-sedge *Rhynchospora alba* and *N. ossifragum.* Bryophyte cover is predominantly Sphagna including *S. capillifolium, S. papillosum, S. palustre, S. cuspidatum.*

Fleet Moss (FM) is an exposed upland area of blanket bog with areas of degradation including gullying and sheet erosion. The area has no conservation designations and is utilised in places as rough grazing for sheep, with evidence of past gripping (drain cutting) to reduce the water table and favour dwarf shrub and grass species. Vegetation is typical of grazed blanket peat with abundant Poaceae amongst a mosaic of *C. vulgaris* and *Eriophorum* spp. Bryophyte cover is locally abundant and diverse with the Sphagna

S. capillifolium, S. cuspidatum, S. papillosum, and *Hypnum jutlandicum, H. cupressiforme, Aulacomnium palustre* and *Racomitrium lanuginosum* occurring frequently throughout.

Thornton Mire (ThM) is a minerotrophic basin mire of around 7.1 ha which has an area of ombrotrophy in the centre (Honeyman, 1985). The site has been fenced off to prevent stock from entering the area and is therefore only subject to minimal grazing by lagomorphs, resulting in a relatively undisturbed bog surface. Vegetation is typically *Calluna – Eriophorum* type, with abundant bryophytes including *S. cuspidatum, S. capillifolium, S. papillosum, S. palustre, H. jutlandicum, Pleurozia schreberi*, and *Rhytidiadelphus squarrosus*. A number of *Salix* spp. trees are growing to maturity at the fen-bog transition zone.

Oxnop Moor (OM) forms part of a larger area of blanket bog covering the upland watershed between Wensleydale and Swaledale. The moor has a mosaic of habitats and bog types, and is part of the North Pennines SAC and Special Protection Area (SPA), and the Lovely Seat – Stainton Moor SSSI complex of over 10128 ha. Areas of *OM* are managed for grouse shooting through gripping and rotational *C. vulgaris* burning, with some livestock grazing. The sampling location lies within a relatively undisturbed area of ombrotrophic blanket bog isolated from groundwater and surface inflow through substrate topography. As well as extensive *C. vulgaris* cover, typical peat-forming vegetation is present; *Eriophorum* spp. and Sphagna including *S. capillifolium, S. papillosum* and *S. cuspidatum.*

Ilkley Moor (IM) occupies around 676 ha and forms a mosaic of blanket bog, acid grassland, mires and flushes, and bracken *Pteridium aquilinum* dominated heath. The entire moor was designated a SSSI in 1994 due to the range of upland vegetation supported, and designated an SPA under the EU Habitats Directive in 1997. The moor is

lightly grazed by sheep, and has a history of traditional grouse moor management practices such as gripping and rotational heather burning. In 2006 a large uncontrolled wildfire destroyed much of the vegetation and peat soils on the upper southern plateau. Sampling was carried out at three blanket peat locations on the moor characterised by contrasting burning regimes, including the site of the wildfire (cf. Turner and Swindles, 2012). Vegetation at the wildfire site comprised pioneer grass species *Poa annua* and bryophytes *Ceratodon purpureus* and *Polytrichum commune* amongst charred *C. vulgaris* tussocks. The second sampling site is a *Calluna – Eriophorum* grouse moor subject to rotational burning, with localised abundances of crowberry *Empetrum nigrum.* The third sampling site is a valley mire complex dominated by *Eriophorum* spp. and *S. fimbriatum* and *S. squarrosum* indicating a more minerotrophic regime, but containing well-developed ombrotrophic *C. vulgaris/E. nigrum* hummocks.

2.2 Contemporary training set

At each site, a number of cross-cutting transects were established to encompass the full range of microtopographical gradients from hummock to hollow, and where present, fen-bog transition. The position of each of the 207 sample points was logged using GPS, and the dominant vegetation type was recorded. A monospecific sample of the surface bryophyte, approximately 10 cm^2 to a depth of 5 cm was removed using a serrated knife. At each location a hand auger was utilised to enable water table depth (WTD) measurement relative to the substrate surface after allowing time for equilibration. High values represent deeper WTD and negative values represent standing water. Water collected from the augered hole or squeezed from *Sphagnum* was used to measure pH and conductivity with field meters. The validity of 'one-shot' WTD measurements has been debated (Bobrov et al., 1999; Booth, 2008), however provided

extreme pre-sampling weather conditions are avoided (prolonged rain, drought) this has been regarded as adequate for transfer function development (Booth, 2008; Charman et al., 2007; Payne et al., 2011; Swindles et al., 2009). Samples were retained in sealed 'Ziploc' type bags, returned to the laboratory and stored at 4°C prior to analysis.

Bryophyte samples were divided, and approximately half was used to determine moisture content (MC) of the sample by weighing and oven drying at 105°C. The other half of the sample was prepared for testate amoebae analysis following a modified version of Charman et al. (2000). Sub-samples were placed in a 500 ml beaker, boiling water added, and agitated for 5 minutes with a spatula. The beaker contents were sieved through 300 μm and back sieved through 15 μm meshes, retaining the fraction between meshes. Back-sieving at 15 μm has been questioned by Payne (2009) who argues that small taxa such as *Cryptodifflugia oviformis* may pass through the smaller sieve skewing community composition results. However in practice, microsieving makes analysis more efficient particularly when processing large numbers of samples often encountered in palaeoclimate studies (Booth et al., 2010) and remains routinely employed (e.g. Elliott et al., in press; Lamentowicz et al., 2010; Payne, 2011; van der Knaap et al., 2011). Due to the large number of samples and the need to improve slide clarity for samples with minerogenic material present, the fine sieve stage was included for both contemporary and palaeo sample preparation to retain consistency. Samples from a peat core from *TM* were prepared following Charman et al. (2000) with some modification; sub-samples of 2 cm^3 were placed in 50 ml centrifuge tubes, 40 ml of hot water added, and agitated for 2 minutes using a 'whirly-mixer'. The contents were

sieved through 300 μm and back sieved through 15 μm meshes, and samples stored in deionised water.

Samples were slide mounted in deionised water and identified under light microscopy at 200 - 400x magnification, reaching a minimum count of 150 individuals. The taxonomic approach taken in this study predominantly follows that of Charman et al. (2000), which groups some ecologically similar morphospecies with intraspecific variability into 'types'. Exceptions to this were; the splitting out of *Assulina scandinavica* (cf. Ogden and Hedley, 1980) from the *Assulina seminulum* group differentiated by larger size (*A. scandinavica* length 90-120 μm; *A. seminulum* 60-80 μm) and distinct sharp tapering of the test from midpoint to the aperture ; the splitting of *Difflugia globulus* (Cash and Hopkinson, 1909) from the *Cyclopyxis arcelloides* type group, differentiated by larger size (*D. globulus* diameter c. 80-110 μm; *C. arcelloides* type c. 30-70 μm) and distinctive test morphology (incorporating large quartz particles and diatom frustules); the inclusion of fen and soil-dwelling taxa not listed in Charman et al. (2000) (*Cyphoderia ampulla, Euglypha cristata, Plagiopyxis callida, Tracheleuglypha dentata*); the classification of '*Euglypha* degraded' for individuals of this genus not identifiable to taxon level due to degradation (Swindles and Ruffell, 2009). Identification was aided with the taxonomic guides of Corbet (1973), Ogden and Hedley (1980) and Charman et al. (2000).

2.3 Data analysis

Absolute testate amoebae numbers were converted to sample percentage in preparation for statistical analysis. The Shannon diversity index (SDI) was calculated for each sample to examine the faunal diversity. The SDI has been shown to indicate relative ecological health of testate amoebae communities and is defined as:

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

where Xi is the abundance of each taxon in a sample, Ni is the total abundance of the sample, and S is equal to the species richness of the sample. Environments are considered to be healthy if the SDI falls between 2.5 to 3.5, in transition between 1.5 and 2.5, and stressed between 0.1 and 1.5. Harsh environments where conditions severely limit species numbers are characterised by low SDI values (Magguran, 1998).

Ordination methods were used to examine contemporary taxa-environment relationships using CANOCO 4.5 (ter Braak, 2002; ter Braak and Smilauer, 2002). Gradient lengths were determined using detrended correspondence analysis (DCA) on the whole percentage-transformed dataset. Gradients were found to be consistently long (> 2σ), suggesting unimodal methods are more appropriate for these data (Birks, 1995). Species data were therefore transformed using Hellinger distance to enable the use of Euclidean based ordination methods over chi-squared distance methods, thus avoiding problems associated with rare species (cf. Legendre and Gallagher, 2001). Redundancy analysis (RDA) was used to explore the relationships between testate amoebae and environmental variables and to identify the dominant controls on testate amoebae community structure. The SDI values were included as a supplementary environmental variable. The relative contribution of each environmental variable was investigated using a series of partial RDAs in order to estimate total variance partitioning and intercorrelations between environmental variables (Borcard and Legendre, 1994). The statistical significance of these analyses was determined with Monte-Carlo permutation tests (999 permutations, under a full model).

