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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.

1. 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

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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, 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.

2. Methods

2.1. 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; Fig. 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.

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 is 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). As a result, 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

Table 1
Site details and meta-data. Only one lat/long position was available for the five sites in Russia of Bobrov et al. (1999) and the four sites in Greece of Payne and Mitchell (2007). Data ordered by author surname/year and sub-divided by country/publication.

Reference/unpublished	Country	Site name	Site code	Lat (° N)	Long (° E/W)	Number of samples
Bobrov et al., 1999	Russia	Tverskaya Reserve	Q	56.4666	33.05 E	5
Bobrov et al., 1999	Russia	Staroselia	STAR	56.4666	33.05 E	10
Bobrov et al., 1999	Russia	Gulnovsky Moch	GUL	56.4666	33.05 E	6
Bobrov et al., 1999	Russia	Katin Moch	KATIN	56.4666	33.05 E	7
Bobrov et al., 1999	Russia	Goltovsky Moch	GOLT	56.4666	33.05 E	2
Charman et al., 2007	Denmark	Lille Vildmose	DK	56.8526	10.1735 E	17
Charman et al., 2007	Germany	Bissendorfer Moor	DM	52.5037	9.6811 E	18
Charman et al., 2007	Estonia	Mannikjarve	ES	58.8752	26.2479 E	18
Charman et al., 2007	Finland	Kontolanrahka	FI	60.7837	22.7874 E	24
Charman et al., 2007	Faroe Islands	Masaklettar	FO	62.1665	6.9000 W	17
Charman et al., 2007	England	Butterburn Flow	GB	55.0759	2.5124 W	18
Charman et al., 2007	Ireland	Ballyduff Bog	IR	53.0807	7.9925 W	16
Lamentowicz et al., 2008a	Poland	Chlebowo	CHL	52.7364	16.7569 E	27
Lamentowicz and Mitchell 2005	Poland	Jelenia Wyspa	JEL	53.6077	17.9560 E	23
Lamentowicz and Mitchell 2005	Poland	Jeziorka Kozie	KOZ	53.6965	17.8974 E	10
Lamentowicz and Mitchell 2005	Poland	Mietlica	MIET	53.8110	17.5153 E	12
Lamentowicz and Mitchell 2005	Poland	Okoniny	OK	53.6740	18.0766 E	3
Lamentowicz et al., 2008a,b	Poland	Jedwabna	JB	52.6144	19.1039 E	10
Lamentowicz et al., 2008b	Poland	Ostrowite	OST	53.7932	17.5880 E	7
Lamentowicz et al., 2008b	Poland	Rybie Oko	RO	53.8140	17.5387 E	19
Lamentowicz et al., 2008b	Poland	Skrzynka	SKR	53.8171	17.5233 E	12
Lamentowicz et al., 2008b	Poland	Stawek	STAW	53.8913	17.5566 E	9
Lamentowicz et al., 2008b	Poland	Stążki	STAZ	54.4241	18.0854 E	10
Lamentowicz et al., 2010	Switzerland	Mauntschas	–	46.4575	9.8561 E	39
Lamentowicz et al., 2010	Switzerland	Lej da Staz	–	46.4972	9.8694 E	11
Lamentowicz et al., 2010	Switzerland	Lej Marsch	–	46.4753	9.8197 E	12
Lamentowicz et al., 2010	Switzerland	Lej Nair	–	46.4703	9.8201 E	10
Lamentowicz et al., 2010	Switzerland	Inn Fen	–	46.4078	9.7027 E	13
Lamentowicz et al., 2010	Switzerland	Maloja Fen	–	46.4053	9.6900 E	8
Lamentowicz et al., 2013b; Jassey et al., 2014	Poland	Kazanie	KAZ	52.4581	17.2981 E	19
Lamentowicz et al., 2013b; Jassey et al., 2014	Poland	Wagowo	WAG	52.4195	17.3647 E	20
Lamentowicz et al., 2013b; Jassey et al., 2014	Poland	Rurzyca	RUR	53.2864	16.7203 E	23
Lamentowicz et al., 2013b; Jassey et al., 2014	Poland	Makąty	MAK	52.6562	15.8721 E	7
Lamentowicz et al., 2013b; Jassey et al., 2014	Poland	Czarci Staw	CZS	53.3797	17.0759 E	15
Lamentowicz et al., 2013b; Jassey et al., 2014	Poland	Czarne	CZAR	52.4742	17.8893 E	18
Lamentowicz et al., 2013b; Jassey et al., 2014	Poland	Wierzchołek	WEK	53.4154	17.2358 E	14
Unpublished (M. Lamentowicz)	Poland	Zamarte	Z	53.5915	17.9670 E	8
Unpublished (M. Lamentowicz)	Poland	Linje	LIN	53.1875	18.3096 E	46
Unpublished (M. Lamentowicz)	Poland	Słowińskie Błoto	SL	54.3624	16.4815 E	25
Unpublished (M. Lamentowicz)	Poland	Kuźnik	K	53.6965	17.8974 E	31
Unpublished (M. Lamentowicz)	Poland	Okoniowe	OKO	53.1884	16.7997 E	5
Unpublished (M. Lamentowicz)	Poland	Kaczory	KACZ	53.1230	16.9155 E	5
Unpublished (M. Lamentowicz)	Poland	Zelgniowo	ZEL	53.1719	16.8701 E	5
Unpublished (M. Lamentowicz)	Poland	Skórki	SKÓ	53.1852	16.9108 E	7
Unpublished (M. Lamentowicz)	Poland	Jeziorki	JEZ	53.1458	16.8594 E	3
Unpublished (M. Lamentowicz)	Poland	Olszowy	OLSZ	53.2126	16.7275 E	4
Unpublished (M. Lamentowicz)	Poland	Bagienny	BAG	53.2140	16.7311 E	4
Unpublished (M. Lamentowicz)	Poland	Gązwa	GAZ	53.8732	21.2232 E	14
Unpublished (M. Lamentowicz)	Poland	Mechacz	MECH	54.3307	22.4422 E	14
Unpublished (G. Mallon)	Sweden	Kortlandamossen	KOR	59.8483	12.2867 E	15
Unpublished (G. Mallon)	Sweden	Gallseredsmossen	GAL	57.1750	12.5975 E	15
Unpublished (G. Mallon)	Sweden	South 3	S3	57.1747	12.6342 E	15
Unpublished (G. Mallon)	Sweden	South 7	S7	57.1725	12.7125 E	15
Unpublished (G. Mallon)	Sweden	South 11	S11	57.1139	12.7811 E	15
Unpublished (G. Mallon)	Sweden	North 1	N1	59.8625	12.3247 E	15
Unpublished (G. Mallon)	Sweden	North 2	N2	59.9239	12.2728 E	15
Unpublished (G. Mallon)	Sweden	North 3	N3	59.8542	12.3031 E	15
Unpublished (G. Mallon)	Sweden	North 4	N4	59.8753	12.2914 E	15
Unpublished (G. Mallon)	Sweden	North 5	N5	59.8728	12.3053 E	15
Mazei and Bubnova 2009	Russia	Karelia	K4	66.5251	32.9386 E	10
Mazei et al., 2009a	Russia	Karelia	K3	66.5225	32.9491 E	9
Mazei et al., 2009b	Russia	Karelia	K2	66.5238	32.9097 E	14
Mazei et al., 2009c	Russia	Karelia	K1	66.5227	32.9185 E	15
Mazei et al., 2009d	Russia	Borok	B1 B4	58.0845	38.2147 E	13
Mazei et al., 2009e	Russia	Penza	P1	52.9325	46.5031 E	5
Mazei and Bubnova 2008	Russia	Penza	P2	52.9575	45.5382 E	5
Mazei and Tsyganov 2007a	Russia	Penza	P3	53.3037	45.1366 E	6
Mazei et al., 2007a	Russia	Penza	P4	52.9857	46.4593 E	5

