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Development of a new pan-European testate amoeba transfer function for reconstructing peatland palaeohydrology

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Abstract

In the decade since the first pan-European testate amoeba-based transfer function for peatland palaeohydrological reconstruction was published, a vast amount of additional data collection has been undertaken by the research community. Here, we expand the pan-European dataset from 128 to 1799 samples, spanning 35° of latitude and 55° of longitude. After the development of a new taxonomic scheme to permit compilation of data from a wide range of contributors and the removal of samples with high pH values, we developed ecological transfer functions using a range of model types and a dataset of ~1300 samples. We rigorously tested the efficacy of these models using both statistical validation and independent test sets with associated instrumental data. Model performance measured by statistical indicators was comparable to other published models. Comparison to test sets showed that taxonomic resolution did not impair model performance and that the new pan-European model can therefore be used as an effective tool for palaeohydrological reconstruction. Our results question the efficacy of relying on statistical validation of transfer functions alone and support a multi-faceted approach to the assessment of new models. We substantiated recent advice that model outputs should be standardised and presented as residual values in order to focus interpretation on secure directional shifts, avoiding potentially inaccurate conclusions relating to specific water-table depths. The extent and diversity of the dataset highlighted that, at the taxonomic resolution applied, a majority of taxa had broad geographic distributions, though some morphotypes appeared to have restricted ranges.

Keywords: Testate amoeba, peatland, water table, transfer function, Europe, spatial scale, data compilation, taxonomy.

Highlights

- A vastly expanded dataset of European peatland testate amoeba samples is compiled;
- A new taxonomic scheme is developed to facilitate data compilation;
- Palaeohydrological transfer functions are tested statistically and against independent data;
- The new model is an effective tool for palaeohydrological reconstruction across Europe;
- Model outputs should be standardised and presented as residual values.

Introduction

Testate amoebae are microscopic, unicellular shelled protozoa that are abundant in a range of wetlands, including peatlands (Mitchell et al., 2008). Early research demonstrated the close ecological coupling between testate amoebae and hydrological parameters such as water-table depth and moisture content in such environments (e.g. Jung, 1936; Schönborn, 1963). Quantitative ecological approaches demonstrated the strength of this relationship and used it to derive reconstructions of hydrological variability from fossil testate amoebae (Warner and Charman, 1994; Woodland et al., 1998). This approach has subsequently been thoroughly developed and extended geographically, using more advanced statistical techniques (e.g. Charman et al., 2007; Booth, 2008; Swindles et al., 2009, 2014, 2015a; Amesbury et al., 2013). Testate amoeba-based hydrological reconstructions are now frequently used as hydroclimate proxies in studies of Holocene climate change (e.g. Charman et al., 2006; Swindles et al., 2010; Elliott et al., 2012; Lamentowicz et al., 2015; Willis et al., 2015). Central to such research is typically the application of a transfer function. These statistical models apply the observed modern ecological preferences of amoebae via a range of mathematical approaches (Juggins and Birks, 2011) to fossil assemblages to quantitatively reconstruct environmental variables of interest, primarily water-table depth in ombrotrophic peatlands, but occasionally other parameters such as pH (Markel et al., 2010; Mitchell et al., 2013). Testate amoeba-based hydrological transfer functions have now been developed in a wide range of locations (e.g. Li et al., 2015; Swindles et al., 2015a, 2014; van Bellen et al., 2014) and wetland types, primarily in bogs, but also in fens (Payne, 2011; Lamentowicz et al., 2013a; Lamentowicz et al., 2013b). Recent debates in this field have focussed on 1) more rigorous analysis of transfer function results, whether via statistical testing (Telford and Birks, 2005, 2009, 2011a, 2011b, Payne et al., 2012, 2016; Amesbury et al., 2013), or by comparison with instrumental data (Swindles et al., 2015b); 2) the appropriateness of varying spatial scales for transfer function development (Turner et al., 2013); and 3) the validity of applying models outside of the geographic range over which they were developed (Turner et al., 2013; Willis et al., 2015), and hence the cosmopolitanism of testate amoeba ecological preferences (Booth and Zygmunt, 2005) across a range of geographical locations (Smith et al., 2008).

When transfer function models developed in one region are applied in a different region where no local model exists, results may theoretically be undermined by a number of factors. These include missing modern analogues, differences in testate amoeba ecology or biogeography between the two regions (Turner et al., 2013), the technique used to measure water-table depth in the calibration data sets (Markel et al., 2010; e.g. long-term mean versus one-off measurement), regionally diverse seasonal variability (Sullivan and Booth, 2011; Marcisz et al., 2014) or vertical zonation (van Bellen et al., 2014) of testate assemblages, or local-scale variability in the response of certain taxa, or even communities, of testate amoebae to environmental variables (e.g. Booth and Zygmunt, 2005). However, in practice, when transfer functions from one region are applied to fossil data from a separate region, even over distances of thousands of kilometres (Turner et al., 2013; Willis et al., 2015), or when regional- and continental-scale models are compared (e.g. Amesbury et al., 2008; Charman et al., 2007; Swindles et al., 2009; Turner et al., 2013), it is largely only the absolute values and magnitude of reconstructed water-table shifts that vary between models, with the timing and direction of change being generally consistent. Given that the absolute values and magnitude of transfer function-reconstructed change in water-table depth have recently been questioned by direct comparison of reconstructed and instrumental water-table depths (Swindles et al., 2015b), it could be argued that a) testate amoeba-based transfer function reconstructions should be viewed as semi-quantitative and interpretation should be based only on the timing and direction of change; and that b) the general ecological cosmopolitanism of testate amoebae (e.g. Mitchell et al., 2000; Booth and Zygmunt, 2005) when studied at coarse taxonomic level (i.e. morphotypes – but see Heger et al., 2013 for an example of cryptic diversity showing geographical patterns) means that regional transfer functions are widely applicable, at least at an intra-continental or even intra-hemispheric scale.

Approaching a decade after the publication of the first testate amoeba-based pan-European transfer function (Charman et al., 2007), which included 128 samples from seven countries, we present a new collaborative effort to vastly extend that dataset, including both published and unpublished data that increases the number of samples to 1799, from a much expanded geographical range covering 18 countries spaced over 35° of latitude and 55° of longitude. In doing so, we develop a new transfer function for peatland testate amoeba palaeohydrological reconstruction and shed new light on the biogeography and cosmopolitanism of testate amoebae and the potential effects of varying spatial scales and supra-regional application on resulting transfer function reconstructions. We rigorously test our newly developed models using a novel combination of statistical validation and checks against independent testate amoeba data with associated instrumental water-table depth measurements. Ultimately, we aim to facilitate more reliable comparisons of spatial and temporal patterns of peatland-derived palaeoclimate records at a continental scale.

Methods

Data compilation and taxonomy

We compiled a full dataset containing 1799 samples from 113 sites in 18 countries from 31 published studies, with contributions of unpublished data from two countries (Table 1; Figure 1). All samples in the dataset had an associated water-table depth value, whereas a reduced number (n=1564) also had an associated pH value.

[INSERT FIGURE 1]

Figure 1: Site locations (see Table 1 for more site details). Sites are coloured by eco-region: Atlantic = red, Scandinavia = green; Continental = blue. For reference to colour, readers are referred to the online version of this article.

Although the potential risks of taxonomic inconsistency, especially in large data compilations with large numbers of analysts, are clear (Payne et al., 2011), the likely effect of using a low taxonomic resolution potentially decreased model performance (in statistical terms) rather than any effect on the timing or direction of major changes in wetness (Mitchell et al., 2014). Due to the high number of data contributors/analysts in this compilation and in order to ensure taxonomic consistency across the merged dataset, we adopted a low-resolution approach to defining an appropriate taxonomic scheme, merging morphologically similar taxa together into a series of newly defined groups. Initial examination of contributed datasets made it clear that different analysts had grouped (or ‘lumped’) or split taxa to varying extents, with many taxa only present in individual datasets. A low-resolution approach to taxonomy was therefore considered to be not only the most parsimonious, but also the only scientifically valid approach to the compilation of such a large dataset, despite genuine variation in water-table optima occurring between taxa within some new groupings (see Results). Individual analysts should not count new samples in line with the low-resolution taxonomic scheme applied here, but rather differentiate between readily identifiable taxa in line with current taxonomies and group taxa together only for statistical analysis. The majority of recently published papers on peatland testate amoebae use Charman et al. (2000) as a standard identification guide, with an increasing number of variations noted in recent years including, most prevalently, the reclassification of *Amphitrema flavum* as *Archerella flavum* (Loeblich and Tappan, 1961), the splitting out of certain ‘type’ groupings into their constituent taxa (e.g. *Cyclopyxis arcelloides* type into *Cyclopyxis arcelloides sensu stricto*, *Phryganella acropodia* and *Difflugia globulosa*; Turner et al., 2013) and more recent reclassifications based on phylogenetic studies (e.g. *Nebela* taxa moving to the genera *Longinebela*, *Planocarina* and *Gibbocarina*; Kosakyan et al., 2016).