2.4 Species-environment modelling

Transfer functions were developed using the C2 software package (Juggins, 2003) employing a number of weighted averaging techniques as these are more robust to autocorrelation (Telford and Birks, 2005); weighted averaging (WA) and tolerance downweighted weighted averaging (WA-Tol) with classical and inverse deshrinking, and weighted averaging partial least squares (WA-PLS) and Maximum Likelihood (ML). Model performance was assessed using the coefficient of determination (r^2) and the root mean square error of prediction (RMSEP) with leave-one-out cross-validation ('jack-knifing'). Transfer function model performance was improved through the removal of 'outliers', samples with residuals above a predetermined value. Hydrological niche statistics (WTD and MC) were calculated for each taxon. The water table transfer function was applied to a fossil sequence from Malham Tarn Moss, applying 1000 bootstrap cycles to generate sample-specific errors of prediction (Line et al., 1994). For comparison, the Northern Ireland water table transfer function of Swindles et al. (2009) and the ACCROTELM water table transfer function (Charman et al., 2007) were also applied to the fossil sequence.

Further comparisons were made between the three transfer functions by using each to predict for the other's contemporary data (testate amoebae community composition and associated WTD measurements) in a series of spatially independent cross-validations (SICVs). Comparisons were evaluated for the six iterations by calculating the percentage of observed values that fall within the bootstrap standard error of the model predicted values, and by Pearson correlation analysis of the observed and model predicted values using SPSS v.19.

3. Results

3.1 Testate amoebae communities and environmental variables

A total of 207 samples were analysed for testate amoebae community composition reaching a count of >150 individuals per sample (cf. Charman, 1999). A total of 67 taxa were recorded. By far the most common, making up 26% of the total count was *Nebela tincta* (Supplementary file 1). *Corythion-Trinema type, Euglypha strigosa, Assulina muscorum* and *Cyclopyxis arcelloides* type were the next four most abundant taxa in decreasing order and altogether these five taxa account for 57% of testate amoebae recorded. There were 51 taxa recorded that occurred in less than 1% of the total count (n = 34609).

During field data collection, a number of sample points on the blanket bog sites had no measurable WTD due to thin peat overlying bedrock; these 48 samples were excluded from water table reconstruction transfer function development, though remain in the MC models. Shannon diversity index values ranged between 0.4 and 3.1 between all 6 sites, though the mean values at each site were not dissimilar at 2.0 ± 0.3 (Fig.2). Samples from Fleet Moss have the lowest SDI mean value and inter-quartile range suggesting that the environmental impacts here (drainage, grazing pressure, erosion) are limiting testate amoebae community diversity. Interestingly, Tarn Moss appears to be the second most stressed environment. Two outliers on the boxplot were samples taken from bog pools dominated by *Arcella discoides* type and therefore understandably less diverse, however the low mean value and limited range is surprising given the nature of this peatland. The higher mean SDI number but limited range for Oxnop Moor could reflect the stable nature of a managed grouse moor where a narrower range of environmental variables were encountered. Swarth Moor has the highest mean value

though the inter-quartile range (i.e. 50% of the samples) is skewed towards a lower diversity. The mean pH values for each site infer the generally ombrotrophic nature of the sites, though outliers indicate that the full environmental gradient was sampled as was intended in this study. Again, the conductivity ranges of 20 to 155 μS cm^{-1} (with an extreme outlier of 359 μS cm^{-1} at Ilkley Moor) suggest that the full range of trophic environments were included in sample collection. The influence of hydrochemistry on testate amoebae community composition should be considered when interpreting relationships with hydrological variables, although it is usually found to be a subordinate to hydrology in most studies, particularly in ombrotrophic systems where non-hydrological variables have a limited range (e.g. Booth, 2002; Booth, 2008; Charman and Warner, 1992; Lamentowicz and Mitchell, 2005).

Constrained ordination using redundancy analysis (RDA) with Hellinger-transformed species data was carried out on the dataset. Samples without WTD data were excluded. RDA axes one (eigenvalue = 0.089) and axes two (eigenvalue = 0.038) explain 12.6% of the variance in the testate amoebae data and 78.8% of the species-environment relationship (Fig.3, Supplementary file 2). Water table depth and moisture content are strongly correlated with axis one (r = -0.752 and r = 0.508 respectively) and occur at opposite ends of the axis, rendering hydrology the primary environmental driver. The associated Monte-Carlo permutation test shows this axis is highly significant ($p < 0.001$, 999 random permutations). The second axis is correlated primarily to pH (r = 0.353) indicating that geochemistry is subordinate to hydrology, consistent with patterns observed in previous studies (Booth, 2008; Charman and Warner, 1997; Charman et al., 2007; Charman and Warner, 1992; Swindles et al., 2009; Tolonen et al., 1992; Warner and Charman, 1994; Woodland et al., 1998).

From the RDA, environmental variables account for 16.0% of the explained variance in the training set, leaving 84.0% unexplained. A series of partial RDAs (pRDA) were performed on each environmental variable with the others as co-variables to apportion the explained variance; 34.4% water table depth ($p < 0.001$), 20.6% moisture content ($p < 0.001$), 15.0% pH ($p < 0.001$), 11.9% conductivity ($p < 0.001$), with the remaining 18.1% related to environmental variable inter-correlations.

3.2 Transfer function development

The RDA shows there is a statistically significant relationship between testate amoebae community composition and hydrology, therefore transfer functions can be developed for water table depth and moisture content. Water table depth and surface moisture content show a significant correlation with each other ($r = -0.546$, $p < 0.01$), however there are a number of samples with lower/higher than expected moisture contents when considering their position in the water table (Fig.4). This is likely due to weather conditions prior to sampling the surface bryophyte and demonstrates the potential short-term flux of surface moisture in relation to precipitation and evapotranspiration. It is also possible that evaporative losses were encountered during sampling and storage. It is therefore proposed that water table depth is a more stable proxy of hydrology than substrate moisture in peatlands in agreement with other studies of this nature (Charman and Warner, 1997; Charman et al., 2007; Charman and Warner, 1992; Swindles et al., 2009; Tolonen et al., 1992; Woodland et al., 1998).

The performance of several models for water table and moisture content were tested, with WA-Tol (inverse deshrinking) found to be the best performing model for water table depth (RMSEP$_{Jack}$ = 15.02 cm, r^2_{Jack} = 0.50). The predictive potential of the WA-Tol Inv and weighted averaging partial least squares (component 2) were very similar (WA-

TOL Inv $RMSEP_{Jack}$ = 9.12%, r^2_{Jack} = 0.42, WAPLS 2 $RMSEP_{Jack}$ = 9.10%, r^2_{Jack} = 0.42) for moisture content (Table.2). Comparison of the water table and moisture content observed and predicted values showed that the models were particularly poor for samples at the extreme ends of the hydrological gradient, either with unusually low moisture contents or deep water tables, or samples of *Sphagnum cuspidatum* collected from deep pools (Fig.5). As outliers have a pronounced effect on ordination methods and transfer function development (Kent, 2012; McCune and Grace, 2002) it is common practice to remove these to a predetermined value, avoiding uncontrolled removal of residuals (e.g. Booth, 2008; Charman et al., 2007; Payne et al., 2006; Woodland et al., 1998). In addition, a number of authors have noted a deterioration in predictive ability in extremely dry environments (Booth, 2002; Payne et al., 2006). Samples with residuals greater than one fifth of the full WTD range were removed from the water table model, plus a number of strongly minerotrophic samples, totalling 23.3% (14.0 cm) of the original dataset leaving 116 samples. Samples with residuals greater than 24.5% of the full MC range (10.0% MC) were removed from the moisture content model, leaving 183 samples. The majority of outliers were associated with extreme xeric conditions or deep pools; however other factors were influencing a number of these outlying samples including vegetation type, pH and conductivity, resulting in unusual community composition. WA-Tol Inv remained the best performing model for water table following residual removal ($RMSEP_{Jack}$ = 7.36 cm, r^2_{Jack} = 0.71), and emerged as the best performing model for moisture content ($RMSEP_{Jack}$ = 3.88%, r^2_{Jack} = 0.58). Optima and tolerance values for each taxon were calculated for the model output (Fig.6, Supplementary file 3).