Table 1 (*continued*)

Reference/unpublished	Country	Site name	Site code	Lat (° N)	Long (° E/W)	Number of samples
Mazei and Tsyganov 2007b	Russia	Penza	P5	52.7218	45.8334 E	4
Mazei et al., 2007b	Russia	Penza	P6	53.3620	46.5856 E	4
Mazei and Bubnova 2007	Russia	Penza	P7	52.8269	46.3535 E	3
Mitchell et al., 1999	France	Les Pontets	–	46.7103	6.1611 E	4
Mitchell et al., 1999	Switzerland	Le Cachot	–	47.0057	6.6652 E	17
Mitchell et al., 1999	Switzerland	Les Pontins	–	47.1275	6.9878 E	16
Mitchell et al., 1999	Switzerland	Le Bois des Lattes	–	46.9729	6.7132 E	11
Mitchell et al., 2000	Finland	Salmisuo Mire	FIN	62.785	30.9481 E	20
Mitchell et al., 2000	Switzerland	La Chaux-des-Breuleux	CDB	47.2268	7.0498 E	20
Mitchell et al., 2000	Sweden	Kopparås Mire	SU	57.1018	14.5381 E	20
Mitchell et al., 2000	Netherlands	Dwingeloo	NL	52.7973	6.3968 E	19
Mitchell et al., 2000	UK	Roundsea bog	UK	54.2141	3.0013 W	10
Payne and Mitchell 2007	Greece	Informal site code	BO	41.4833	24.3166 E	9
Payne and Mitchell 2007	Greece	Informal site code	DE	41.4833	24.3166 E	24
Payne and Mitchell 2007	Greece	Informal site code	KB	41.4833	24.3166 E	11
Payne and Mitchell 2007	Greece	Informal site code	XE	41.4833	24.3166 E	13
Payne et al., 2008	Turkey	Surmene Agacbasi Yaylasi	–	40.1000	40.5666 E	42
Payne et al., 2010a	Scotland	Moidach More	MOMO	57.4487	3.6346 W	150
Payne 2010	Scotland	Moss of Achnacree	–	56.4517	5.3930 W	50
Payne et al., 2010b	Israel	Hula	MAC	33.0666	35.5833 E	42
Swindles et al., 2009	Northern Ireland	Dead Island	DI	54.8875	6.5475 W	30
Swindles et al., 2009	Northern Ireland	Slieveanorra	SL	55.0728	6.2108 W	32
Swindles et al., 2009	Northern Ireland	Moninea	MO	54.1417	7.5425 W	32
Swindles et al., 2015a	Sweden	Stordalen	S	68.3563	19.0477 E	40
Swindles et al., 2015a	Sweden	Eagle	E	68.3657	19.5831 E	6
Swindles et al., 2015a	Sweden	Craterpool	P	68.3196	19.8572 E	7
Swindles et al., 2015a	Sweden	Instrument	I	68.0312	19.7656 E	6
Swindles et al., 2015a	Sweden	Railway	R	68.0869	19.8311 E	7
Swindles et al., 2015a	Sweden	Marooned	M	67.9566	19.9865 E	7
Swindles et al., 2015a	Sweden	Crash	C	67.8243	19.7322 E	4
Swindles et al., 2015a	Sweden	Electric	L	67.8656	19.3683 E	6
Swindles et al., 2015a	Sweden	Nikka	N	67.8671	19.1778 E	6
Tolonen et al., 1992	Finland	Kaurastensuo	KS	61.0319	24.9839 E	5
Tolonen et al., 1992	Finland	Laaviosuo	LS	61.0348	23.9423 E	2
Tolonen et al., 1992	Finland	Heinisuo	HS	61.0333	25.0333 E	4
Tolonen et al., 1992	Finland	Suurisuo	SS	60.9833	24.6666 E	5
Tolonen et al., 1992	Finland	Lakkasuo	LK	61.7833	24.2943 E	32
Tolonen et al., 1992	Finland	Kuivajärvi suo	KJ	63.3274	25.2930 E	2
Tsyganov and Mazei 2007	Russia	Penza	P8	53.3319	46.8190 E	6
Turner et al., 2013	England	Fleet Moss	FM	54.2464	2.2094 W	25
Turner et al., 2013	England	Ilkley Moor	IM	53.8942	1.8241 W	17
Turner et al., 2013	England	Thornton Moor	ThM	54.0964	2.175 W	30
Turner et al., 2013	England	Oxnop Moor	OM	54.3456	2.0961 W	30
Turner et al., 2013	England	Swarth Moor	SM	54.1214	2.2975 W	20
Turner et al., 2013	England	Malham Tarn Moss	TM	54.2781	2.0713 W	37
					TOTAL:	**1799**

(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 (Fig. 2). Transfer function