Across all 1799 samples in the full dataset, a total of 186 individual taxa were identified, with the final taxonomic scheme containing a reduced 60 taxa, of which 41 were ‘type’ groupings (38 newly defined) that each contained between two and 11 taxa with similar morphological features (Table 2). These groups were defined with reference to a range of identification keys and source literature (Cash and Hopkinson, 1905, 1909, Cash et al., 1915, 1918; Ogden and Hedley, 1980; Meisterfeld, 2000a, 2000b) as well as using the expertise and experience of the authors. Our treatment of the two *Euglypha* groups – *E. ciliata* type and *E. rotunda* type – provides an example of the low resolution approach we adopted. These groups contained 11 and eight individual taxa respectively that had been identified by individual analysts in the originally contributed datasets. However, the only morphological characteristic that we could identify as consistently applied across all datasets was size, with several datasets only defining *E. tuberculata* (i.e. larger type >45 μm) and *E. rotunda* (i.e. smaller type <45 μm). Since the presence/absence of spines (e.g. *E. strigosa* vs. *E. tuberculata*) may be biased by taphonomic processes (Payne et al., 2011), we therefore defaulted to a two-taxon system for this family.

When all data were compiled using this new taxonomy, taxa which occurred in <18 samples (i.e. 1% of the data) were excluded as rare taxa (n=8; Table 3), resulting in a total of 52 taxa in the 'edited' dataset. With the exception of *Cyphoderia* sp., *Placocista* sp. and *Trigonopyxis* sp., which were included in *Cyphoderia ampulla* type, *Placocista spinosa* type and *Trigonopyxis arcula* type respectively (groupings which contained all potential examples of these genera), all individuals defined only to the family level were also excluded from the dataset. Where this process resulted in a total assemblage <90% of the original total count, we excluded whole samples from the full dataset (n=24, Table 4), resulting in a total of 1775 samples in an 'edited' dataset (Figure 2). Transfer function development proceeded from this 'edited' dataset. Hereafter, this 'edited' dataset will be referred to as the full dataset.

[INSERT FIGURE 2]

Figure 2: Percentage distribution of all taxa. Taxa are ordered from 'wet' on the left to 'dry' on the right based on the taxa optima from the WA-Tol (inv) model of the full dataset (n = 1775).

Statistics

Since the full dataset contained samples from a range of different peatland types on a continuum between more oligotrophic bogs to more eutrophic fens (range in pH values of 2.5 – 8.1), and in light of the overarching aim of this study to produce a transfer function for palaeohydrological reconstruction, we initially used exploratory ordination analyses (non-metric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity) to objectively reduce the dataset to those samples more representative of the nutrient poor, ombrotrophic peatlands commonly used in palaeoclimate research. We applied a high pH cut-off based on NMDS axis one scores and k-means cluster analysis (for additional details see ordination results). All analyses were carried out in R version 3.2.2 (R Core Team, 2015) using the packages vegan (Oksanen et al., 2015) for NMDS and cluster analysis and pvclust for significance testing between clusters (Suzuki and Shimodaira, 2014).

Transfer function development was also carried out in R (R Core Team, 2015) using the package rioja (Juggins, 2015), applying four commonly used model types, namely: weighted averaging (WA; with and without tolerance downweighting (WA-Tol)), weighted average partial least squares (WAPLS), maximum likelihood (ML) and the modern analogue technique (MAT). In each case, only results of the best performing (judged by root mean square error of prediction (RMSEP) and R^2) model within each type are shown. RMSEP values were calculated using the standard leave-one-out ($RMSEP_{LOO}$) technique, as well as leave-one-site out ($RMSEP_{LOSO}$; Payne et al., 2012) and segment-wise ($RMSEP_{SW}$; Telford and Birks, 2011b) approaches. Spatial autocorrelation tests were calculated in the R package palaeoSig (Telford, 2015) using the 'rne' (random, neighbour, environment) function.

To test the applicability of the new model, we applied it to 1) downcore independent test data from a long-term (~6000 years) record from Tor Royal Bog (TRB) in Dartmoor, UK (Amesbury et al., 2008), 2) a simulated palaeo dataset developed from surface samples with associated automated instrumental water-table depth measurements, ordered to 'create' two major shifts in water-table depth (Swindles et al., 2015b) and 3) downcore independent test data from a short-term record from Männikjärve Bog, Estonia with associated automated instrumental water-table data (Charman et al., 2004). For test sets 2 and 3, we used annual and summer (JJA) mean water-table depth values in each case, calculated from multiple daily measurements (for full details see source publications). Sample-specific errors for the transfer function reconstructions were based on 1000 bootstrapping cycles. We compared our reconstructions with output from the previous European transfer function (Charman et al., 2007) and tested the significance of the new reconstructions using the 'randomTF' function in palaeoSig (Telford, 2015).

We used the programme PAST (version 3.10; Hammer et al., 2001) to run one-way PERMANOVA tests (9999 iterations) of the differences between samples from different countries and three assigned eco-regions (Atlantic, n=461; Scandinavia, n=341; Continental, n=500; Figure 1) that represented broadly different climate zones and degrees of oceanicity/continentality.

Results

Ordination

NMDS of the full dataset (n=1775; Figure 3A and B) showed that the primary environmental variable explaining species distribution along axis 1 was pH, as opposed to water-table depth, illustrating the influence of the peatland type gradient (i.e. ombrotrophic to minerotrophic). A distinct group of samples formed an outlying cluster with high NMDS axis 1 scores. To determine an appropriate pH cut-off to reduce the dataset to those containing nutrient poor, ombrotrophic peatlands, we used results of k-means cluster analysis, forcing the data into two clusters (Figure S1; i.e. lower pH values in Group 1, higher pH values in Group 2). 5.4 was the highest pH where the majority of samples fell in Group 1 and 5.5 was the lowest pH where the majority of samples fell in Group 2. We therefore removed all samples with pH≥5.5. This division was supported by plotting NMDS scores against pH, which showed an abrupt jump to higher axis 1 values at this point in the pH range (Figure S1) and also by general peatland ecology: *Sphagnum* moss, the dominant peat-forming species in Northern Hemisphere ombrotrophic peatlands is known to actively acidify its environment (van Breemen, 1995) and therefore ombrotrophic bogs are typically dominated by pH ranges of 3.0 – 4.5, with *Sphagnum*-dominated poor fens having marginally higher pH (4.5 – 5.5; Lamentowicz and Mitchell, 2005). Using this cut-off resulted in the removal of 370 samples with pH values 5.5 – 8.1 and the removal of all samples from France, Greece and Israel. We re-ran NMDS ordination on the reduced, low-pH dataset (n=1405, including samples without a pH measurement (n=211); Figure 3C and D) which then showed that water-table depth was the primary environmental variable explaining species variation along axis 1 (p<0.001 using the 'envfit' function in vegan), providing a statistical foundation to proceed with transfer function development. Despite water-table depth being the primary explanatory variable after removal of high pH samples, there is still considerable variability along NMDS axis two (Figure 3D) that reflects previous axis one variability (Figure 3B), potentially driven by samples without pH values that may in reality be from sites with pH≥5.5. In particular, a group of nine taxa (*Tracheleuglypha dentata* type, *Gibbocarina (Nebela) penardiana* type, *Arcella gibbosa* type, *Quadrulella symmetrica*, *Microclamys patella*, *Lesquereusia spiralis* type, *Cyphoderia ampulla* type, *Arcella dentata* type and *Pyxidicula operculata* type) fall outside of the main cluster of variability with more negative axis two scores (Figure 3D), potentially suggesting that these taxa may be less reliable water table indicators, associated more with nutrient enrichment (Payne, 2011; Lamentowicz et al., 2013a). Following the removal of high pH samples, all nine are relatively rare taxa, occurring in < 5% of the 1405 samples. Five of the taxa are defined as rare based on previously defined criteria (i.e. < 1% of the dataset, or n=14; *A. dentata* type, *C. ampulla* type, *L. spiralis* type, *M. patella*, *P. operculata* type) and so these were excluded from further analyses, reducing the number of taxa in the dataset for transfer function development to 47. The number of samples in which the remaining four taxa (*A. gibbosa* type, *G. (N.) penardiana* type, *Q. symmetrica*, *T. dentata* type) were present was reduced by > 50% in all cases with the removal of high pH samples (e.g. by 52% for *A. gibbosa* type but up to a reduction of > 80% for *Q. symmetrica*), so water-table depth reconstructions based on fossil assemblages containing significant proportions of these taxa should be treated with caution.

[INSERT FIGURE 3]

Figure 3: NMDS plots before (A and B) and after (C and D) the removal of samples from the dataset with high pH values. A and C show sample positions, coded by country. B and D show taxa positions for same data as in A and B (but note different axis lengths). Vectors on all plots show influence of environmental drivers. Some taxa positions in B and D have been marginally altered to improve legibility of the figure, but relative positions remain intact. Full names for species abbreviations can be found in Table 2. For reference to colour, readers are referred to the online version of this article.