3.3 Application to fossil sequence

The sub-fossil testate amoebae diagram for the top 100 cm of Malham Tarn Moss (present to 389 – 210 cal. BC) is shown together with WTD reconstructions using the Northern England (NE), Northern Ireland (NI) and ACCROTELM transfer functions (Fig.7). The three reconstructions are shown separately on the same WTD axis in Fig.8 for clarity. Water table depth transfer functions were applied with 1000 bootstrap cycles to generate sample-specific error ranges. Past changes in bog surface wetness are interpreted through marked changes in testate amoebae assemblages. Taxa associated with wetter conditions represented in the training set include *Amphitrema wrightianum, Archerella flavum* and *Arcella discoides* type*.* Dry-indicator taxa include *Trigonopyxis arcula* type*, Assulina muscorum* and *Hyalosphenia subflava.* The three transfer functions compare well in terms of direction, particularly over major wet shifts, although the NE reconstruction appears to display more noise in the data. In terms of magnitude, the abrupt wet shifts at 12 cm, 60 cm and 78 cm compare favourably, however there is some disparity in magnitude throughout much of the rest of the reconstruction profile, with the NI model consistently predicting deeper water table depths than the NE and ACCROTELM models where *H. subflava* dominates the record. Between 36 cm and 58 cm the ACCROTELM model predicts higher water tables than the NE model, however this trend is reversed from 82 – 100 cm. Both the NI and ACCROTELM models show a dip toward wetter conditions at the surface, however the NE model shows a rapid dry shift from an inferred WTD of 13 cm to 26 cm following a more pronounced wet phase due to the presence of *A. discoides* type. *A. wrightianum* is a particularly consistent indicator of higher water tables throughout the reconstructions, with comparable direction and magnitude for all models. Water table

reconstructions appear more uniform in magnitude when *T. arcula* type replaces *H. subflava* in the record, reflecting the consistency in WTD optima of this xerophilous taxon in all three contemporary training sets.

4. Discussion

This study shows that testate amoebae in Northern England peatlands have comparable environmental controls to those from other regions across the globe (e.g. Bobrov et al., 1999; Booth, 2002; Booth et al., 2008; Charman, 1997; Charman et al., 2007; Payne et al., 2008; Payne et al., 2006). The strongest relationships to testate amoebae community composition and distribution are hydrological; water table depth and/or substrate moisture content. pH is the second most influential environmental control, though is consistently subordinate to hydrology in ombrotrophic peatlands. Key indicator taxa (*A. wrightianum, A. flavum, A. discoides* type*, H. subflava, T. arcula* type and *A. muscorum*) occupy similar positions along the hydrological gradient to other studies (Booth et al., 2008; Charman, 1997; Charman et al., 2007; Charman and Warner, 1992; Lamentowicz and Mitchell, 2005; Mitchell et al., 2001; Swindles et al., 2009; Tolonen, 1986; Tolonen et al., 1994; Woodland et al., 1998) reinforcing the suitability of these organisms as bioindicators of hydrological change and application to transfer function-based palaeoclimate studies.

The predictive capability of the Northern England water table depth training set (RMSEP$_{Jack}$ = 7.36 cm, r^2_{Jack} = 0.71) does not perform quite as well as the Northern Ireland (RMSEP$_{Jack}$ = 4.99 cm, r^2_{Jack} = 0.76) or the ACCROTELM (RMSEP$_{Jack}$ = 5.65 cm, r^2_{Jack} = 0.71) which may be due to the much greater number of samples in the training set, the length of the sampled gradient and associated increased error (NI n = 94; ACCROTELM n = 119; NE n = 159). The performance of the moisture content transfer

function is more comparable (RMSEP$_{Jack}$ = 3.88%, r^2_{Jack} = 0.54). On application to the *TM* fossil sequence, the NE inferred WTD reconstruction is strongly correlated to both the NI (r = 0.703, p < 0.01) and ACCROTELM (r = 0.618, p < 0.01), however the NI and ACCROTELM reconstructions show the greatest correlation (r = 0.887, p < 0.01). The water table predictions from the three models are very similar in direction and, to a lesser extent magnitude, particularly for higher water tables. The NI model consistently predicts deeper water tables where the fossil assemblage contains higher numbers of *Hyalosphenia subflava* due mainly to the higher optimum water table value in the NI training set (29.0 cm, n = 7) compared to this study (15.9 cm, n = 17) and the ACCROTELM (17.8 cm, n = 18). This dissimilarity in the NI dataset could be due to hydrological conditions during the time of sampling, as 2003 and 2004 were unusually dry years (Met Office, 2012) , or that certain taxa (including *H. subflava*) are mostly from very large hummocks (Swindles, 2006).

Spatially independent cross-validation (SICV) of the three transfer functions shows some considerable variability in the performance of the individual training sets when applied to independent data (Fig. 9, Supplementary file 4). Performing reconstructions on independent contemporary testate amoebae data sets is a robust method of assessing performance without the problem of positive spatial autocorrelation and removes the unquantifiable effects of test preservation affecting palaeoecological samples. The NE and ACCROTELM models perform similarly when inferring water table depth for the other two sets of contemporary data; 73.95% of the observed water table depths fall within the standard error range of the model predicted values when the NE model is applied to the ACCROTELM training set and 75.74% when the ACCROTELM predicts for the NE data. The proportions of observed values drier and wetter than

predicted are also reasonably similar and evenly spread (10.06 – 12.56% drier; 13.49 – 14.2% wetter). Both the NE and ACCROTELM models perform less well when inferring water tables for the NI training set with only 66.81 and 67.37% of the observed water tables depths falling within the model predicted standard error range respectively. Although all the SICVs are significant at $p < 0.01$, the NE model has the lowest correlation coefficient ($r = 0.588$ for the ACCROTELM reconstruction and $r = 0.550$ for the NI reconstruction). The best performing data combination is for the NI model predicting for ACCROTELM data, with 80.34% of the observed water table values falling within the model predicted values and these are highly correlated ($r = 0.827$, $p < 0.01$). However, a much greater proportion of the values falling outside the predicted value standard error are wetter than predicted (17.09% versus 2.56% drier). This is an effect of the very dry observed values for *H. subflava* encountered in the NI training set. It is worth noting that the ACCROTELM transfer function was originally tested using the British training set of Woodland et al. (1998) as independent data. The ACCROTELM model predicted well for the British data, but not in reverse which was attributed to testate amoebae community variance at a sub-continental scale, or much narrower hydrological ranges in the British data due to annual-average hydrological data. The SICV tests for the NE transfer function observed here appear to support the community variance hypothesis and may well extend to the sub-regional as well as sub-continental scale.

The hydrological optima and tolerance values for many taxa from the Northern England region equate well with the NI and ACCROTELM figures with regard to wet indictor taxa such as *Amphitrema wrightianum, Difflugia lucida* type, *Nebela carinata* and *Difflugia bacillifera* type. Toward the drier end of the scale *Trigonopyxis arcula* type, *Bullinularia*

indica and *Corython-Trinema* type also compare favourably. *Hyalosphenia subflava* consistently resides at the driest end of the scale in terms of moisture content in all three studies, though occupies a less extreme position in the water table in the Northern England data (Supplementary file 5). The position of *Difflugia pulex* at the driest end of both water table and moisture content ranges in the Northern England data is highly questionable as this taxon was present in only a single sample retained in both models. The ACCROTELM and NI species-environment models found this taxon to occupy a moderate position in the water table and moderate-to- dry in the moisture content models, and represents the best modern analogues to date due to the high numbers of samples containing *D. pulex* (ACCROTELM WTD $n = 26$, MC $n = 36$; NI WTD $n = 16$, MC $n = 18$).

The Northern England transfer functions have produced hydrology optima and tolerance statistics for a number of taxa not published from the NI or ACCROTELM studies, giving a broader understanding of species-environment relationships in British peatlands (Table 3). From the species RDA (Fig.3), a number of these taxa are associated with higher pH environments (*C. ampulla*, *L. spiralis*, *N. lageniformis*, *Q. symmetrica*, *S. lenta*, *T. dentata*, *T. lineare*) and reflect more minerotrophic conditions, substantiating the findings of Lamentowicz and Mitchell (2005) from North-West Poland. Notable taxa not encountered in the contemporary study are *Amphitrema stenostoma* and *Nebela marginata.* Two taxa, *Assulina scandinavica* and *Difflugia globulus* were lower taxonomic divisions from the grouping approach of Charman et al. (2000), the implications of which are discussed later.

4.1 Improving analogues

A prominent taxon in the NE transfer function is *Hyalosphenia subflava* which has up until this point been scarce in the UK training sets (Charman et al., 2007; Woodland et al., 1998) but frequently encountered in fossil assemblages from British palaeoenvironmental studies (e.g. Barber and Langdon, 2007; Blundell and Barber, 2005; Langdon et al., 2003). The hydrological data generated for this taxon here are much needed in the British training set as the NI and ACCROTELM water table depth optima-tolerance figures are dissimilar which is likely due to localised site variance. In the NE training set, *H. subflava* was encountered at all sites except Swarth Moor and therefore the hydrological preference data should be robust, providing significantly better modern analogues for Northern England and for *H. subflava* dominated palaeoecological samples from the region.