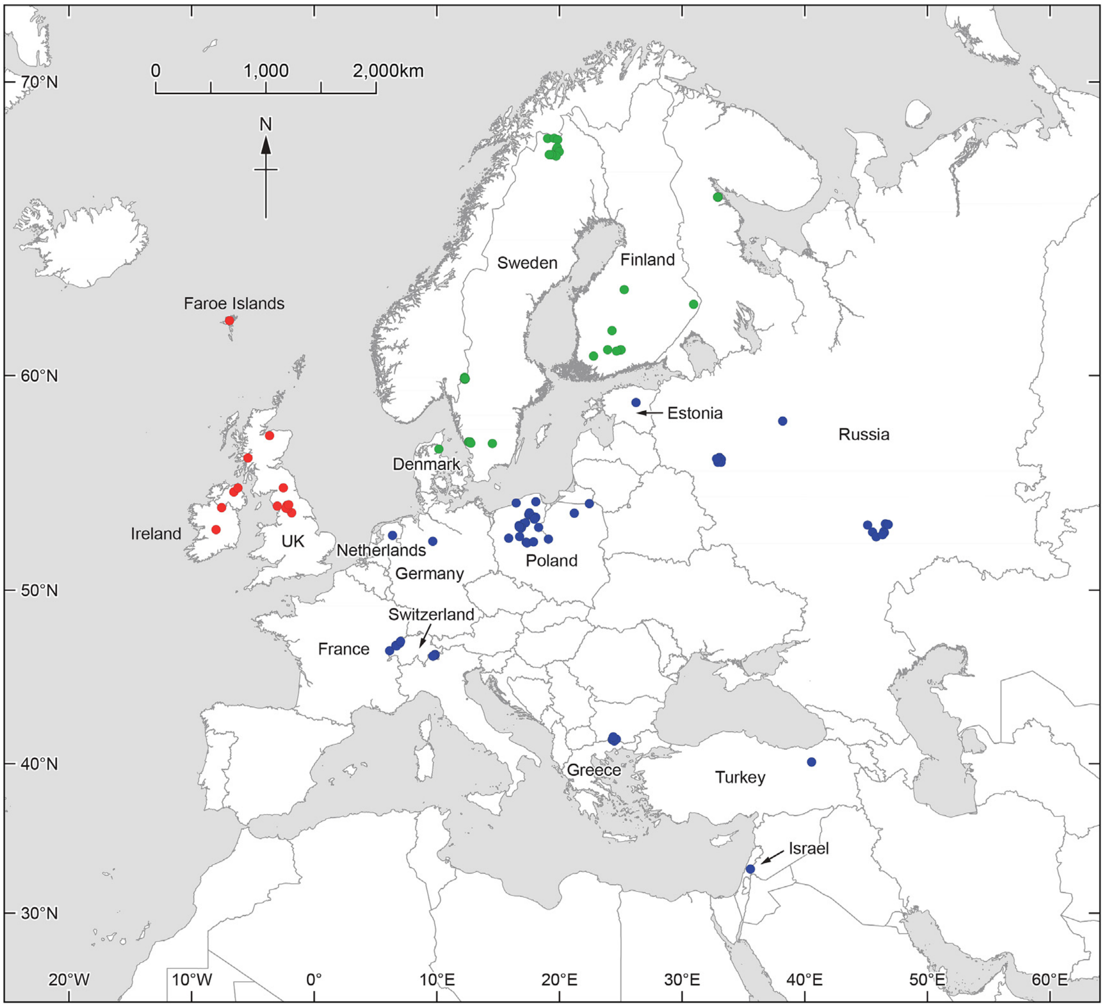


Fig. 1. Site locations (see Table 1 for more site details). Sites are coloured by eco-region: Atlantic = red, Scandinavia = green; Continental = blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

development proceeded from this ‘edited’ dataset. Hereafter, this ‘edited’ dataset will be referred to as the full dataset.

2.2. 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

Table 2
Summary of taxonomic scheme developed for and applied in this study, resulting in 41 ‘type’ groupings (38 newly defined) and 19 individual taxa. Taxon codes are included.