Transfer function development and statistical assessment

Before proceeding with transfer function development, we removed 12 further samples with extreme measured water table values (Table S1), resulting in a dataset for transfer function development of 1393 samples. These 12 samples fell below the 0.5th (i.e. representing deep surface ponding, n=2) and above the 99.5th (i.e. representing extreme deep water tables, n=10) percentiles of water-table depth and were removed to avoid the large increase in water-table depth range that would result from their inclusion and the subsequent effect on removal of samples with high residual values. In addition, the removal of extreme deep water-table depth samples is supported by Swindles et al., 2015b, who showed a disconnect between testate amoebae and water table in such circumstances. In keeping with standard practice, we then ran two iterations of models, the first using all samples and the second having removed samples with residual values greater than 20% of the range of water-table values in the dataset (min = -10 cm, max = 85 cm, range = 95 cm, 20% range = 19 cm) (e.g. Amesbury et al., 2013; Booth, 2008; Charman et al., 2007; Payne et al., 2006; Swindles et al., 2009). Residuals removed in the second iteration of model runs were specific to each model type and therefore varied in number (Table 5). The effect of removing residual samples is shown in Figure 4 for the best performing versions of the four model types under investigation (WA-Tol (inv) = weighted average tolerance downweighting with inverse deshrinking; WAPLS C2 = second component of weighted averaging partial least squares; WMAT K5 = weighted mean modern analogue technique with five nearest neighbours). Results for WAPLS C2 are included but fell marginally outside the recommended cut-off for acceptance (5% at $p<0.05$; Birks, 1998); the second component provided a 4.71% improvement ($p=0.001$) over the first component (i.e. simple weighted averaging). Residual error plots show that the majority of samples with high residual values fell at the 'dry' end of the water table gradient and that, in general, all models tended to under-predict at the dry end of the gradient (i.e. negative residual value) and over-predict at the wet end of the gradient (i.e. positive residual value). Biplots of observed and predicted water-table depths show that, particularly for both weighted average models and WMAT K5 but not so ML, models tended to reach a plateau of predicted values at around 40 – 50 cm regardless of the observed value. In contrast to previous studies (e.g. Amesbury et al., 2013) which found larger water table tolerances correlated with drier optima, tolerance ranges for the WA-Tol (inv) model were similar throughout the water table gradient (Figure 5), potentially as a result of the 'averaging out' effect of taxonomic groupings, although a small group of hydrophilous taxa did have narrower tolerances. The ordering of taxa water table optima (Figure 5) reflected the positioning of taxa along NMDS axis one (Figure 3).

[INSERT FIGURES 4 AND 5]

Figure 4: Biplots of observed and predicted (leave-one-out cross-validated) water-table depth (left) and residual error plots (right) for the best performing versions of the four model types under investigation. WA-Tol (inv) = weighted averaging with tolerance downweighting and inverse deshrinking; WAPLS C2 = second component of weighted averaging partial least squares; WMAT K5 = weighted mean modern analogue technique with five nearest neighbours; ML = maximum likelihood. Red points are model runs with all data, black points are model runs after the removal of samples with high residual values. For reference to colour, readers are referred to the online version of this article.

Figure 5: Water-table depth optima and tolerances (cm) for 57 taxa based on the WA-Tol (inv) model after the removal of outlying samples (n=1302).

Performance statistics (Table 5; principally, RMSEP and R^2) before the removal of outlier samples were generally poor, though equivalent to some published models (e.g. Swindles et al., 2015a; van Bellen et al., 2014). After the removal of outlier samples with high residual values (Figure 4), $RMSEP_{LOO}$ values for the WA-Tol (inv), WAPLS C2 and WMAT K5 models fell in the range 7 – 8 cm, equivalent to that generally seen in other published transfer functions (Booth, 2008; Markel et al., 2010; Amesbury et al., 2013; Lamarre et al., 2013; Li et al., 2015; Swindles et al., 2015a) and, notably, similar to the ACCROTELM European model (Charman et al., 2007). $RMSEP_{LOSO}$ values showed a mean relative decrease in performance of only 0.068 (mean of 0.036 without WMAT K5) compared to $RMSEP_{LOO}$, less than that in Payne et al. (2012; mean decrease in performance of 0.141). Calculation of $RMSEP_{SW}$ (Figure S2; single value for $RMSEP_{SW}$ is a mean of all individual segment RMSEPs) resulted in a decrease in performance compared to $RMSEP_{LOO}$ for all models with the exception of ML, which supports previous research that found ML outperformed MAT- and WA-based models on unevenly sampled gradients (Telford and Birks, 2011b). There was a prevalence of samples in the water-table depth range 0 – 35 cm, with water-table depths <0 cm and >35 cm less well represented (although it should be noted that due to the high overall number of samples in the dataset, even the lowest frequency segment, 45 – 49.5 cm still contained 15 – 18 samples, depending on model type). Individual segment RMSEP values generally increase where sampling frequency is lower, particularly at the 'dry' end of the water table gradient, in keeping with expectation (Telford and Birks, 2011b), except for ML, which shows more consistent RMSEP values across all segments, driving the observed relative improvement in $RMSEP_{SW}$ against other model types. In all cases, RMSEP values, however calculated, remained lower than the standard deviation of all water table measurements (Table 5), suggesting all models have a degree of predictive ability (*cf.* Amesbury et al., 2013; Mitchell et al., 2013). All models display a degree of spatial autocorrelation (Figure S3), given that r^2 values decline more steeply when geographically proximal, as opposed to random, samples are removed (Telford and Birks, 2009). For all models to some extent, but for WMAT K5 in particular, the decline in r^2 over the first 100 km is similar to the decline for the most environmentally similar samples, indicating that geographically proximal samples are also the most environmentally similar across the dataset. Coupled to the general similarity of R^2 from 100 – 1000 km, this reflects the spatial structure of the data whereby each individual data contribution (Table 1) tended to include multiple sites/samples, with individual study locations being widely distributed across Europe (Figure 1).

Testing model efficacy

In addition to statistical assessment of model performance, we used three independent data sets, two with associated instrumental water table measurements, to test the new models. Broadly speaking, reconstructions using the four different model types under consideration (WA-Tol (inv), WAPLS-C2, ML, WMAT-K5) showed similar patterns of change to either alternative published transfer function reconstructions or instrumentally recorded water table fluctuations, although water-table depth ranges were more variable (Figure 6). For the Tor Royal Bog test set, all model types reconstructed generally drier conditions and ranges of reconstructed water-table depths were much higher for all model types when compared to a published reconstruction (Figure 6A; Amesbury et al., 2008), particularly for ML. However, when viewed as residual plots (Figure 6B; Swindles et al., 2015b), all models show extremely similar patterns of change over the ~6000 year record. For the simulated shifts in water-table depth (Figure 6C and D; Swindles et al., 2015b), all models again produced comparable reconstructions with the exception of ML. All models reconstructed the simulated shifts in water table with the correct frequency and direction of change, but reconstructed shifts were more abrupt, occurring over 2 – 3 samples, with simulated shifts more gradual, occurring over 6 – 10 samples. Whereas the wet and dry ends of the simulated shifts were single point extremes, modelled reconstructions exhibited more rapid, threshold-type switches in water table interspersed with plateaux of more consistently wet or dry conditions. Reconstructions of monitored water-table depth at Männikjärve fell between the annual and summer mean values for water-table depth (Figure 6E), but when viewed as residual values (Figure 6F), differences were evident in the patterns of change over the c. 50 year record, with the comparatively smooth reconstructions suggesting a broadly drier period during the 1970s and 1985 – 1995, with wetter conditions before and after, whereas instrumental data show that water table varied over shorter time scales throughout the period of monitoring.

[INSERT FIGURE 6]

Figure 6: Comparison of transfer function reconstructions from four model types (WA-Tol (inv), WAPLS-C2, ML, WMAT-K5) with independent test sets. A, C & E are raw water-table depth values; B, D & F are residual z-scores. A and B: reconstructions from Tor Royal Bog, Dartmoor, UK (Amesbury et al., 2008) compared (panel A only) with a published reconstruction using a European transfer function (black line; Charman et al., 2007). C and D: reconstructions of simulated wet and dry shifts derived from reordered surface samples with associated instrumental water table measurements (black line = annual mean water-table depth, grey line = summer (JJA) mean water-table depth; Swindles et al., 2015b). Y-axis (not shown) is randomly ordered surface sample codes. E and F: reconstructions of near-surface fossil data from Männikjärve Bog, Estonia with associated long-term instrumental water table measurements (black line = annual mean water-table depth, grey line = summer (JJA) mean water-table depth; Charman et al., 2004). For reference to colour, readers are referred to the online version of this article.

All reconstructions were subject to significance testing against transfer functions built on randomly generated data (Table 6; Telford and Birks, 2011a). This methodology has recently been tested (Payne et al., 2016), with a substantial majority of reconstructions unexpectedly found to be non-significant. In addition, the risks of misapplying (e.g. over-simplified decision making) or over-relying on (e.g. lack of real-world context) *p*-value cut-offs, are clear (Wasserstein and Lazar, 2016). However, the significance testing technique does provide a method of statistical assessment that can be used as part of a wider toolkit to evaluate model performance. P-values varied between model types and test sets. Only WMAT-K5 reconstructions consistently met the $p<0.05$ criterion across all test sets. WA-Tol (inv) and WAPLS-C2 reconstructions were consistently $p>0.05$ though for the Tor Royal Bog and simulated test sets, were consistently $p<0.08$. ML reconstructions showed the greatest degree of variability, ranging from $p=0.274$ for the Tor Royal Bog test set to $p=0.031$ for the Männikjärve test set.