When considering the exclusion of particular taxa from transfer function training sets to improve model performance, some thought must be given to the question of test preservation. *Euglypha strigosa* was the third most common taxon encountered in the contemporary training set ranging from 0 to 46% of sample abundance, however the idiosomic tests of the this genus are rarely encountered below the acrotelm (aerobic zone) in many palaeoecological studies (cf. Mitchell et al., 2008b). The optima-tolerance statistics for the taxon in this study shows it favours the drier end of the hydrological gradient, though it occupies a more neutral position in the Northern Ireland and ACCROTELM species-environment models. Swindles et al. (2009) addressed the problem by running a palaeohydrological reconstruction with *Euglypha* spp. removed from dataset and found the water table reconstruction to be virtually identical to the one based on the entire fossil dataset. However, this only addresses presence-absence

in the entire profile rather than the degradation-depth issue. Therefore the presence of *E. strigosa* in the upper few centimetres of the Malham Tarn Moss peat sequence palaeohydrological reconstruction may falsely skew the water table reconstruction. We tested this hypothesis by selecting, in rank order, the top ten samples from the NE training set with the highest abundance of *Euglypha* taxa present. We used the NI and ACCROTELM transfer functions to predict the WTD for these ten samples, then re- ran the models on adjusted percentage values following removal of *Euglypha* taxa. The results show that there is little change in the prediction as bootstrap ranges overlap, and the predictions are highly correlated with each other (ACCROTELM $r = 0.979$, $p < 0.001$; NI $r = 0.910$, $p < 0.001$). However, the inferred WTD with *Euglypha* taxa removed were consistently slightly drier in this case.

An interesting observation with regard to the taxon classification of '*Euglypha* degraded' is apparent in the RDA data. The position of this 'taxon' in the species biplot suggests that test degradation within the genus *Euglypha* is associated with drier conditions (lower moisture content in particular). Previous work on the dissolution effects of increased acidity (Swindles and Roe, 2007) and increased acidity and desiccation (Payne, 2007) found that the genus *Euglypha* to be particularly susceptible to preferential degradation. The results of the RDA ordination may suggest that exposure to aerobic conditions (and therefore increased microbial activity) is a significant causal factor of degradation.

In this study, the sampling of wider environmental gradients than those usually associated with raised bogs has allowed modern analogues to be established for several less-common taxa. Such taxa may be important for interpreting bog development trajectories including pathways to ombrotrophy. Payne et al. (2011) offer a number of

recommendations to improve clustered training sets that we have followed here; 1) a large number of sites should ideally be sampled to reduce autocorrelation problems; 2) sites should be similar in terms of climate and vegetation; 3) there should be sufficient diversity in the sample sites to ensure there are good analogues for all fossil samples in the training set; 4) the full environmental gradient is sampled at each site. However, the encompassment of the full hydrological gradient at each site is somewhat weakened by the need to exclude outlier samples to achieve greater predictive power. Despite this, this study has examined the hydrological optima-tolerance values for a number of taxa not previously reported, particularly *Assulina scandinavica* (cf. Ogden and Hedley, 1980) and *Difflugia globulus* which have customarily been grouped in the morphospecies approach of Charman et al. (2000). In other studies, *Assulina seminulum* have shown hydrological optima of ~ 19 cm WTD and ~ 90% MC (Charman et al., 2007), ~ 20 cm WTD and 89% MC (Swindles et al., 2009), 20.4 cm WTD and 91.7% MC , (Bobrov et al., 1999), 28.1 cm WTD (Lamentowicz and Mitchell, 2005), ~ 6.5cm WTD and ~ 92% MC (Woodland et al., 1998). In this study the hydrological optima for *A. seminulum* was comparable with 20.1cm WTD and 90.2% MC, however *A. scandinavica* optima were 8.2 cm WTD and 94.7% MC demonstrating a very different hydrological niche to all other findings except Woodland et al. (1998) (however the proportion of *A. scandinavica* counted as *A. seminulum* in Woodland et al.'s dataset is unknown). The consequences of this in terms of palaeoecological reconstruction using the transfer function approach could well be a subtle one, nevertheless the environmental preferences of this taxon should be not be discounted.

Of perhaps greater importance is the inclusion of *Difflugia globulus* in the *Cyclopyxis arcelloides* type group. These taxa are grouped together as their key differentiating

identification feature is the pseudopodia of the living amoebae rather than test characteristic, and therefore problematic to separate in sub-fossil remains. Whilst the grouping of these taxa is therefore justified in palaeoecological studies, the extreme differences in hydrological niche of these taxa cannot be ignored; in this study *C. arcelloides* type optima are 23.6 cm WTD and 86.6% MC, whereas *D. globulus* optima are -3.9 cm WTD and 97.1% MC. This taxonomic problem may partly explain the contradictory environmental preference data for *C. arcelloides* type in published material (Booth, 2001; Charman, 1997; Lamentowicz and Mitchell, 2005; Tolonen et al., 1992, 1994; Woodland et al., 1998). The complications of isolating *D. globulus* from the *C. arcelloides* type group in palaeoecological samples may in part be addressed by reference to Bobrov et al. (1999) and test size related to moisture availability, and division to lower taxonomic level. Recent papers have sought to improve transfer functions through both the sampling and statistical methodology (Payne et al., 2011; Telford and Birks, 2011). While these approaches may well improve the precision of transfer function-based reconstructions it is unlikely that enhanced accuracy of these models is convincing while the fundamental issue of unclear taxonomy remains (Mitchell and Gilbert, 2010). Research on contemporary testate amoebae ecology should adopt a cautious approach to 'over-grouping' taxa and be clear about the application of the results. Care should be exercised when interpreting the assemblage when encountering this taxon in palaeohydrological reconstruction, and close attention should be given to the other taxa sharing the spatio-temporal location.

Despite the critical assessment of the use of testate amoebae-based transfer functions to quantitatively reconstruct palaeohydrology in ombrotrophic peatlands presented here, they nevertheless remain a very powerful tool in understanding past climatic changes.

Comparisons of the NE, NI and ACCROTELM models demonstrate that although there are differences in the performance statistics of the models, the reconstructed direction and magnitude of hydrological changes from palaeoecological data are broadly similar (within reconstruction errors) and strongly correlated. Although the biogeography of testate amoebae has been shown to be broadly comparable on a supra-regional scale, site-specific factors clearly play a significant role in testate amoebae community composition and remain challenging to unravel. The use of contemporary data from one region to infer hydrological values at another should be approached with caution, and wherever practicable local data should be included in the transfer function. Equally, it is possible that for older fossil samples, the best analogues may be found in contemporary samples from other regions, where the modern climate and conditions are more similar to those under which the peat originally formed. Comparing outputs from different independent transfer functions is a useful way of assessing reconstruction robustness. Quantifying the effects of testate amoebae taxonomy and preservation in fossil assemblages remains problematic and fundamental to the accuracy and precision of transfer function output.

Peatland water table response to changes in climate is complex and dependant on a number of variables including, but not limited to; hydraulic conductivity, peat mass, porosity, dry bulk density, water table height, bog surface height, decay rate, underlying substrate and lateral extent (Baird et al., 2008; Baird et al., 2011; Frolking et al., 2010; Holden, 2005; Morris et al., 2011). There has recently been a concerted effort to improve the understanding of raised bog hydrological response to climatic factors through manipulation of a peatland development model (Swindles et al., submitted). This work suggests that peatland water table reconstructions should not be considered

as a simple metric of climate. Despite this, clear spatially-replicated relationships between testate amoebae and hydrology have been found and the consistency of hydrological direction (wet shift/dry shift) is robust and remains a powerful tool for understanding Holocene climatic changes.

5. Conclusions

1. A contemporary testate amoebae training set and associated environmental variables from six peatland sites within a similar climatic region of Northern England were analysed using multivariate methods. In agreement with other studies, hydrology (water table depth and surface moisture content) was found to be the dominant environmental control.

2. Transfer functions to predict water table depth and moisture content were developed and rigorously tested using spatially independent data from Northern Ireland and Europe. It was demonstrated that palaeoenvironmental reconstructions from the three models were consistent in terms of wet/dry shifts. Variation in the magnitudes of reconstructed water tables suggest site-specific errors are difficult to eliminate and therefore caution should be used when applying transfer function reconstructions from one region to another.

3. The inclusion of a wide range of mire sites and environmental variables in Northern England has increased the range of modern analogues for this region. Previously unquantified taxa, in terms of hydrological preference include *Arcella gibbosa* type, *Arcella hemispherica*, *Assulina scandinavica*, *Cryptodifflugia oviformis*, *Cyphoderia ampulla*, *Difflugia globulus*, *Difflugia lanceolata*, *Hyalosphenia ovalis, Lesquereusia spiralis, Nebela lageniformis, Quadrulella symmetrica, Sphenoderia lenta, Tracheleuglypha dentata*, and *Trinema lineare.* Of

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particular value is the large number of samples containing *Hyalosphenia subflava* as this taxon was not previously reported from British sites and is often found in palaeoecological samples in the UK. This study provides the best modern analogue for this taxon in Britain to date.