Taxa defined in new groups	
New taxon name	*Includes*
Amphitrema wrightianum type (ARC WRI)	*Amphitrema wrightianum* *Amphitrema stenostoma*
Arcella arenaria type (ARC ARE)	*Arcella artocrea* *Arcella arenaria* *Arcella catinus* type *Arcella polypora*
Arcella discoides type (ARC DIS)	*Arcella discoides* type *Arcella megastoma*
Arcella gibbosa type (ARC GIB)	*Arcella bathystoma* *Arcella conica* *Arcella costata* *Arcella gibbosa* type *Arcella intermedia*
Arcella hemisphaerica type (ARC HEM)	*Arcella hemisphaerica* *Arcella rotundata*
Arcella vulgaris type (ARC VUL)	*Arcella crenulata* *Arcella vulgaris* type
Argynnia dentistoma type (ARG DEN)	*Nebela dentistoma* *Nebela vitraea* type
Assulina muscorum type (ASS MUS)	*Assulina muscorum* *Assulina minuta* *Valkanovia elegans*
Assulina seminulum type (ASS SEM)	*Assulina scandinavica* *Assulina seminulum*
Centropyxis aculeata type (CEN ACU)	*Centropyxis aculeata* type *Centropyxis aculeata* var. *minor* *Centropyxis aculeata* var. *oblonga* *Centropyxis aculeata* var. *gibbosa* *Centropyxis gibba* *Centropyxis hirsuta* *Centropyxis spinosa*
Centropyxis aerophila type (CEN AER)	*Centropyxis aerophila* *Centropyxis cassis* type *Centropyxis constricta* *Centropyxis minuta* *Centropyxis orbicularis* *Centropyxis platystoma* type *Centropyxis sphagnicola* *Centropyxis sylvatica*
Centropyxis ecornis type (CEN ECO)	*Centropyxis laevigata* *Centropyxis latior* *Centropyxis laevigata* *Centropyxis ecornis* type *Centropyxis ecornis* var. *quadripannosa*
Corythion-Trinema type (COR TRI)	*Corythion dubium* *Corythion pulchellum* *Corythion-trinema* type *Trinema complanatum* *Trinema enchelys* *Trinema lineare* type *Trinema penardi*
Cryptodifflugia oviformis type (CRY OVI)	*Cryptodifflugia oviformis* *Cryptodifflugia compressa* *Difflugiella (Cryptodifflugia) apiculata* *Difflugiella (Cryptodifflugia) minuta* *Difflugiella (Cryptodifflugia) oviformis*
Crypotodifflugia sacculus type (CRY SAC)	*Crypodifflugia sacculus* *Difflugia pulex* *Hyalosphenia minuta* type
Cyclopyxis arcelloides type (CYC ARC)	*Centropyxis eurystoma* *Cyclopyxis arcelloides* type *Cyclopyxis eurystoma* *Cyclopyxis kahli* *Difflugia globulosa* type *Difflugia microstoma* *Phryganella acropodia* *Phryganella hemispherica* *Phryganella nidulus* *Phryganella paradoxa*
Cyphoderia ampulla type (CYP AMP)	*Cyphoderia ampulla* *Cyphoderia calceolus* *Cyphoderia* sp.
Difflugia acuminata type (DIF ACU)	*Difflugia acuminata* type *Difflugia bacilliarium* type *Difflugia elegans*
Difflugia gramen type (DIF GRA)	*Difflugia gramen* *Difflugia brevicolla*
Difflugia labiosa type (DIF LAB)	*Difflugia amphora* *Difflugia oviformis/labiosa* *Netzelia tuberculata*
Difflugia lucida type (DIF LUC)	*Difflugia avellana* *Diffguia glans* *Difflugia lithophila* *Difflugia lucida* type *Difflugia mica* *Difflugia penardi* *Difflugia pristis* type *Difflugia viscidula* *Pseudodifflugia fascicularis* *Pseudodifflugia fulva* type
Difflugia oblonga type (DIF OBL)	*Difflugia bacillifera* type *Difflugia gassowski/longicollis* *Difflugia lacustris* *Difflugia lanceolata* *Difflugia oblonga* type *Difflugia parva* *Difflugia paulii* *Difflugia pyriformis* *Difflugia rubescens*
Euglypha ciliata type (EUG CIL)	*Euglypha acanthophora* *Euglypha ciliata* *Euglypha ciliata* var. *glabra* *Euglypha ciliata/compressa* *Euglypha compressa* type *Euglypha cristata* *Euglypha dolioformis* *Euglypha filifera* *Euglypha scutigera* *Euglypha strigosa* type *Euglypha tuberculata* type
Euglypha rotunda type (EUG ROT)	*Euglypha alveolata* *Euglypha anadonta* *Euglypha cuspidata* *Euglypha denticulata* *Euglypha hyalina* *Euglypha laevis* *Euglypha rotunda* type *Euglypha simplex*
Heleopera petricola type (HEL PET)	*Awerintzewia cyclostoma* *Heleopera petricola* *Heleopera sphagni* *Heleopera petricola* var *amethystea* *Heleopera picta*
Lesqueresia spiralis type (LES SPI)	*Lesquereusia modesta* type *Lesquereusia spiralis* type
Planocarina (Nebela) carinata type (PLA CAR)	*Planocarina (Nebela) carinata* *Gibbocarina (Nebela) galeata* *Gibbocarina (Nebela) maxima* *Planocarina (Nebela) marginata*

Table 2 (*continued*)

Taxa defined in new groups	
New taxon name	*Includes*
Nebela collaris type (NEB COL)	*Nebela collaris* type *Nebela tincta* var. *major* *Hyalosphenia ovalis*
Gibbocarina (Nebela) penardiana type (GIB PEN)	*Gibbocarina (Nebela) penardiana* type *Gibbocarina (Nebela) gracilis* *Gibbocarina (Nebela) tubulosa* type
Nebela tincta type (NEB TIN)	*Nebela tincta* *Nebela parvula* *Nebela bohemica* type
Padaungiella lageniformis type (PAD LAG)	*Nebela lageniformis* *Nebela wailesi* type *Nebela tubulata*
Physochila griseola type (PHY GRI)	*Nebela griseola* type *Nebela tenella*
Placocista spinosa type (PLA SPI)	*Placocista spinosa* type *Placocista lens* *Placocista gracilis* *Placocista jurassica* *Placocista* sp.
Plagiopyxis type (PLAGIO)	*Plagiopyxis callida* *Plagiopyxis declivis*
Pyxidicula operculata type (PYX OPE)	*Pyxidicula declivis* *Pyxidicula operculata*
Sphenoderia type (SPHENO)	*Sphenoderia fissirostris* *Sphenoderia lenta* *Sphenoderia lenta/fissirostris*
Tracheleuglypha dentata type (TRA DEN)	*Tracheleuglypha dentata* *Tracheleuglypha acolla*
Trigonopyxis arcula type (TRI ARC)	*Trigonopyxis arcula* type *Trigonopyxis minuta* type *Trigonopyxis* sp.
Individual taxa/types not redefined	
Arcella dentata (ARC DEN) *Arcella mitrata* (ARC MIT) *Archerella flavum* (ARC FLA) *Bullinularia indica* (BUL IND) *Campascus minutus* (CAM MIN) *Difflugia leidyi* (DIF LEI) *Difflugia urceolata* type (DIF URC) *Heleopera rosea* (HEL ROS) *Heleopera sylvatica* (HEL SYL) *Hyalosphenia elegans* (HEL ELE) *Hyalosphenia papilio* (HYA PAP) *Hyalosphenia subflava* (HYA SUB) *Lagenodifflugia vas* (LAG VAS) *Lesquereusia epistomum* (LES EPI) *Longinebela (Nebela) militaris* type (NEB MIL) *Microclamys patella* (MIC PAT) *Nebela flabellulum* (NEB FLA) *Nebela minor* type (NEB MIN) *Paraquadrula irregularis* (PAR IRR) *Pontigulasia elisa* (PON ELI) *Pseudodifflugia gracilis* (PSE GRA) *Quadrulella symmetrica* (QUA SYM)	

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).

Table 3
Taxa occurring in <18 samples (<1% of the full dataset) excluded from the full dataset, resulting in an edited dataset of 52 taxa.

Taxon code	n	Full name
ARC MIT	2	*Arcella mitrata*
CAM MIN	11	*Campascus minutus*
DIF URC	12	*Difflugia urceolata* type
LAG VAS	8	*Lagenodifflugia vas*
LES EPI	14	*Lesquereusia epistomum*
PLAGIO	8	*Plagiopyxis* type
PON ELI	1	*Pontigulasia elisa*
PSE GRA	3	*Pseudodifflugia gracilis*

Table 4
Samples with total %s < 90 when 'sp.' taxa were deleted which were excluded from the full dataset, resulting in an edited dataset of 1775 samples.