Spatial scales and regional variability

To further investigate the potential effects of varying spatial scales and supra-regional application on resulting transfer function reconstructions, we subdivided our data into three eco-regions (Figure 1); Atlantic (n=461), Scandinavia (n=341) and Continental (n=500). We developed individual transfer functions for each region and applied them to the same three independent test-sets as for the full European-scale models. These three datasets include data from all three eco-regions (Tor Royal Bog in the UK; simulated test set from the UK and Finland; Männikjärve from Estonia) so provide a test of the effects in within- and supra-regional model application (Turner et al., 2013). Given the broad similarity of reconstructions between model types (Figure 6), especially when presented as standardised water-table depth residual values (Swindles et al., 2015b), only one model type (WA-Tol (inv)) was used for this exercise. This model type has been frequently applied in previous studies (e.g. Amesbury et al., 2013; Swindles et al., 2015a, 2009) and in this study, compared favourably to other model types in terms of reported performance statistics, with low $\text{RMSEP}_{\text{LOO}}$ and $\text{RMSEP}_{\text{LOSO}}$ values (Table 5). Performance statistics ($\text{RMSEP}_{\text{LOO}}$, R^2) for the regional models (Table 7) were comparable to, or better than, the full European model (Table 5), potentially suggesting the presence of regional differences in biogeography strong enough to influence model performance. Reconstructed water-table depth profiles for the Atlantic and Continental models for all three independent test sets are broadly similar (Figure 7) and comparable to the WA-Tol (inv) reconstruction using the full European dataset. However, in all three test sets, the Scandinavian model tended to result in notably different profiles. For the Tor Royal Bog and simulated test sets, the Scandinavian model predicted similar patterns of change but overall wetter conditions (Figure 7A and B) whereas for the Männikjärve test set, drier overall conditions were predicted. The Scandinavian model contained the lowest number of samples of all three regions (n=341), but still more than many published models. Scandinavian samples also recorded the highest (i.e. wettest) mean water-table depth of the three regional models (Table 7; Figure S4; 12.7 cm compared to 16 cm for the Atlantic and Continental models) and the lowest range of water-table depth values (Table 7; Figure S4; range of 55 cm, compared to 57 cm and 62 cm for the Atlantic and Continental models respectively).

[INSERT FIGURE 7]

Figure 7: Comparison of three regional transfer function reconstructions to the full European model for the same three independent test sets as Figure 6. All reconstructions use the WA-Tol (inv) model type. A: Tor Royal Bog (black line uses the established ACCROTELM European transfer function of Charman et al., 2007); B: simulated changes in water-table depth (black (annual) and grey (summer) lines are instrumental water table measurements); C: Männikjärve Bog, Estonia (black (annual) and grey (summer) lines are instrumental water table measurements). For reference to colour, readers are referred to the online version of this article.

To provide additional insight into the differences between regional models, we examined the prevalence of individual taxa across the three regions to identify whether taxa were cosmopolitan, or tended to have skewed distributions, favouring a particular region (Figure 8; Figure S5). One-way PERMANOVA tests on both individual countries as well as the three eco-regions showed that there were significant differences between both factor countries and regions (p<0.0001 for both, F=29.87 for countries, F=45.6 for regions, assessed by Bray Curtis distance). Twenty-six taxa, including the three most abundant in the dataset (i.e. *Assulina muscorum* (n=1180), *Euglypha ciliata* type (n=1145), *Nebela tincta* type (n=1022)), were evenly distributed across all sub eco-regions within the wider European study zone (Figure S5). However, a large number (n=19) had skewed distributions that suggested taxa were more abundant in particular regions, especially in continental Europe (n=14; Figure 8).

[INSERT FIGURE 8]

Figure 8: Taxa with uneven distributions across the three regions (n = 19). Taxa with all occurrences < 5% abundance have been excluded (n = 2; DIF GRA and PAR IRR). Region codes on x-axes: 1 = Atlantic, 2 = Scandinavia, 3 = continental Europe. For full taxa abbreviations, see Table 2. Number of occurrences in 1302 samples shown in brackets after taxon code. Red dots indicate complete absence from a particular region. For reference to colour, readers are referred to the online version of this article.

Discussion

Data compilation

A low-resolution approach to taxonomy was necessitated in this work by the large number of data contributors (see Methods). It has been shown that reducing taxonomic resolution may have a detrimental effect on model performance (Mitchell et al., 2014; as determined by RMSEP and R^2) but with only limited effects on patterns of reconstructed water-table depth. Our data support this view. For example, directional shifts reconstructed by all model types tested showed the same patterns as a European transfer function based on a higher resolution taxonomy (Charman et al., 2007) when applied to a record from Tor Royal Bog, Dartmoor, UK (Figure 6). Particularly when records were standardised (Swindles et al., 2015b) to remove variability in specific reconstructed water-table depth values (Figure 6B), the reconstructions at different taxonomic resolutions were indistinguishable, strongly supporting the view that the necessary reduction in taxonomic resolution applied here has not had a detrimental effect on the potential interpretation of inferred water-table depth profiles from the new model. Indeed, it should be noted that even the highest taxonomic resolution that is practically applicable to light microscopy studies corresponds to a relatively crude resolution in reality given the demonstrated existence of high cryptic and pseudo-cryptic diversity (e.g. Oliverio et al., 2014; Kosakyan et al., 2016), so some degree of taxonomic parsimony will always be necessary.

Spatial scales and regional variability

The debate surrounding the degree of cosmopolitanism exhibited in free-living microorganisms, of which testate amoebae provide a good model group, is well established and on-going (e.g. Heger et al., 2009). Conflicting views assume universal ubiquity (Finlay and Clarke, 1999; Finlay et al., 1999, 2001) or (occasional) limited geographical distribution of microorganisms (i.e. the 'moderate endemicity model'; Foissner, 2008, 2006, 1999). An increasing number of studies focussing on the distribution of testate amoebae have observed taxa which do not appear to support the theory of ubiquity (Smith and Wilkinson, 2007; Smith et al., 2008; Heger et al., 2009, 2011; Yang et al., 2010; Turner et al., 2013; Fournier et al., 2015; Lara et al., 2016).

Europe possesses relatively few of the physical, climatic and biological barriers typically associated with ecological endemism (Kier et al., 2009) and the passive distribution of testate amoebae should, therefore, be comparatively uninhibited. As a result, it could be argued that any evidence of regionally restricted distributions of testate amoebae in Europe, which cannot be explained by other ecological factors, such as peatland type and trophic status, is supporting evidence of the moderate endemicity model.

By compiling data from across Europe, we were able to examine the distributions of taxa across the continent. The majority (41 of 47) of taxa were found in all regions (Figures 8 and S5), arguing strongly that the continental transfer function can be readily applied to individual core locations within its geographical extent. The Continental region was the most taxonomically diverse, with only *Nebela flabellulum* (strongly skewed to the Atlantic region; Figure 8) completely absent. A similar strongly oceanic distribution has been noted for this taxon in Canadian peatlands (Charman and Warner, 1997), which was the most common to be completely absent from any one region (n=246 samples). Three taxa were present only in the Continental region, being completely absent from both the Atlantic and Scandinavian regions (*Centropyxis ecornis* type, *Difflugia gramen* type, *Paraquadrula irregularis*), however these were rare taxa, with *C. ecornis* type the most common (n=26; Figure 8) and *D. gramen* and *P. irregularis* never occurring >5% in any one sample (n=1 and n=2 respectively). In addition, *P. irregularis* is a calcareous taxon found predominately in rich fens (e.g. Lamentowicz et al., 2013a), which were mainly sampled in the Continental region and were included in the model due to having no associated pH measurement. *Difflugia labiosa* type was absent from the Atlantic region and present at very low abundance (0.7%) in only one sample in the Scandinavian region. *Arcella hemisphaerica* type was completely absent from the Atlantic region, whereas *Amphitrema wrightianum* type was strongly skewed towards it, also in common with findings from Canada (Charman and Warner, 1997). For nutrient poor, ombrotrophic peatlands, given the number and range of sites included in the dataset, it is likely that these regional patterns represent genuine geographical restrictions of these taxa, rather than a lack of appropriate habitats (Smith and Wilkinson, 2007; Smith et al., 2008; Yang et al., 2010). Patterns relating to taxa commonly associated with other site types (e.g. rich fens; *P. irregularis,* Lamentowicz et al., 2013a) should be viewed with more caution since only limited numbers of geographically restricted samples from such site types were included in the model as a result of their lacking associated pH measurements.

Twenty-six of 47 taxa showed distributions that were relatively evenly distributed across the three defined eco-regions (Figure S5). These included all of the most common taxa (e.g. *A. muscorum*, *E. ciliata* type, *N. tincta* type). Of the taxa shown to have uneven distributions across Europe (Figure 8), the majority (n=14) were found in greater abundance in the taxonomically diverse Continental region. Water table optima of these 14 taxa are evenly distributed (Figure 5) with taxa indicative of wetter (e.g. *Arcella discoides* type, *Arcella vulgaris* type), intermediate (e.g. *Centropyxis arcelloides* type, *Heleopera petricola* type) and drier (e.g. *Bullinularia indica*, *Centropyxis ecornis* type) conditions all represented. In contrast, a much smaller number of taxa had distributions skewed to the Atlantic or Scandinavia regions (Figure 5). Given the similar mean values and water-table depth ranges of all regions, particularly Atlantic and Continental (Table 6; Figure S4), the higher number of taxa skewed to Continental, which include key hydrological indicator taxa commonly found in fossil studies (e.g. *A. discoides* type, *B. indica*) is intriguing. Skewed distributions do not preclude cosmopolitan distributions for many of these taxa, but may relate more to either the general condition or trophic status (e.g Booth and Zygmunt, 2005) of peatlands within each region, or to gradients of oceanicity/continentality. In addition, while differences in the numbers of taxa skewed to particular regions may relate partly to genuine biogeographical differences, they may also be an effect of the different taxonomic knowledge and skill of individual analysts and therefore a reflection of the research design.