4. There remains a level of complexity to testate amoebae taxonomy and grouping is valid for certain taxa, nevertheless it has been shown that further splitting of some taxa is possible and indeed warranted when such disparate ecological niches are apparent.

Acknowledgments

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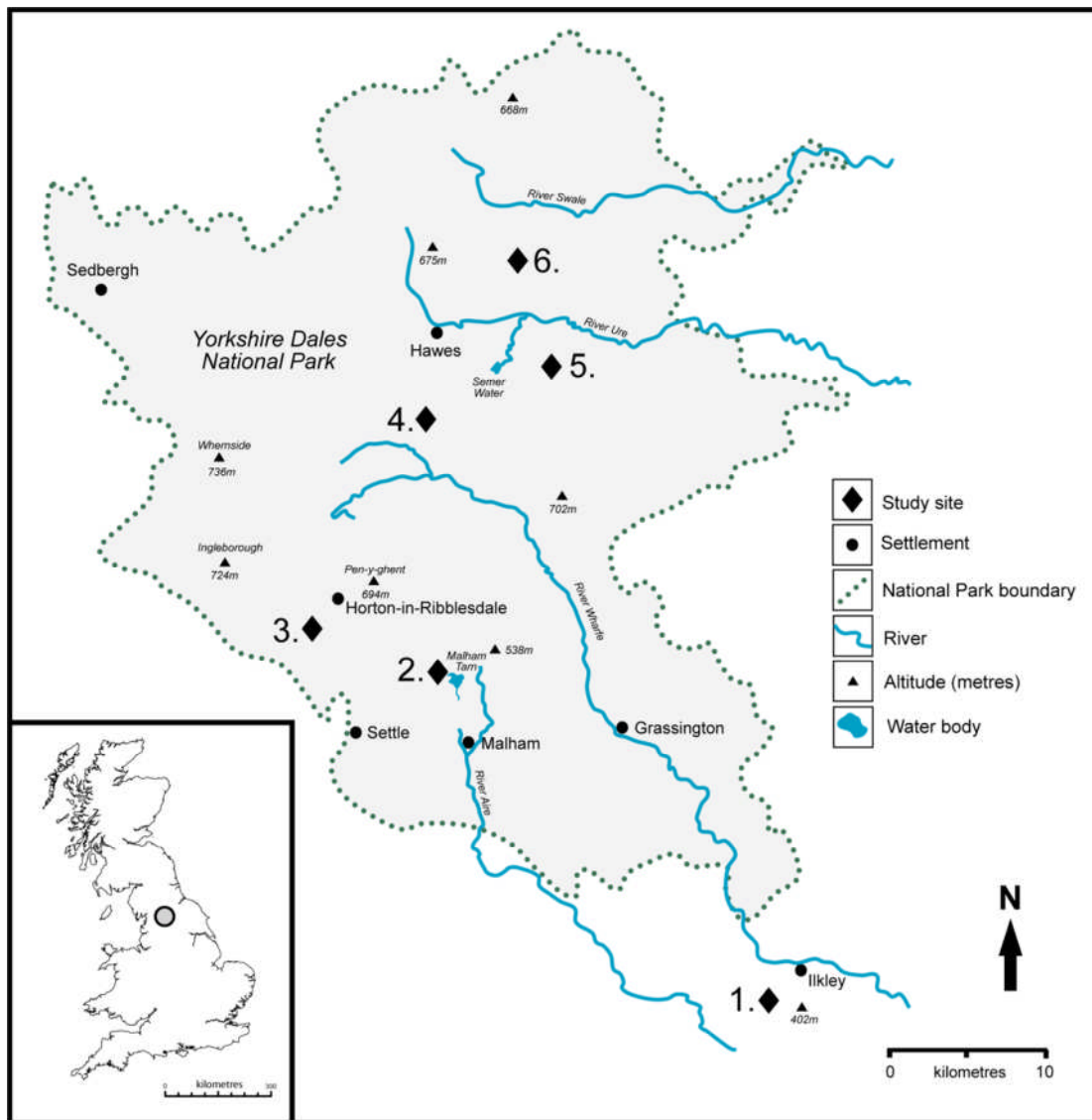
Fig.1. Map showing the location of study sites: 1. Ilkley Moor; 2. Malham Tarn Moss; 3. Swarth Moor; 4. Fleet Moss; 5. Thornton Mire; 6. Oxnop Moor.
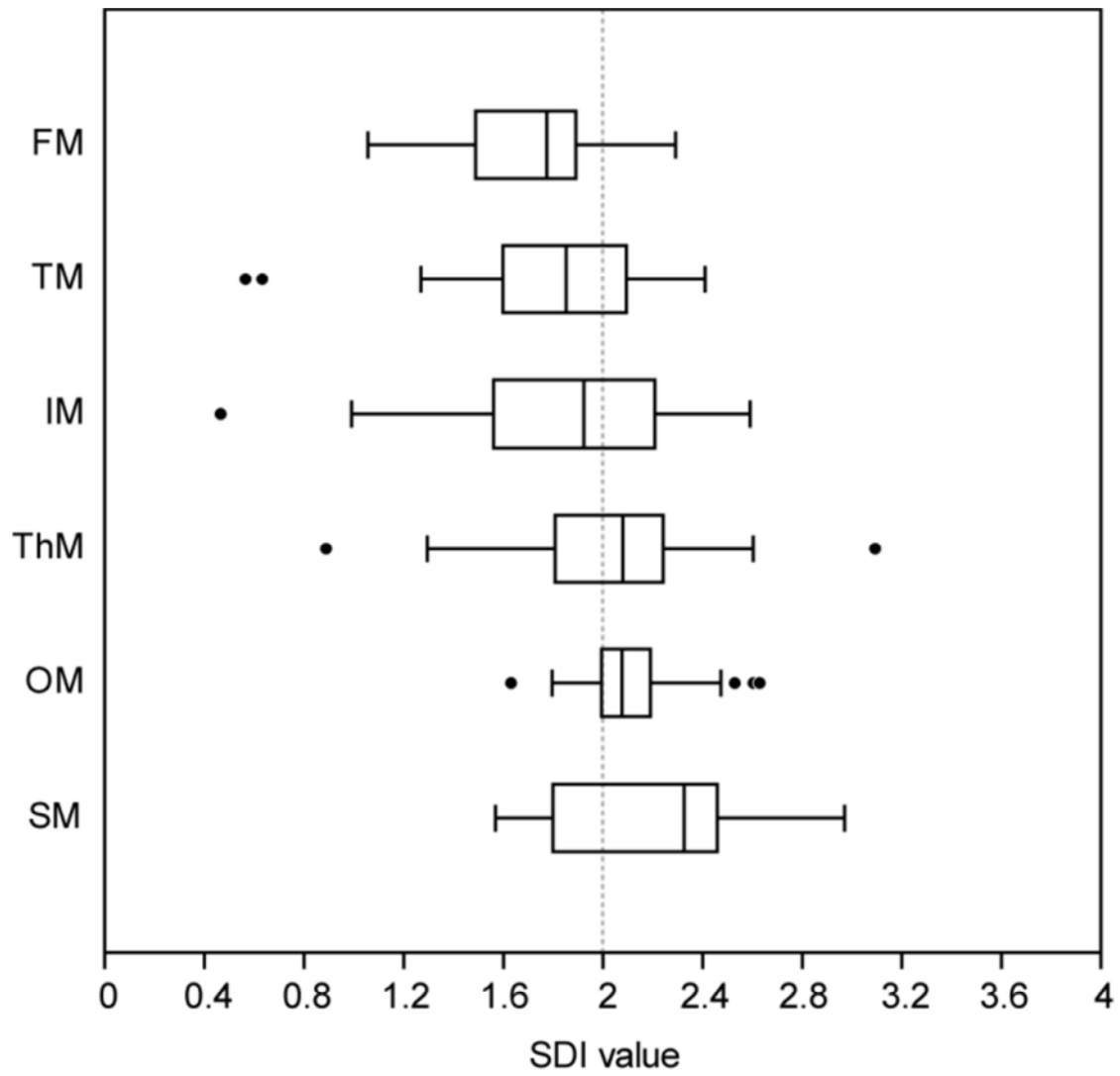
Fig. 2. Boxplots of Shannon diversity index values by site. *FM*: Fleet Moss, *TM*: Tarn Moss, *IM*: Ilkley Moor, *ThM*: Thornton Mire, *OM*: Oxnop Moor, *SM*: Swarth Moor.