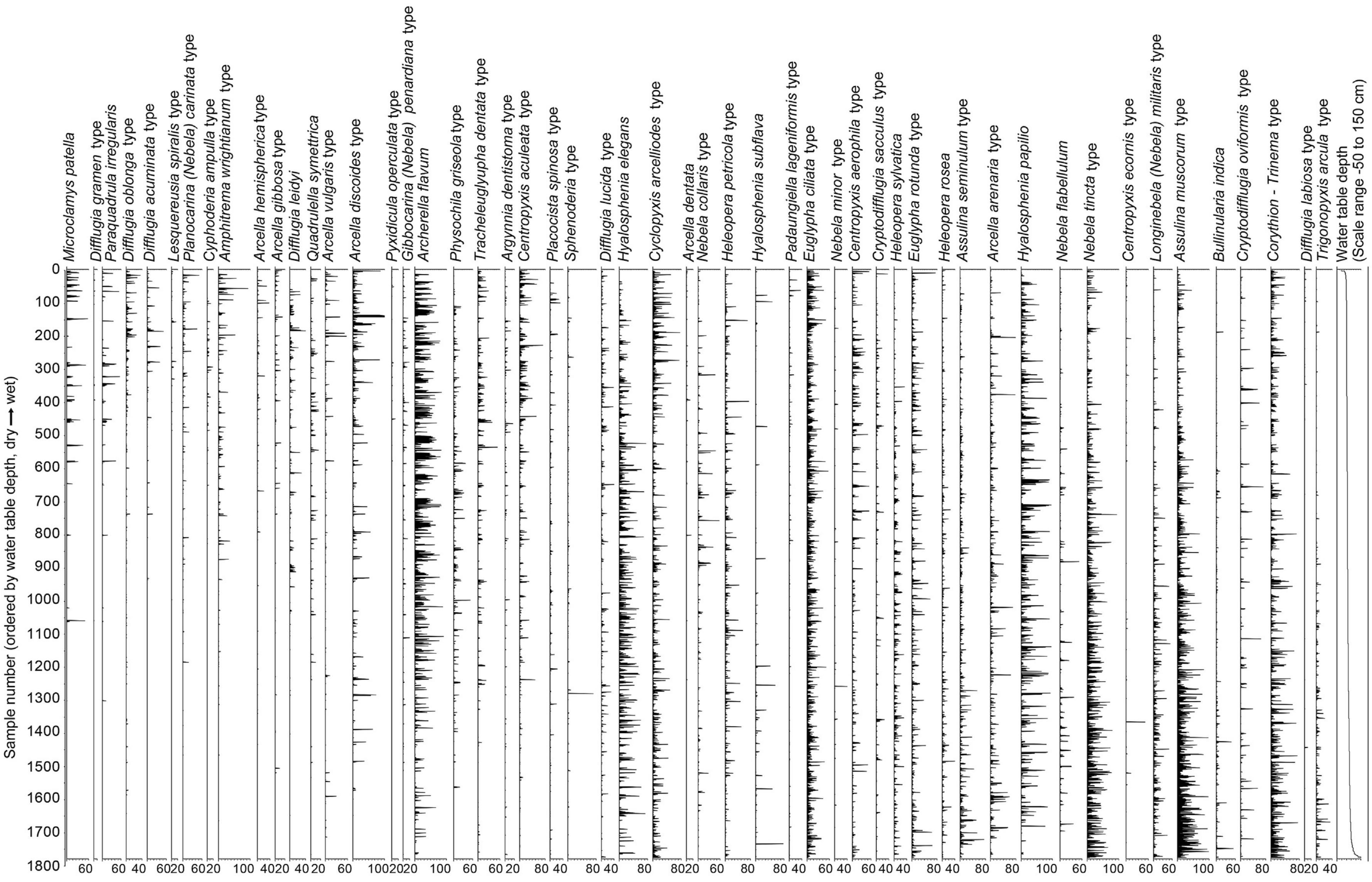
Country	Site name	Code	% Removed
Sweden	Gallseredsmossen	GAL_3	14.3
Sweden	South	S11_8	11.0
Poland	Chlebowo	CHL1	19.6
Poland	Chlebowo	CHL7	27.4
Poland	Chlebowo	CHL22	31.2
Poland	Chlebowo	CHL23	40.9
Poland	Kuźnik	K07	10.3
Poland	Kuźnik	K09	10.9
Poland	Kuźnik	K14	13.0
Poland	Rurzyca	RUR23	23.1
Poland	Wierzchołek	WEK2	28.2
Poland	Wierzchołek	WEK5	19.6
Poland	Wierzchołek	WEK6	40.4
Poland	Wierzchołek	WEK11	40.2
Poland	Wierzchołek	WEK12	12.8
Poland	Wierzchołek	WEK13	19.3
Poland	Wierzchołek	WEK14	46.2
Russia	Tverskya Reserve	Q92_1	11.4
Russia	Staroselia	STAR_1	19.0
Russia	Katin Moch	KATIN_5	10.3
Russia	Katin Moch	KATIN_6	44.4
Russia	Katin Moch	KATIN_7	19.3
England	Thornton Moor	TM85	14.5
Sweden	Crash	C1	17.6

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; Fig. 1) that represented broadly different climate zones and degrees of oceanicity/continentality.

3. Results

3.1. Ordination

NMDS of the full dataset (n = 1775; Fig. 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 (Fig. 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



All taxa proportions are % values

Fig. 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).