The use of local transfer functions to reconstruct water-table depth from other regions should be approached with caution (Turner et al., 2013), but by including a high number of analogues from a wide geographic region and long water table gradient and by using a relatively coarse taxonomic resolution, we show here that continental-scale models may be just as effective in reconstructing local changes as local-scale models specific to the core data location. A large scale regional model such as that presented here will contain more analogues and therefore provide a more robust approach to reconstructing past hydrological variability than the use of smaller data sets collected from individual sites or small regions.

A way forward for interpreting transfer function-based palaeohydrological reconstructions?

Due to the complexity of peatland water table and testate amoeba ecological responses, both moderated by a range of differing factors, it is becoming clear that transfer function reconstructions should not be seen as simple metrics of past climate (Turner et al., 2013). In addition, the apparent inaccuracy of reconstructed water-table depth values, particularly towards the ‘dry’ end of the gradient where both methodological and ecological problems are exacerbated, suggests that reconstructions should be displayed as residuals or standardised values and interpreted primarily as metrics of directional shifts between wetter and drier conditions, an approach in which context reconstructions from a range of models have shown to be robust (Swindles et al., 2015b). Our own data (e.g. Figures 6 and 7) show that, with some exceptions, our models are relatively consistent in performance for reconstructed water-table depth values across the full gradient. This is likely as a result of the high number of samples characterising all water table segments (Figure S2); although there are relatively fewer samples in drier water table segments, a common problem identified in other studies (Amesbury et al., 2013; Swindles et al., 2015a). Segment-specific n values are still high (lowest is n=25 for 45 – 49.5 cm, mean for all water-table depth segments >20 cm is n=70), highlighting the value of the large compiled dataset. However, the variability present between both model types and regions, despite an unprecedented training set size, argues that reconstructions should be standardised and presented as residual values (*sensu* Swindles et al., 2015b) in order to focus interpretation on secure directional shifts, avoiding potentially inaccurate conclusions relating to specific water-table depths.

There has been a recent recognition that testing transfer functions against independent instrumental data may be a more powerful test than relying purely on statistical methods (Swindles et al., 2015b). Here, by applying both approaches, we are able to rigorously validate our new models and show that, despite a low-resolution taxonomic approach, our new pan-European transfer function provides a reliable tool for reconstruction of Holocene hydroclimatic shifts across Europe. In particular, we highlight the potential limitations of applying statistical tests alone. The use of the 'randomTF' function, which tests the significance of reconstructions against models trained on randomly generated data has recently been reviewed (Payne et al., 2016), with >80% reconstructions tested found to be insignificant (*p*>0.05), with no correlation between significance and model performance. Our results question the efficacy of this method as different model types applied in different regions (Table 6) showed only 4 out of 12 'significant' reconstructions and resulted in a range of *p* values from 0.001 to 0.274, despite all reconstructions being trained on the same data and showing what could be interpreted as the same reconstructed patterns of change (Figure 7). Coupled with recent guidance that warns against the use of seemingly arbitrary *p*-value cut-offs and stresses the need for contextual information in decision making rather than a binary 'yes/no' approach (Wasserstein and Lazar, 2016), a multi-faceted approach to model assessment is clearly supported. While statistical validation remains an important and useful indicator of model performance alongside tests against independent data, we advocate a balanced approach to model efficacy taking into account both lines of evidence as well as the role of contextual information. For example, some 'insignificant' reconstructions (Table 6) performed well in tests against independent data (Figure 7). In addition, the similarity of reconstructions across model types and/or regions presented here (Figures 6 and 7), despite variations in performance statistics, suggests that the high number of training set samples has resulted in a wider range of modern analogues and therefore a better representation of testate amoeba ecology in the model.

Conclusions and guidelines for the application of the transfer function

We developed and validated a new pan-European peatland testate amoeba-based transfer function for palaeohydrological reconstruction using a vastly expanded dataset of 1799 samples and a newly developed low resolution taxonomic scheme to accommodate the large number of data contributors. Following the removal of samples with high pH values, we developed water-table depth transfer functions using a range of model types. These were tested using a combination of statistical validation and comparison to independent test sets with associated instrumental water table measurements. Taxonomic resolution did not impair model performance, which was comparable to other published models. We conclude that the new model provides an effective tool for testate amoeba-based palaeohydrological reconstruction in ombrotrophic peatlands throughout Europe. Model output should be standardised and presented as residual values to focus interpretation on directional shifts and avoiding potential misinterpretation of absolute water-table depth values. The extent and diversity of the dataset highlighted that, at the taxonomic resolution applied, a majority of taxa had broad geographic distributions, though some morphotypes appeared to have restricted ranges.

To facilitate future research, we provide the full compiled dataset, along with R code to allow the free application of our transfer function to fossil data by individual users, as supplementary online material. The R code facilitates the application of the WA-Tol (inv) model and conversion of reconstructed water-table depth values to standardised residual z-scores. The WA-Tol (inv) model type has been commonly applied in other studies (e.g. Amesbury et al., 2013; Swindles et al., 2015a, 2009) and compared favourably to other model types in terms of reported performance statistics in this study. Users are free to alter the R code as appropriate to apply other model types, but should justify these changes in their work.

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Figure captions:

Figure 1: Site locations (see Table 1 for more site details). Sites are coloured by eco-region: Atlantic = red, Scandinavia = green; Continental = blue. For reference to colour, readers are referred to the online version of this article.

Figure 2: Percentage distribution of all taxa. Taxa are ordered from ‘wet’ on the left to ‘dry’ on the right based on the taxa optima from the WA-Tol (inv) model of the full dataset (n = 1775).

Figure 3: NMDS plots before (A and B) and after (C and D) the removal of samples from the dataset with high pH values. A and C show sample positions, coded by country. B and D show taxa positions for same data as in A and B (but note different axis lengths). Vectors on all plots show influence of environmental drivers. Some taxa positions in B and D have been marginally altered to improve legibility of the figure, but relative positions remain intact. Full names for species abbreviations can be found in Table 2. For reference to colour, readers are referred to the online version of this article.

Figure 4: Biplots of observed and predicted (leave-one-out cross-validated) water-table depth (left) and residual error plots (right) for the best performing versions of the four model types under investigation. WA-Tol (inv) = weighted averaging with tolerance downweighting and inverse deshrinking; WAPLS C2 = second component of weighted averaging partial least squares; WMAT K5 = weighted mean modern analogue technique with five nearest neighbours; ML = maximum likelihood. Red points are model runs with all data, black points are model runs after the removal of samples with high residual values. For reference to colour, readers are referred to the online version of this article.

Figure 5: Water-table depth optima and tolerances (cm) for 57 taxa based on the WA-Tol (inv) model after the removal of outlying samples (n=1302).

Figure 6: Comparison of transfer function reconstructions from four model types (WA-Tol (inv), WAPLS-C2, ML, WMAT-K5) with independent test sets. A, C & E are raw water-table depth values; B, D & F are residual z-scores. A and B: reconstructions from Tor Royal Bog, Dartmoor, UK (Amesbury et al., 2008) compared (panel A only) with a published reconstruction using a European transfer function (black line; Charman et al., 2007). C and D: reconstructions of simulated wet and dry shifts derived from reordered surface samples with associated instrumental water table measurements (black line = annual mean water-table depth, grey line = summer (JJA) mean water-table depth; Swindles et al., 2015b). Y-axis (not shown) is randomly ordered surface sample codes. E and F: reconstructions of near-surface fossil data from Männikjärve Bog, Estonia with associated long-term instrumental water table measurements (black line = annual mean water-table depth, grey line = summer (JJA) mean water-table depth; Charman et al., 2004). For reference to colour, readers are referred to the online version of this article.

Figure 7: Comparison of three regional transfer function reconstructions to the full European model for the same three independent test sets as Figure 6. All reconstructions use the WA-Tol (inv) model type. A: Tor Royal Bog (black line uses the established ACCROTELM European transfer function of Charman et al., 2007); B: simulated changes in water-table depth (black (annual) and grey (summer) lines are instrumental water table measurements); C: Männikjärve Bog, Estonia (black (annual) and grey (summer) lines are instrumental water table measurements). For reference to colour, readers are referred to the online version of this article.

Figure 8: Taxa with uneven distributions across the three regions (n = 19). Taxa with all occurrences < 5% abundance have been excluded (n = 2; DIF GRA and PAR IRR). Region codes on x-axes: Atl. = Atlantic, Scan. = Scandinavia, Cont. = continental Europe. For full taxa abbreviations, see Table 2. Number of occurrences in 1302 samples shown in brackets after taxon code. Red dots indicate complete absence from a particular region. For reference to colour, readers are referred to the online version of this article.

Supplementary Figure captions:

Figure S1: NMDS axis 1 scores plotted against pH for all samples (n = 1775). Inset: pH of two k-means cluster analysis groups. Red line in both plots shows cut-off applied of pH ≥ 5.5.

Figure S2: Sampling distribution for the full dataset (n=1393) divided into 12 segments. Lines show segment-wise RMSEP for the best performing versions of the four model types under investigation. Full names for species abbreviations can be found in Table 2 of the manuscript.

Figure S3: Spatial autocorrelation plots for the best performing versions of the four model types under investigation. Full names for species abbreviations can be found in Table 2 of the manuscript. Plots show effect on r^2 by deleting sites at random (open circles), from the geographical neighbourhood of the test site (filled circles) or that are most environmentally similar (crosses) during cross-validation. Note y-axes are on different scales.

Figure S4: Boxplots for water-table depth values for the three regional transfer functions.