Fig.3. Redundancy analysis biplot of (a) taxa and environmental variables; (b) samples and environmental variables. Species data are Hellinger-transformed. Species codes: **AMWR** *Amphitrema wrightianum*; **ARAR** *Arcella artocrea*; **ARCA** *Arcella catinus* type; **ARDI** *Arcella discoides* type; **ARGI** *Arcella gibbosa* type; **ARHE** *Arcella hemispherica*; **ARVU** *Arcella vulgaris*; **ARFL** *Archerella flavum*; **ASMU** *Assulina muscorum*; **ASSC** *Assulina scandinavica*; **ASSE** *Assulina seminulum*; **BUIN** *Bullinularia indica*; **CEAC** *Centropyxis aculeata* type; **CECA** *Centropyxis cassis* type; **CEPL** *Centropyxis platystoma* type; **COTR** *Corythion-Trinema* type; **CROV** *Cryptodifflugia oviformis*; **CYAR** *Cyclopyxis arcelloides* type; **CYAM** *Cyphoderia ampulla*; **DIAC** *Difflugia acuminata*; **DIBM** *Difflugia bacillariarum*; **DIBA** *Difflugia bacillifera*; **DIGL** *Difflugia globulus*; **DILA** *Difflugia lanceolata*; **DILE** *Difflugia leidyi*; **DILU** *Difflugia lucida* type; **DIOB** *Difflugia oblonga* type; **DIPR** *Difflugia pristis* type; **DIPU** *Difflugia pulex*; **DIRU** *Difflugia rubescens*; **DIUR** *Difflugia urceolata*; **EUCI** *Euglypha ciliate*; **EUCO** *Euglypha compressa*; **EUCR** *Euglypha cristata*; **EURO** *Euglypha rotunda* type; **EUST** *Euglypha strigosa*; **EUTU** *Euglypha tuberculata* type; **EUDE** *Euglypha* type degraded; **HEPE** *Heleopera petricola*; **HERO** *Heleopera rosea*; **HESP** *Heleopera sphagni*; **HESY** *Heleopera sylvatica*; **HYEL** *Hyalosphenia elegans*; **HYOV** *Hyalosphenia ovalis*; **HYPA** *Hyalosphenia papilio*; **HYSU** *Hyalosphenia subflava*; **LESP** *Lesquereusia spiralis*; **NECA** *Nebela carinata*; **NECO** *Nebela collaris*; **NEFL** *Nebela flabellulum*; **NEGR** *Nebela griseola* type; **NELA** *Nebela lageniformis*; **NEMI** *Nebela militaris*; **NEMR** *Nebela minor*; **NEPA** *Nebela parvula*; **NETI** *Nebela tincta*; **NETU** *Nebela tubulata*; **NETB** *Nebela tubulosa* type; **NEVI** *Nebela vitraea* type; **PLSP** *Placocista spinosa* type; **PLCA** *Plagiopyxis callida*; **PSFU** *Pseudodifflugia fulva* type; **QUSY** *Quadrulella symmetrica*; **SPLE** *Sphenoderia lenta*; **TRDE** *Tracheleuglypha dentate*; **TRAR** *Trigonopyxis arcula* type; **TRLI** *Trinema lineare.*
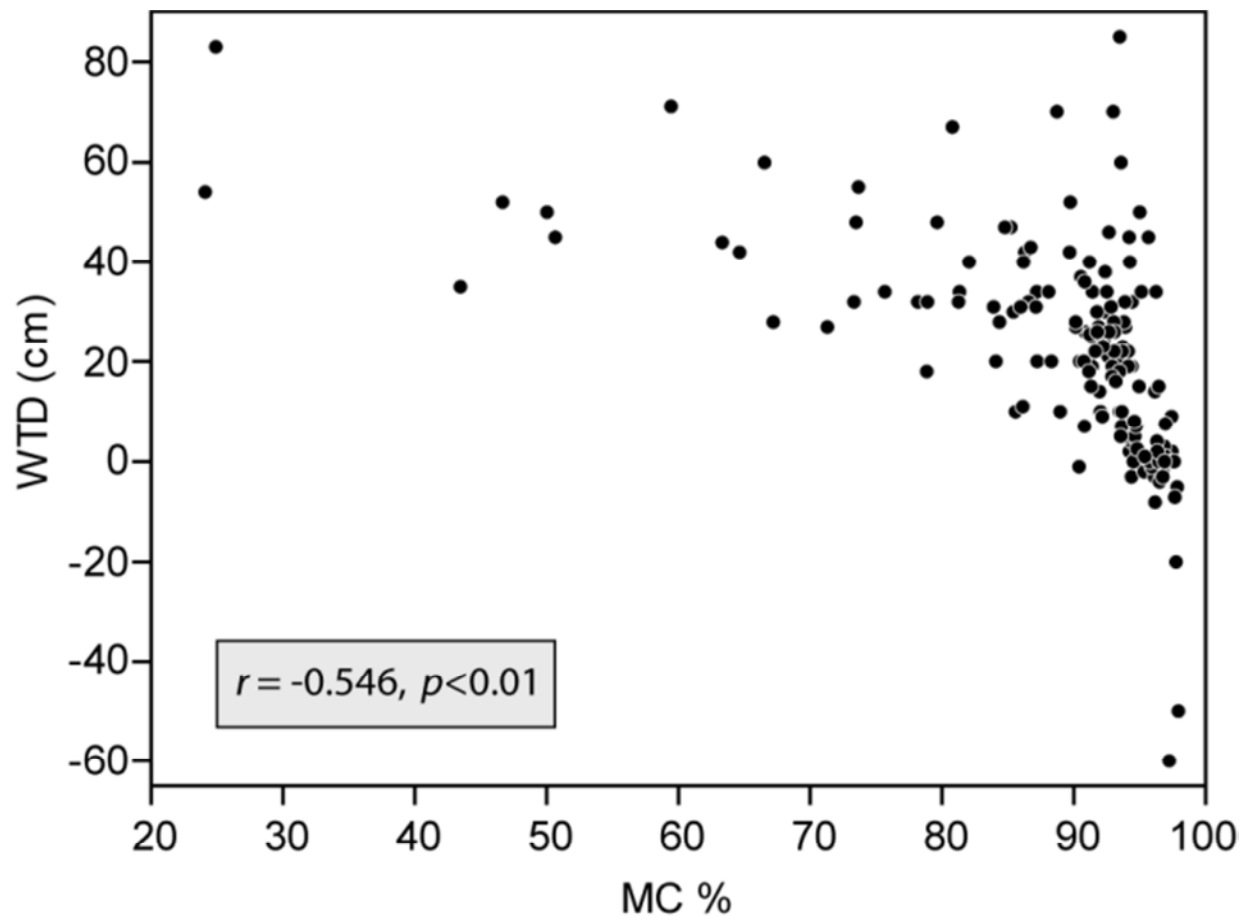
Fig. 4. Scatterplot of percentage moisture content against water table depth for each sample in the Northern England training set. The relation of a Pearson correlation analysis of the two variables is shown.
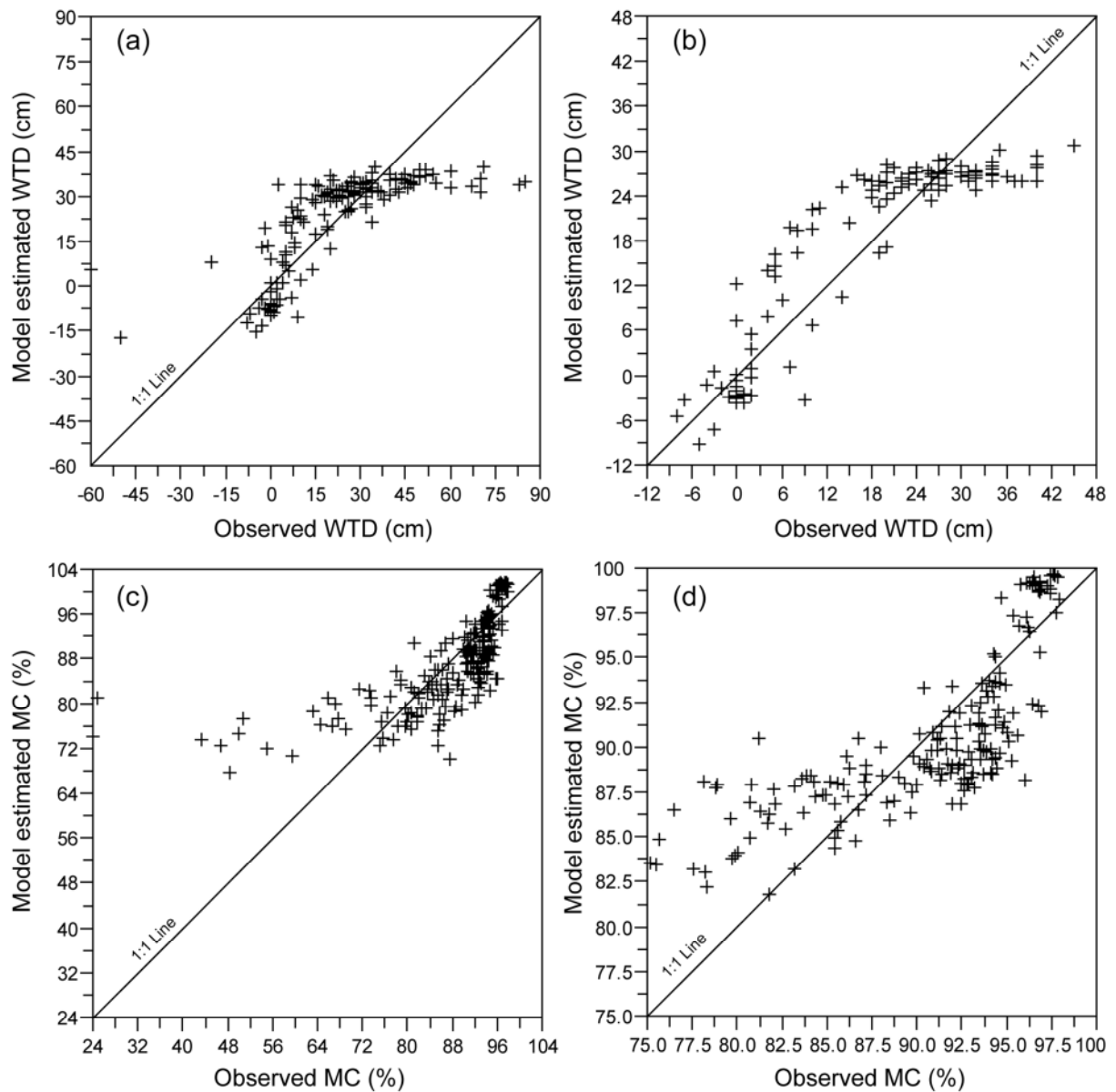
Fig. 5. Observed against model-estimated water table and moisture content values in the Northern England transfer function; (a) original water table data; (b) screened water table data; (c) original moisture content data; (d) screened moisture content data). The 1:1 relationship line is shown in each case.
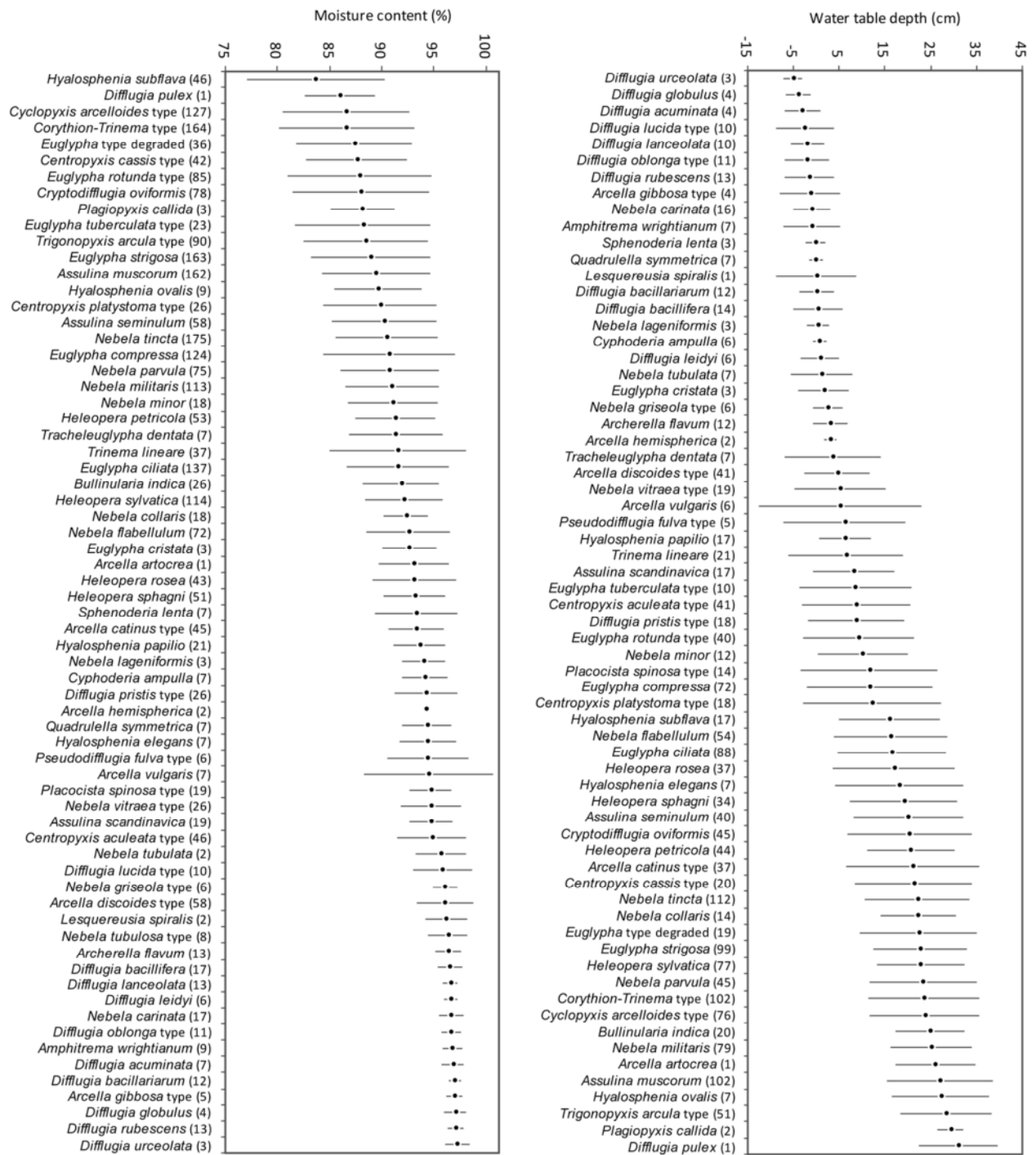
Fig. 6. Tolerance and optima statistics for taxa in the Northern England transfer function; (a) water table depth (cm); (b) moisture content (%). The number of samples containing the taxa are shown in brackets.