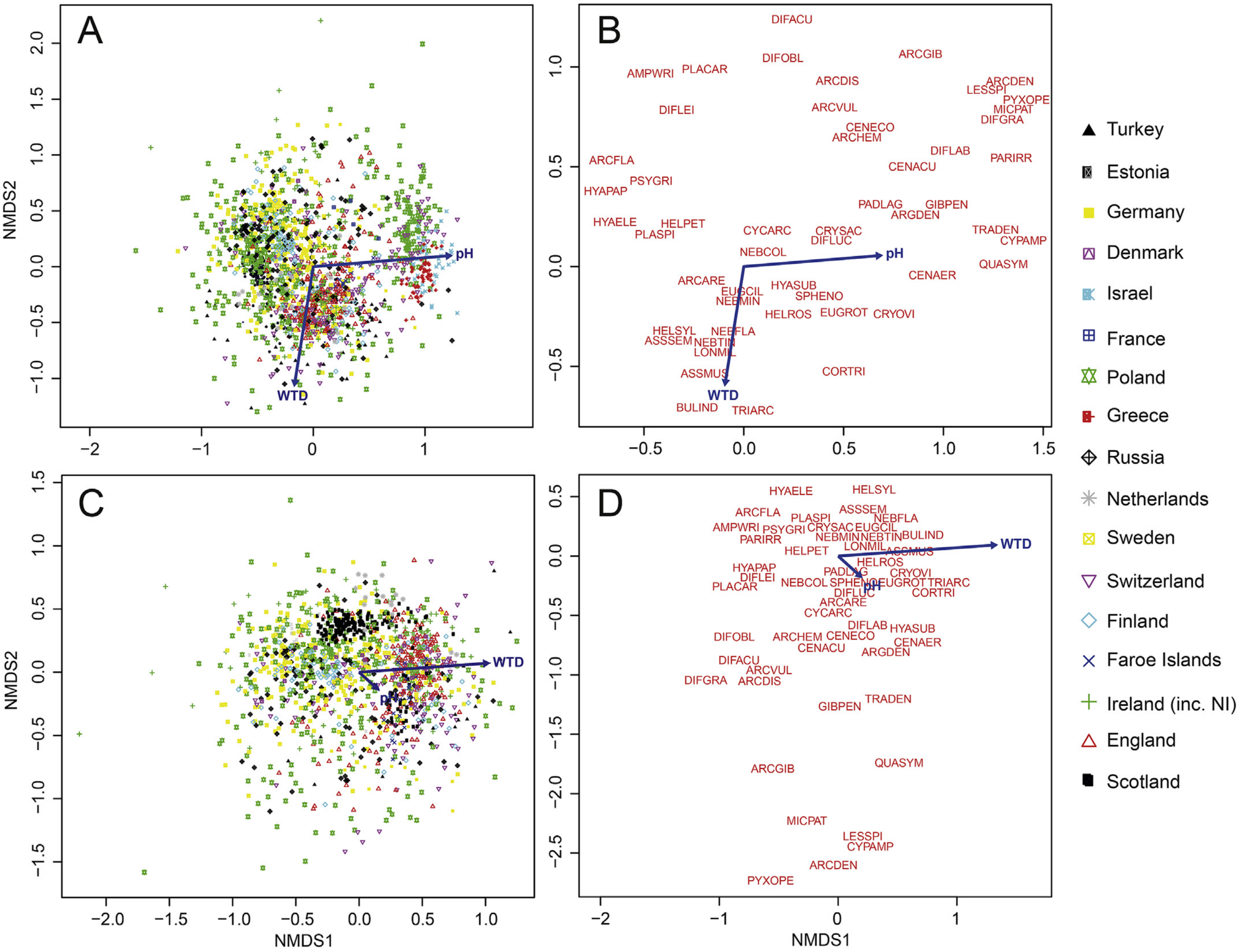


Fig. 3. NMDS plots before (A and B) and after (C and D) the removal of samples from the dataset with high pH values. A and C show sample positions, coded by country. B and D show taxa positions for same data as in A and B (but note different axis lengths). Vectors on all plots show influence of environmental 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.

abrupt jump to higher axis 1 values at this point in the pH range (Fig. 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 ($n = 211$); Fig. 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 (Fig. 3D) that reflects previous axis one variability (Fig. 3B), potentially driven by samples without pH values that may in reality be from sites with pH $\geq$ 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 (Fig. 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.

3.2. 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 Fig. 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 (Fig. 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 (Fig. 5) reflected the positioning of taxa along NMDS axis one (Fig. 3).

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 (Fig. 4), RMSEP$_{\text{LOO}}$ 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$_{\text{LOSO}}$ values showed a mean relative decrease in performance of only 0.068 (mean of 0.036 without WMAT K5) compared to RMSEP$_{\text{LOO}}$, less than that in Payne et al. (2012; mean decrease in performance of 0.141). Calculation of RMSEP$_{\text{SW}}$ (Fig. S2; single value for RMSEP$_{\text{SW}}$ is a mean of all individual segment RMSEPs) resulted in a decrease in performance compared to RMSEP$_{\text{LOO}}$ 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$_{\text{SW}}$ 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 (Fig. 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 to 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 (Fig. 1).

3.3. Testing model efficacy

In addition to statistical assessment of model performance, we

Table 5
Performance statistics for all transfer function models for water-table depth based on leave-one-out (RMSEP$_{\text{LOO}}$), leave-one-site-out (RMSEP$_{\text{LOSO}}$) and segment-wise (RMSEP$_{\text{SW}}$) cross validation methods. Results are in order of performance as assessed by RMSEP$_{\text{LOO}}$ of the first model run. Results for RMSEP$_{\text{LOO}}$ are given for both pre- and post-removal of samples with high residual values (see text for details; figures in parentheses show performance statistics after the removal of outlier samples). Results for RMSEP$_{\text{LOSO}}$ and RMSEP$_{\text{SW}}$ are only given for post-outlier removal models. SD is the standard deviation of all water-table measurements included in each model after the removal of outliers. Results are only shown for the best performing version of each model type. WA.inv.tol = weighted averaging with tolerance downweighting and inverse deshrinking; WAPLS C2 = second component of weighted averaging partial least squares; ML = maximum likelihood; WMAT K5 = weighted averaging modern analogue technique with five nearest neighbours.

Model type	RMSEP$_{\text{LOO}}$	R^2 (LOO)	Maximum bias (LOO)	Average bias (LOO)	RMSEP$_{\text{LOSO}}$	RMSEP$_{\text{SW}}$	Number of outlier samples removed	n for post-outlier removal model	SD
WA-Tol (inv)	10.87 (7.72)	0.46 (0.59)	58.45 (18.66)	0.03 (0.01)	7.95	9.93	91	1302	12.00
WAPLS C2	11.05 (7.66)	0.44 (0.59)	57.86 (17.57)	0.05 (0.02)	8.04	9.10	92	1301	12.03
WMAT K5	11.05 (7.22)	0.39 (0.67)	52.07 (13.25)	−1.07 (−0.8)	8.44	8.69	143	1250	12.44
ML	17.58 (8.73)	0.42 (0.71)	49.27 (4.07)	−5.01 (−1.95)	8.95	8.70	294	1099	13.64