Figure S5: Taxa with even distributions across the three regions (n = 26). Region codes on x-axes: Atl. = Atlantic, Scan. = Scandinavia, Cont. = continental Europe. For full taxa abbreviations, see Table 2. Number of occurrences in 1302 samples shown in brackets after taxon code.

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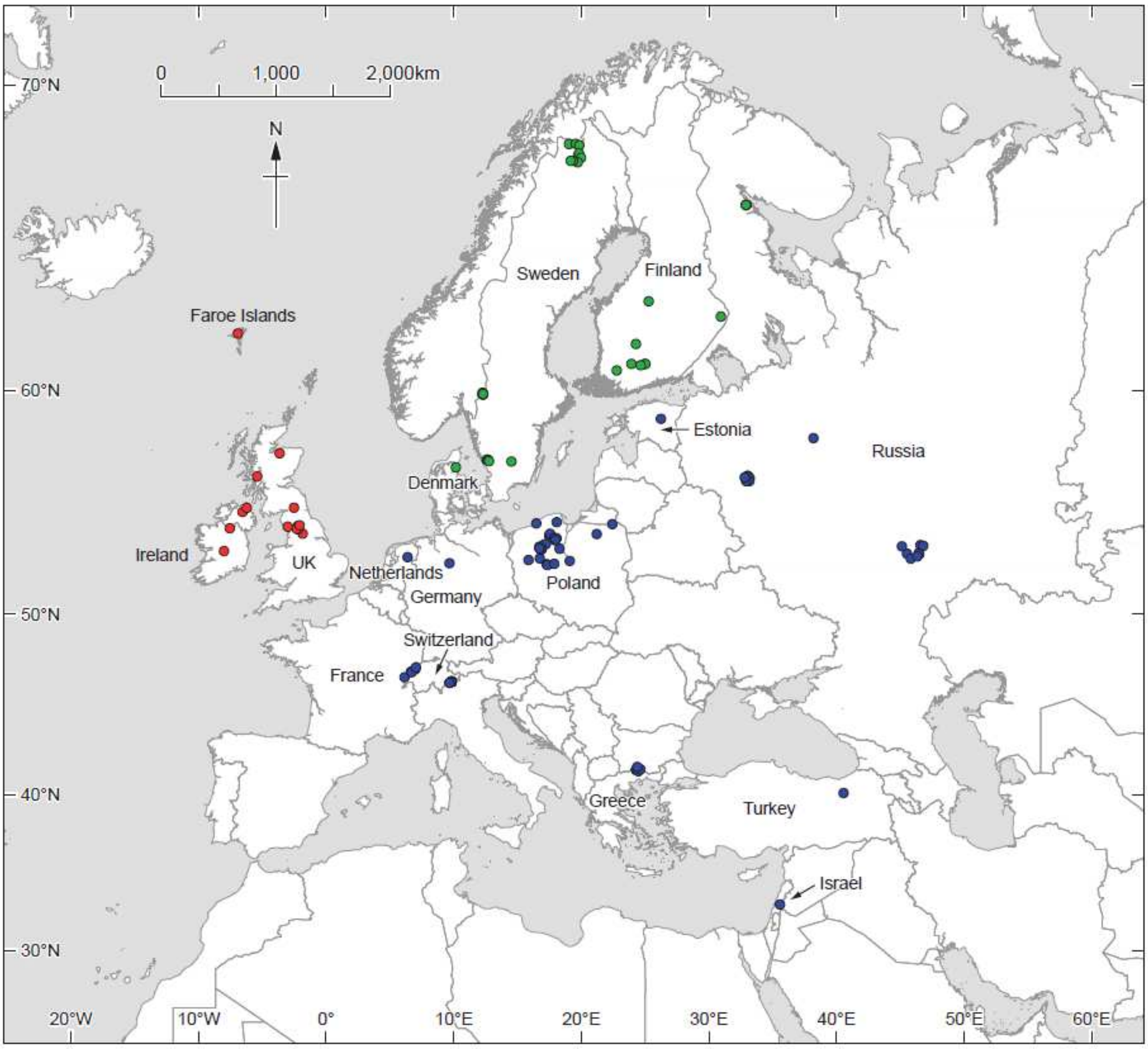
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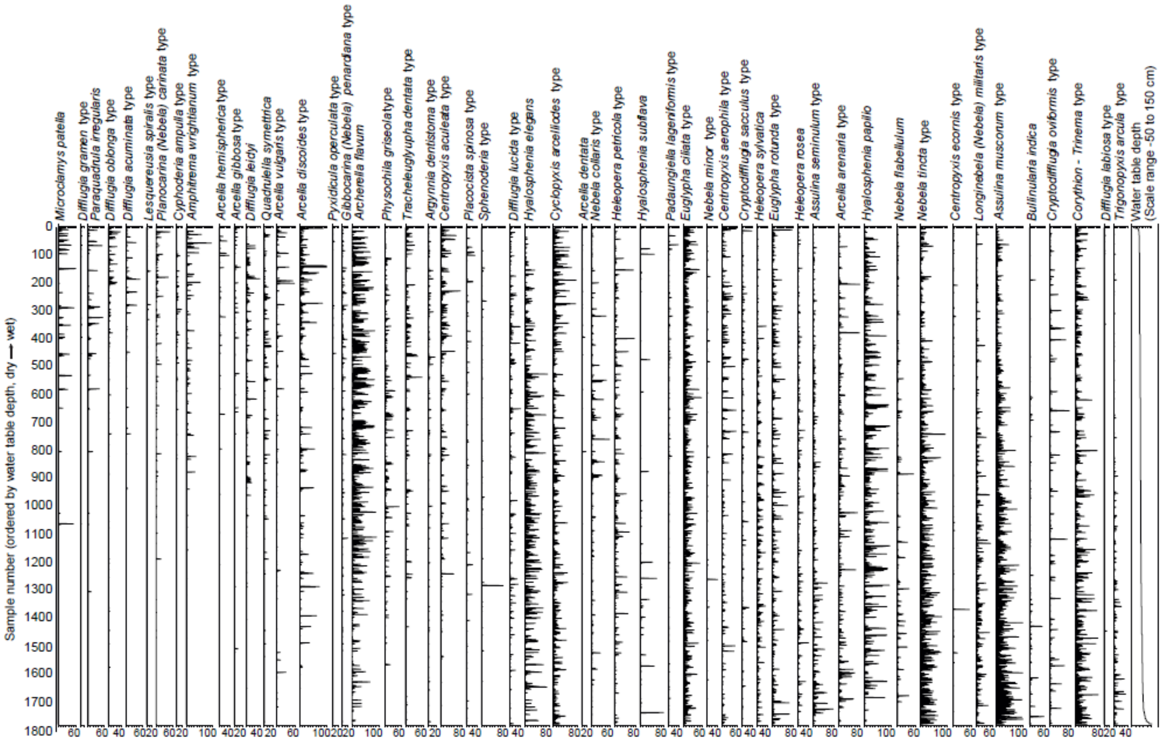
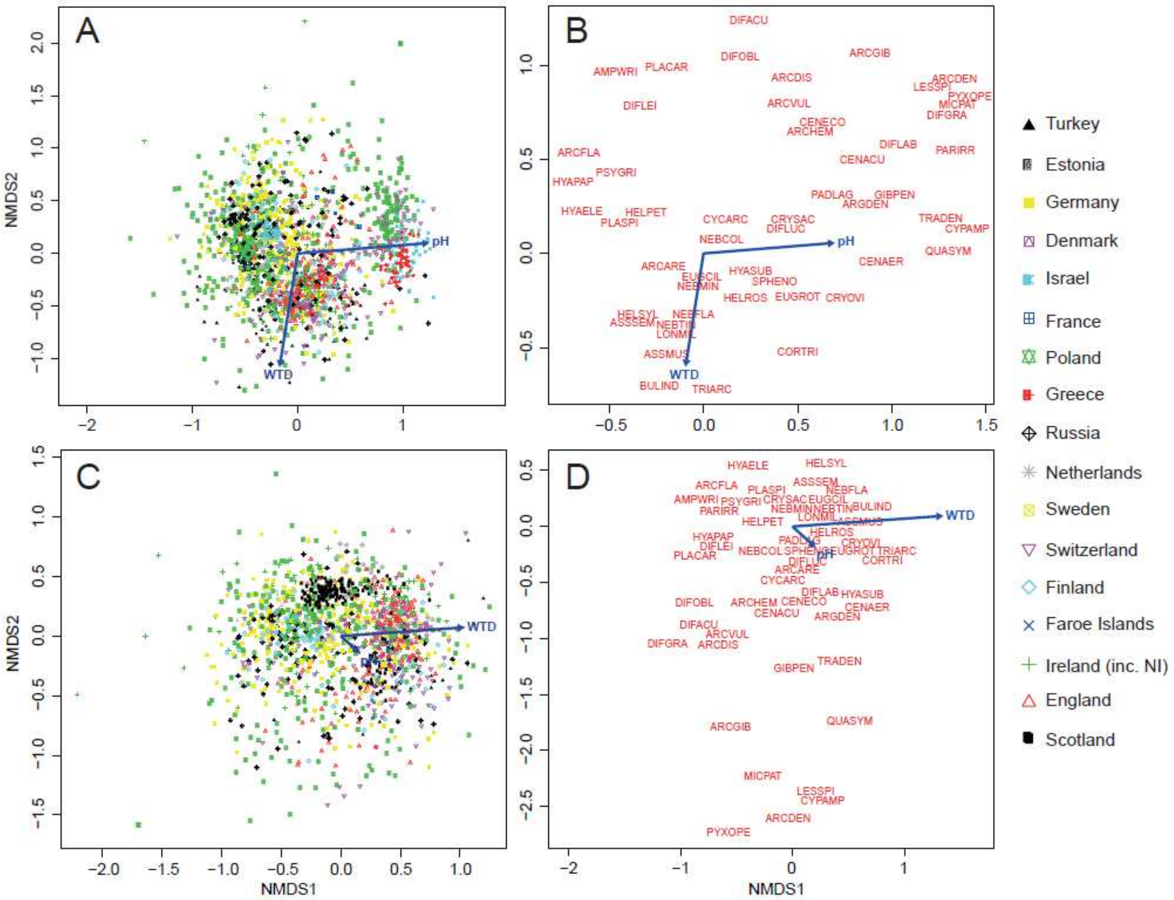