Fig. 7. Percentage testate amoebae diagram from Malham Tarn Moss, Northern England. Water table reconstructions based on the Northern England, Northern Ireland and ACCROTELM European transfer functions are shown. Sample-specific errors are based on 1,000 bootstrap cycles.
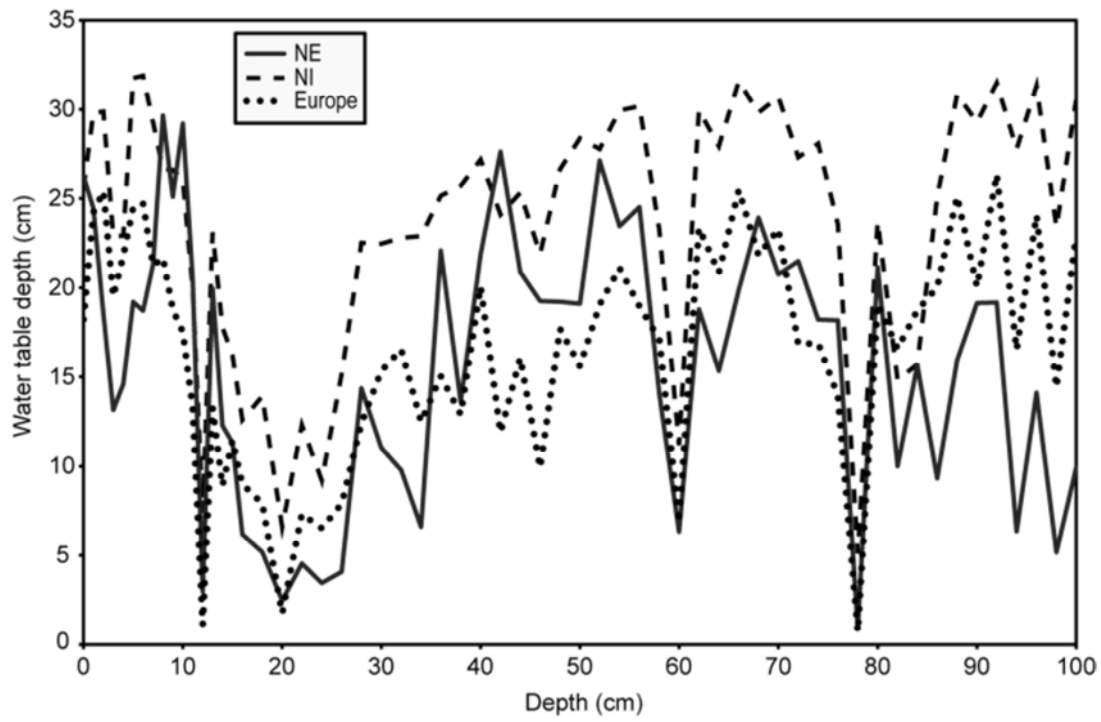
Fig. 8. Water table reconstruction based on Northern England, Northern Ireland and Accrotelm transfer functions plotted on the same axis. Error ranges have been removed for diagrammatic clarity.
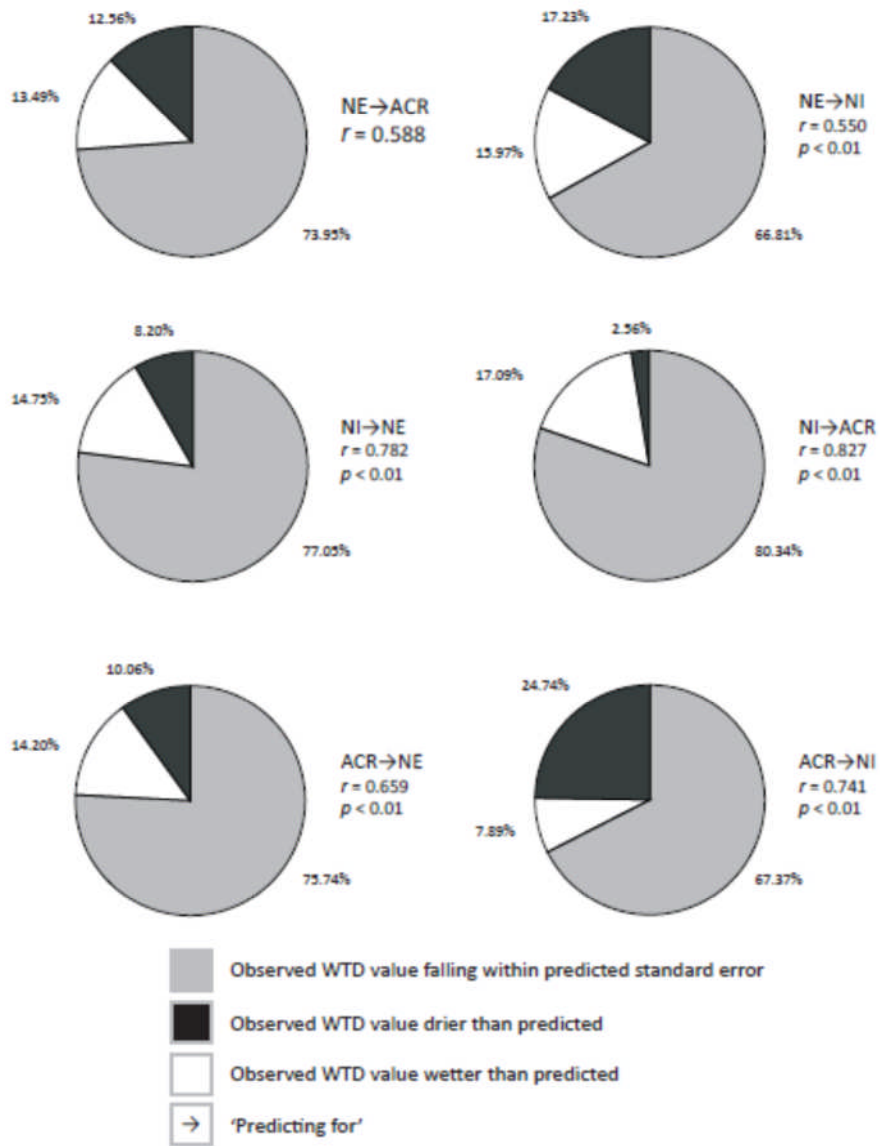
Fig. 9. Pie charts showing the results of the spatially independent cross-validation (SICV) analysis.