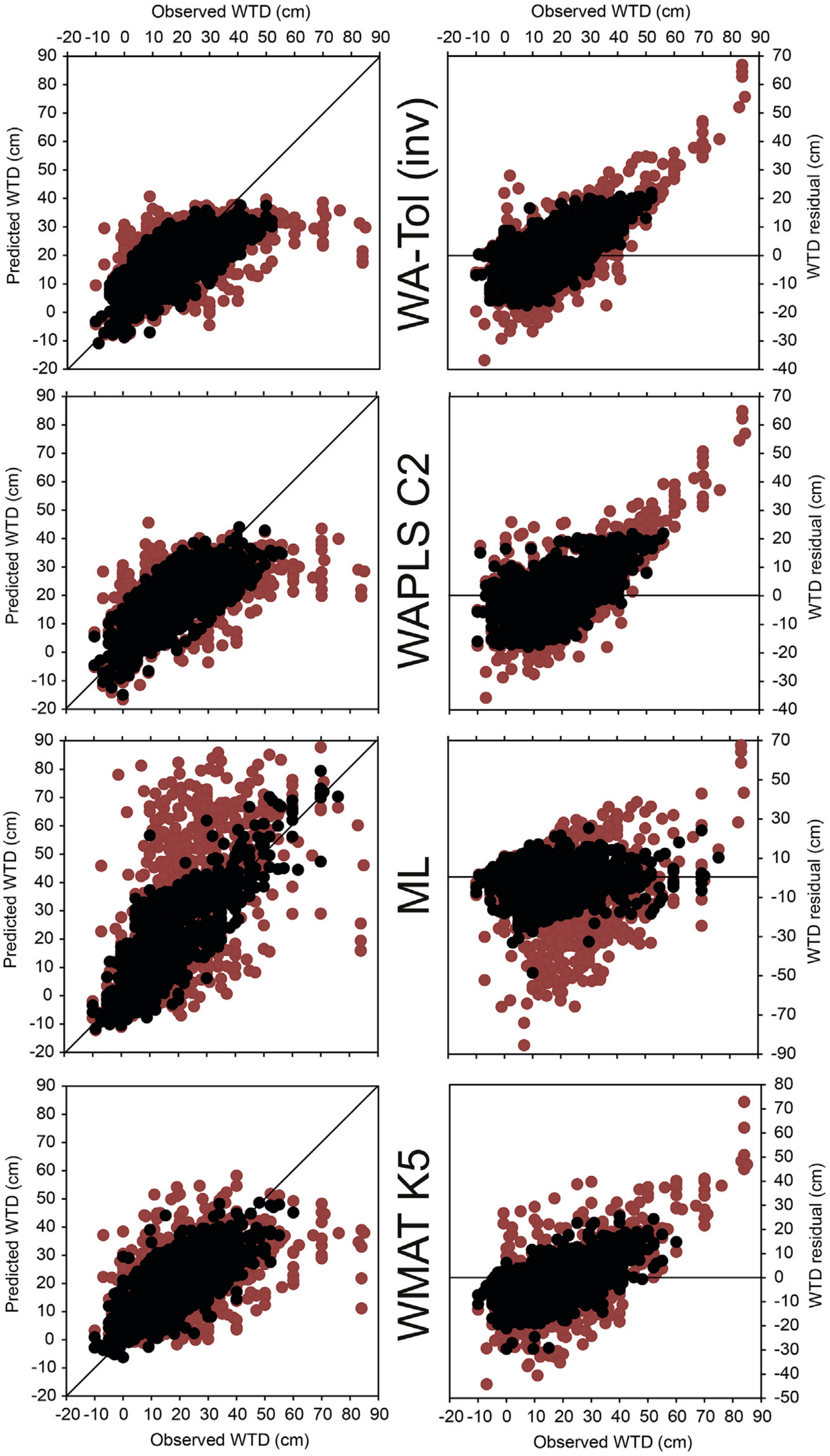


Fig. 4. Biplots of observed and predicted (leave-one-out cross-validated) water-table depth (left) and residual error plots (right) for the best performing versions of the four model types under investigation. WA-Tol (inv) = weighted averaging with tolerance downweighting and inverse deshrinking; WAPLS 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 interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 (Fig. 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