All taxa proportions are % values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NEBMINNEBTIN
Turkey
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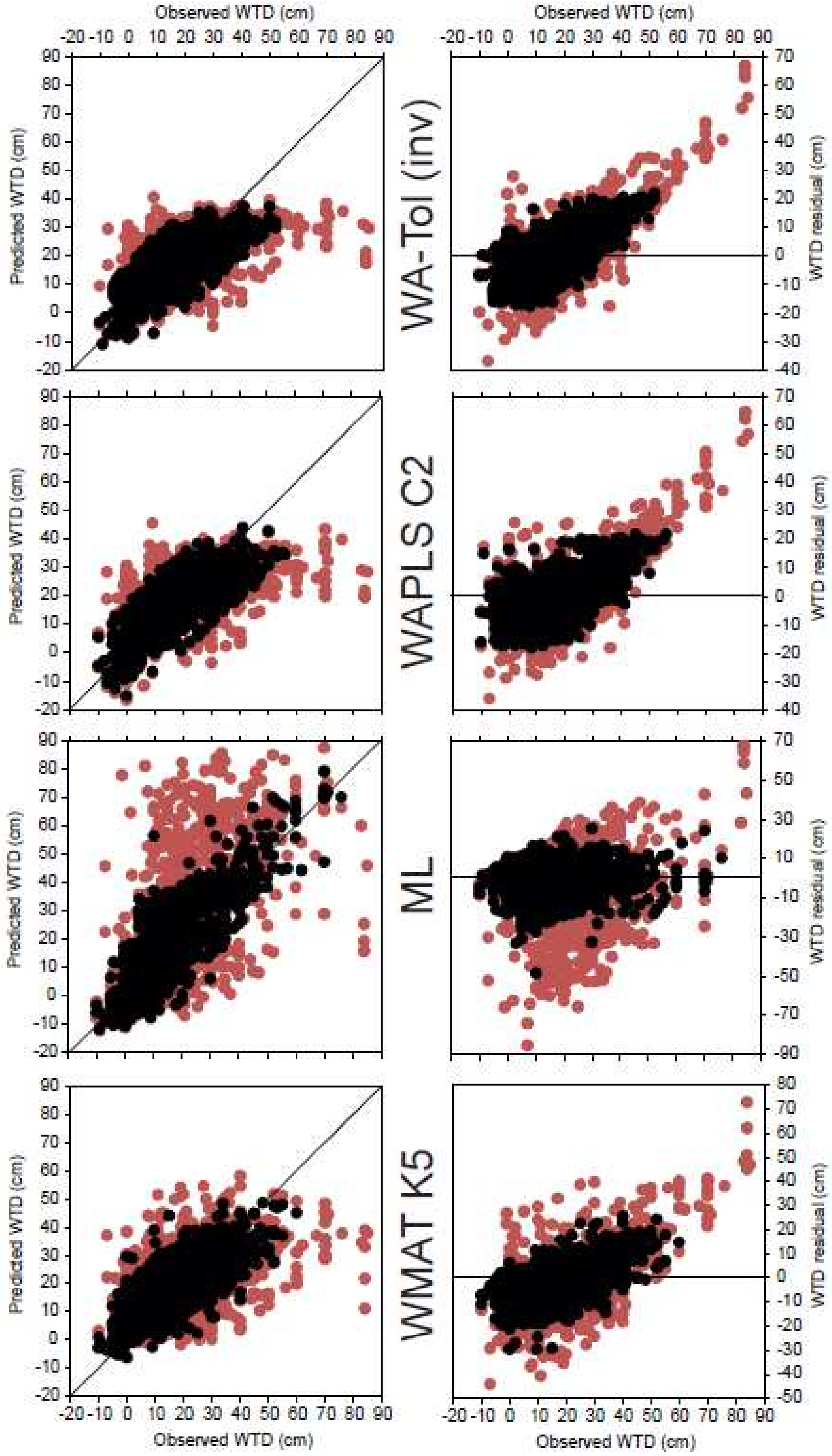
Observed WTD (cm)
Predicted WTD (cm)
WTD residual (cm)
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WAPLS C2
ML
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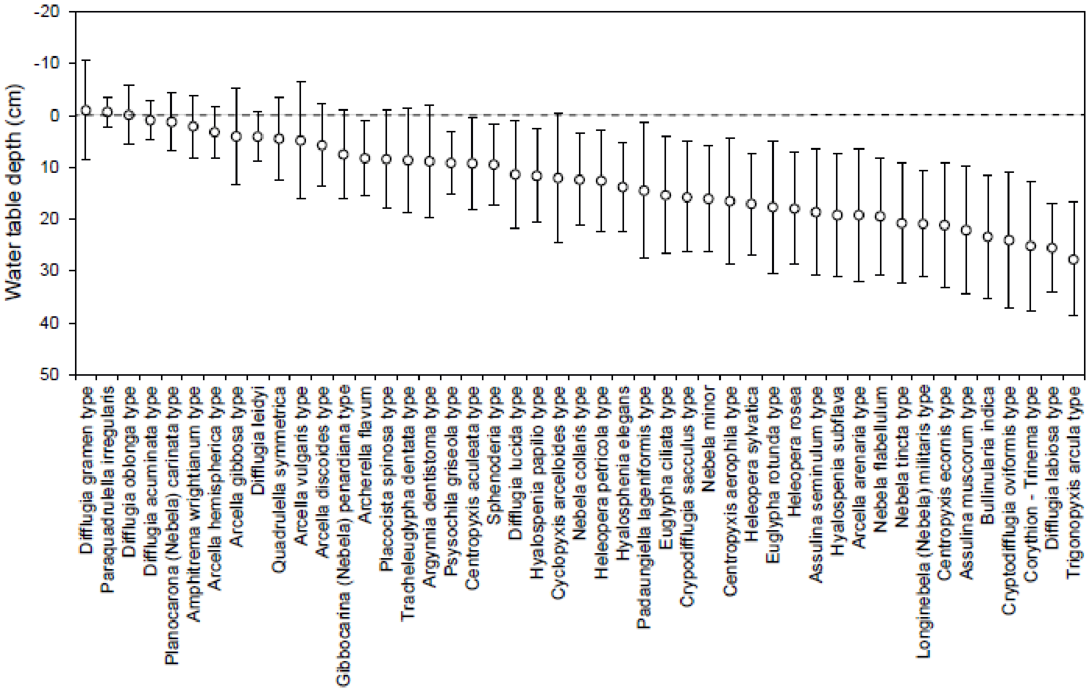
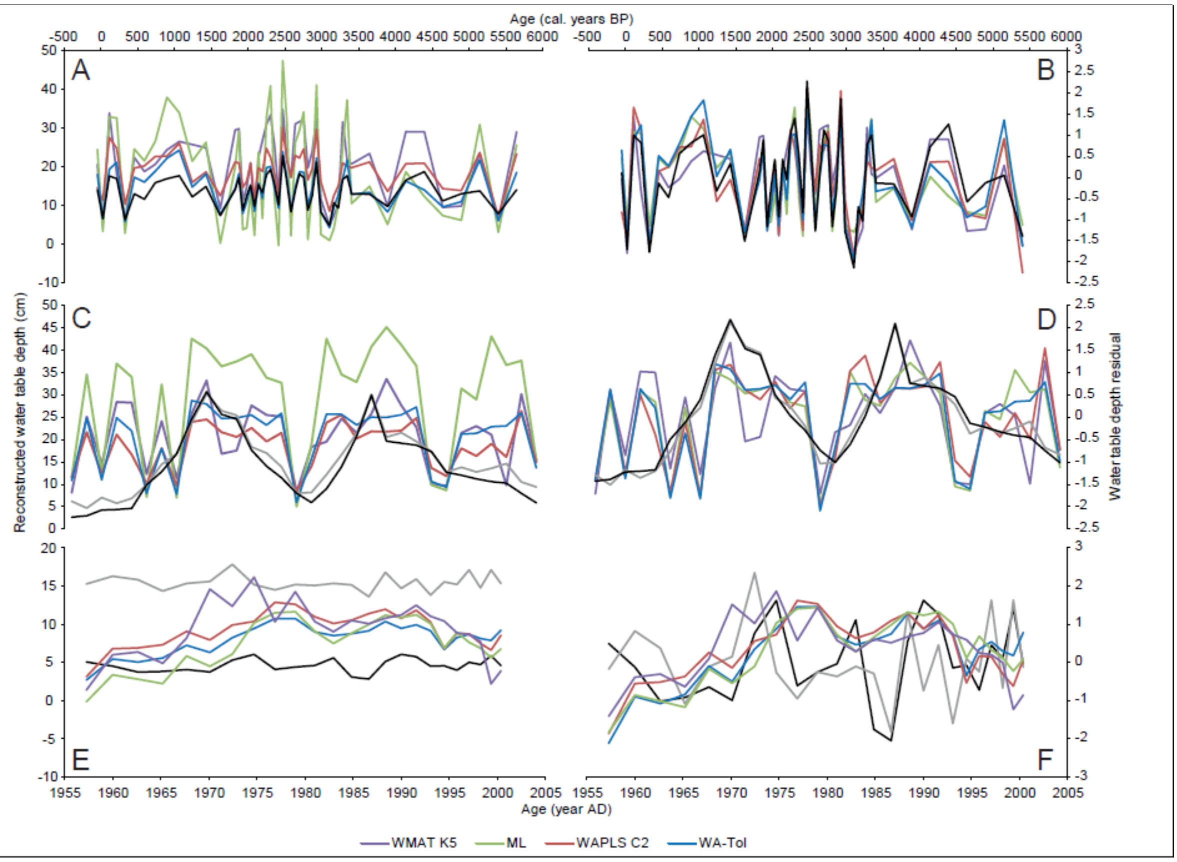