Table.1. Characteristics of study sites.

Site	Classification	Latitude	Longitude	Altitude m (ASL)	Surface samples (n)	Water table depth (cm)	Moisture content (%)	pH range (mean)	Conductivity (μS cm^{-1} at 20°C)
Fleet Moss	Blanket bog	54°14′47″N	002°12′34″W	560	30	-7 to 70	50.0 to 97.6	3.8 to 5.5 (4.4)	20 to 125
Ilkley Moor	Blanket bog	53°53′39″N	001°49′27″W	402	60	-3 to 20	48.4 to 96.7	3.6 to 7.3 (4.4)	27 to 359
Malham Tarn Moss	Raised bog	54°05′47″N	002°10′30″W	380	37	-60 to 83	24.1 to 98.0	3.7 to 6.4 (4.5)	31 to 155
Oxnop Moor	Blanket bog	54°20′44″N	002°05′46″W	505	30	0 to 44	63.3 to 96.7	3.4 to 4.1 (3.7)	71 to 121
Swarth Moor	Raised bog	54°07′17″N	002°17′51″W	227	20	-8 to 48	79.6 to 97.8	4.2 to 5.6 (4.9)	36 to 126
Thornton Mire	Raised bog	54°16′41″N	002°04′17″W	390	30	-3 to 85	43.4 to 97.6	3.8 to 5.4 (4.4)	36 to 133

Table.2. Transfer function performance statistics.

Water table depth (cm)

Total dataset

n = 159	WA Inv	WA Cla	**WA-Tol Inv**	WA-Tol Cla	WAPLS 1	WAPLS 2	WAPLS 3	ML
Jack r^2	0.48	0.48	**0.50**	0.51	0.48	0.50	0.45	0.57
Jack Average bias	-0.08	-0.11	**0.51**	0.95	-0.08	0.02	0.34	-3.36
Jack Maximum bias	49.70	35.55	**51.40**	35.01	49.72	48.06	54.83	44.74
RMSEP	15.43	20.13	**15.02**	19.96	15.42	15.21	16.23	15.61

Residuals > 14cm removed

n = 116	WA Inv	WA Cla	**WA-Tol Inv**	WA-Tol Cla
Jack r^2	0.62	0.63	**0.71**	0.71
Jack Average bias	0.14	0.23	**0.61**	0.82
Jack Maximum bias	14.38	10.38	**13.62**	11.02
RMSEP	8.27	9.83	**7.36**	8.32

Moisture content (%)

Total dataset

n = 207	WA Inv	WA Cla	WA-Tol Inv	WA-Tol Cla	WAPLS 1	**WAPLS 2**	WAPLS 3	ML
Jack r^2	0.36	0.37	0.42	0.42	0.36	**0.42**	0.41	0.50
Jack Average bias	-0.02	-0.04	-0.23	-0.49	-0.02	**-0.04**	-0.12	1.61
Jack Maximum bias	57.34	49.23	55.04	46.65	57.34	**55.51**	54.06	40.82
RMSEP	9.55	14.93	9.12	13.03	9.55	**9.10**	9.23	9.75

Residuals > 10% removed

n = 183	WA Inv	WA Cla	**WA-Tol Inv**	WA-Tol Cla
Jack r^2	0.49	0.49	**0.54**	0.54
Jack Average bias	-0.03	-0.05	**-0.07**	-0.12
Jack Maximum bias	9.77	5.71	**9.42**	5.51
RMSEP	4.07	5.52	**3.88**	5.00

Table.3. Optima-tolerance figures for previously unpublished taxa.

Taxon	WTD opt (cm)	WTD tol (±)	*n* (WTD)	MC opt (%)	MC tol (±)	*n* (MC)
Arcella gibbosa type	-1.4	6.7	5	97.0	0.7	4
Arcella hemispherica	3.0	1.4	2	94.3	0.1	2
Assulina scandinavica	8.2	9.0	19	94.7	2.0	17
Cryptodifflugia oviformis	20.3	13.5	78	88.0	6.5	45
Cyphoderia ampulla	0.7	1.5	7	94.1	2.1	6
Difflugia globulus	-3.9	2.7	4	97.1	1.0	4
Difflugia lanceolata	-2.0	3.7	13	96.6	0.7	10
Hyalosphenia ovalis	27.1	10.6	9	90.0	4.1	7
Lesquereusia spiralis	0.0	8.7	2	96.2	1.9	1
Nebela lageniformis	0.3	2.4	3	94.0	2.0	3
Placocista spinosa type	11.6	14.9	19	94.7	1.9	14
Quadrulella symmetrica	-0.1	1.6	7	94.3	2.3	7
Sphenoderia lenta	-0.2	2.2	7	93.2	3.9	3
Tracheleuglypha dentata	3.5	10.5	7	91.3	4.4	7
Trinema lineare	6.4	12.4	37	91.5	6.5	21

Supplementary file 1. Percentage abundances of testate amoebae and environmental data for each site.

Supplementary file 2. Table of RDA results.

Supplementary file 3. Transfer function model C2 file.

Supplementary file 4. SICV tests.

Supplementary file 5. Tolerance and optima model comparisons.

CONTACT AUTHOR FOR SUPPLEMENTARY MATERIAL