Water table depth (cm)
-20
-10
0
10
20
30
40
50
Difflugia gramen type
Paraquadrulella irregularis
Difflugia oblonga type
Difflugia acuminata type
Planocarona (Nebela) carinata type
Amphitrena wrightianum type
Arcella hemispherica type
Arcella gibbosa type
Difflugia leidyi
Quadrulella symmetrica
Arcella vulgaris type
Arcella discoides type
Gibbocarina (Nebela) penardiana type
Archerella flavum
Placocista spinosa type
Tracheleuglypha dentata type
Argynnia dentistoma type
Psysochila griseola type
Centropyxis aculeata type
Sphenoderia type
Difflugia lucida type
Hyalospenia papilio type
Cyclopyxis arcelloides type
Nebela collaris type
Heleopera petricola type
Hyalosphenia elegans
Padaungiella lageniformis type
Euglypha ciliata type
Crypodifflugia sacculus type
Nebela minor
Centropyxis aerophila type
Heleopera sylvatica
Euglypha rotunda type
Heleopera rosea
Assulina seminulum type
Hyalospenia subflava
Arcella arenaria type
Nebela flabellulum
Nebela tincta type
Longinebela (Nebela) militaris type
Centropyxis ecornis type
Assulina muscorum type
Bullinularia indica
Cryptodifflugia oviformis type
Corythion - Trinema type
Difflugia labiosa type
Trigonopyxis arcula type


Age (cal. years BP)
-500
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
A
B
C
D
E
F
Reconstructed water table depth (cm)
Water table depth residual
50
40
30
20
10
0
-10
45
35
25
15
5
-5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
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-2
-2.5
-3
1955
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Age (year AD)
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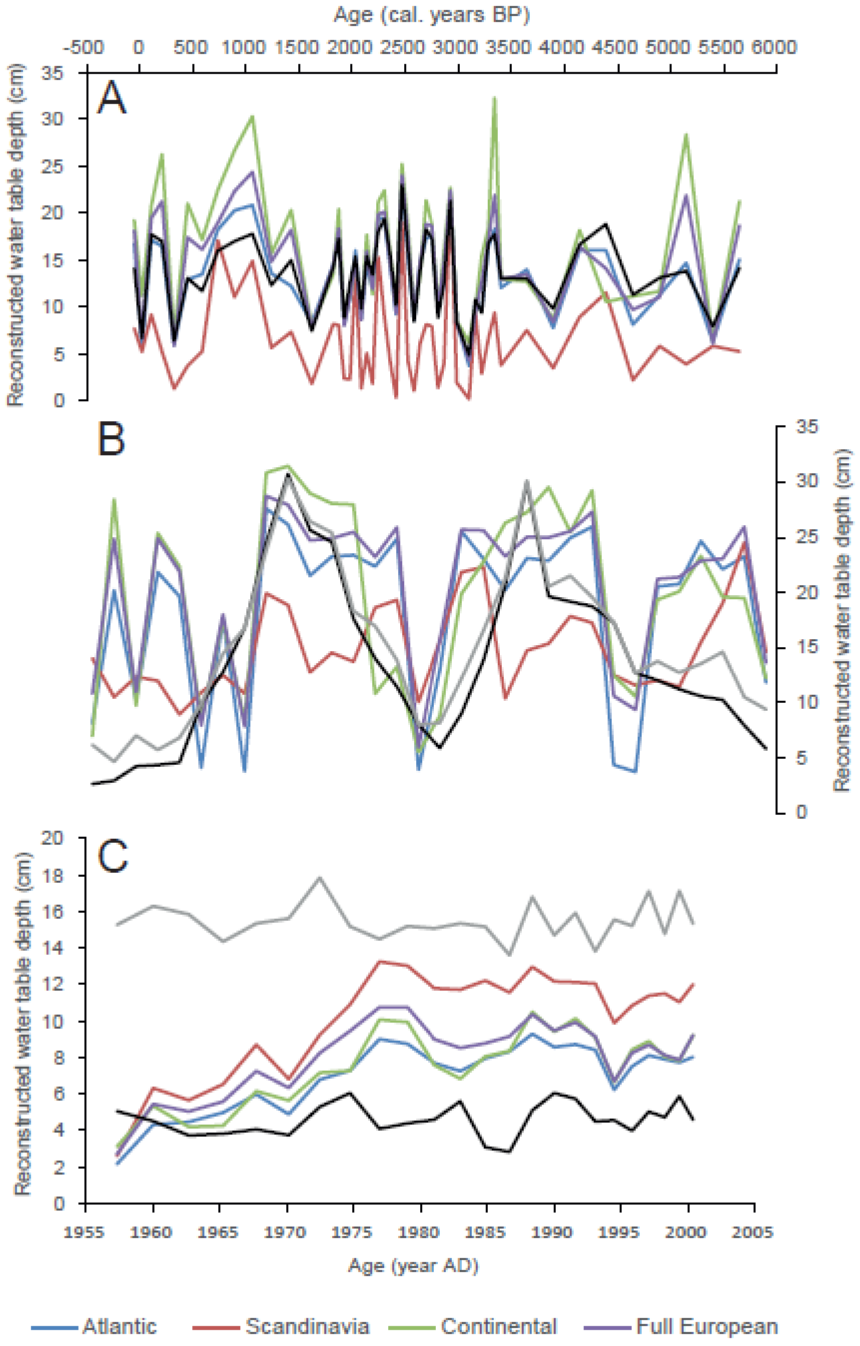
Age (cal. years BP)
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Reconstructed water table depth (cm)
35
30
25
20
15
10
5
0
A
B
Reconstructed water table depth (cm)
35
30
25
20
15
10
5
0
C
Reconstructed water table depth (cm)
20
18
16
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8
6
4
2
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Age (year AD)
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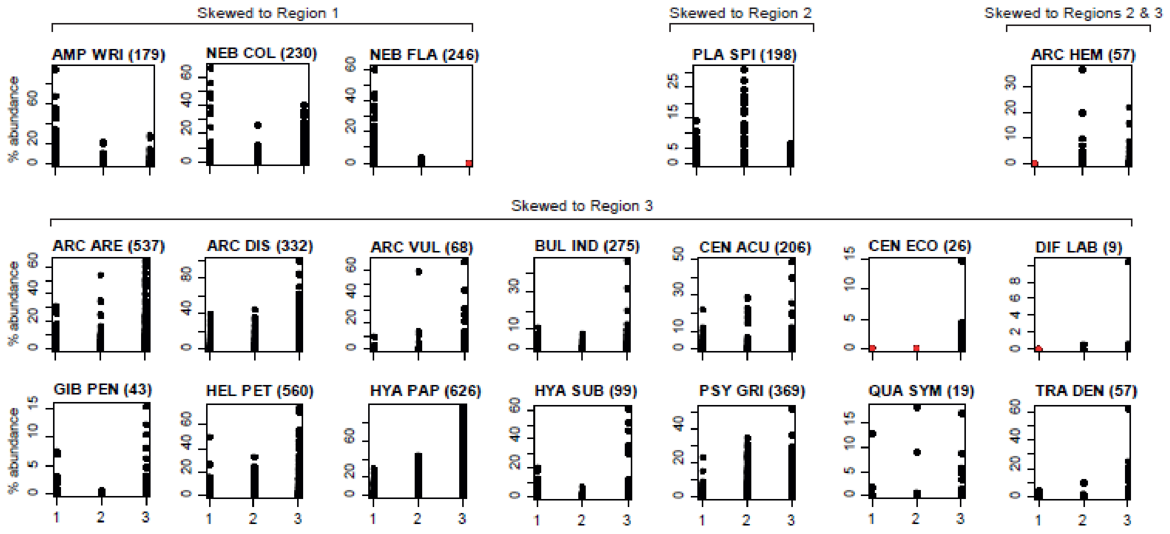
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Skewed to Region 2
Skewed to Regions 2 & 3
AMP WRI (179)
NEB COL (230)
NEB FLA (246)
PLA SPI (198)
ARC HEM (57)
Skewed to Region 3
ARC ARE (537)
ARC DIS (332)
ARC VUL (68)
BUL IND (275)
CEN ACU (206)
CEN ECO (26)
DIF LAB (9)
GIB PEN (43)
HEL PET (560)
HYA PAP (626)
HYA SUB (99)
PSY GRI (369)
QUA SYM (19)
TRA DEN (57)
% abundance
1
2
3