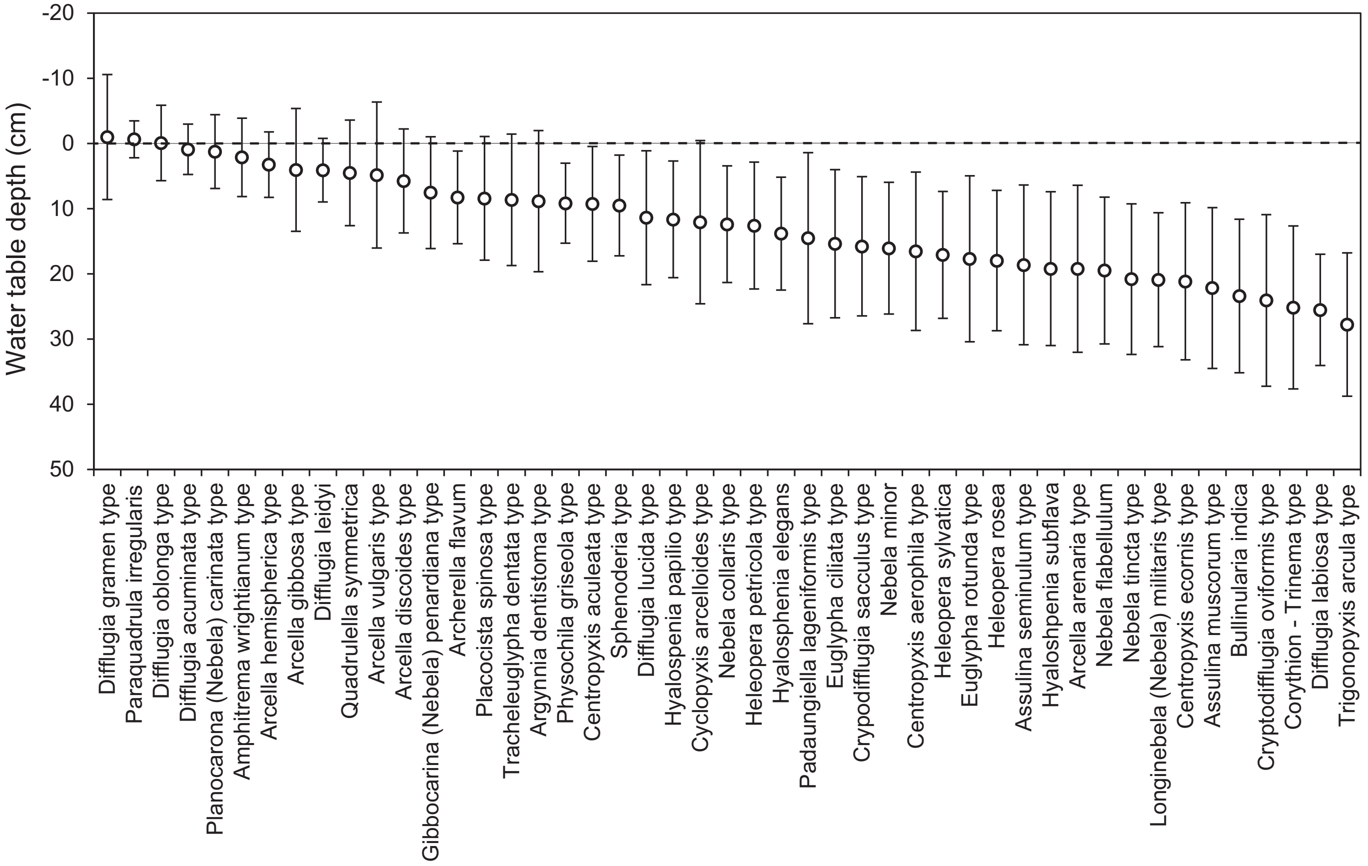


Fig. 5. Water-table depth optima and tolerances (cm) for 57 taxa based on the WA-Tol (inv) model after the removal of outlying samples (n = 1302).

when compared to a published reconstruction (Fig. 6A; Amesbury et al., 2008), particularly for ML. However, when viewed as residual plots (Fig. 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 (Fig. 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 (Fig. 6E), but when viewed as residual values (Fig. 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.

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.

3.4. 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 (Fig. 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 (Fig. 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

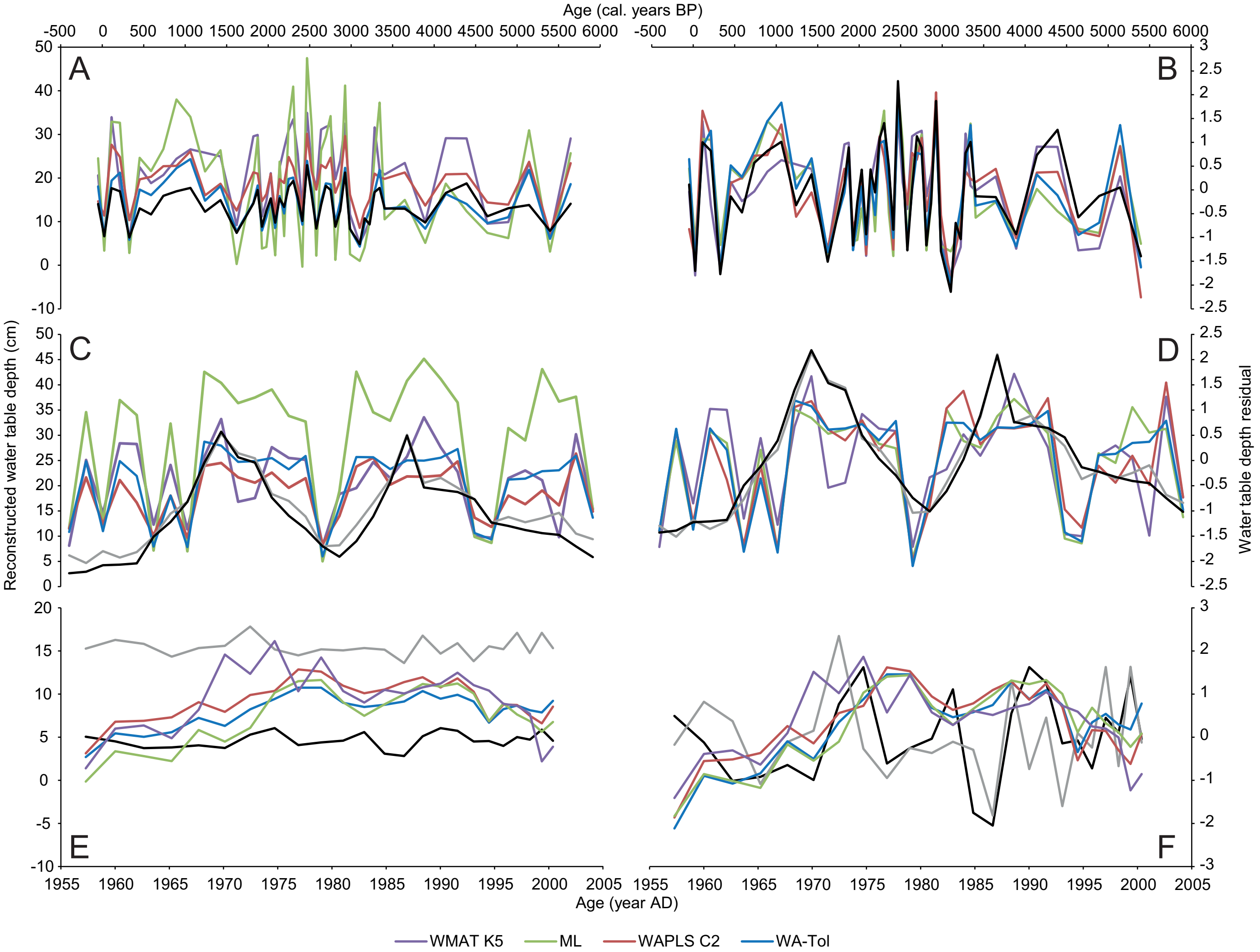


Fig. 6. Comparison of transfer function reconstructions from four model types (WA-Tol (inv), WAPLS-C2, ML, WMAT-K5) with independent test sets. A, C & E are raw water-table depth values; B, D & F are residual z-scores. A and B: reconstructions from Tor Royal Bog, Dartmoor, UK (Amesbury et al., 2008) 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).

Table 6
PalaeoSig p values for the significance of reconstructions against models based on randomly generated data for four model types and three independent test sets.

Test set	Reference	WA-Tol (inv)	WAPLS-C2	ML	WMAT-K5
Tor Royal Bog	Amesbury et al., 2008	0.077	0.075	0.274	0.003
Simulated	Swindles et al., 2015a,b	0.051	0.076	0.056	0.011
Männikjärve	Charman et al., 2004	0.154	0.15	0.031	0.001

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

Table 7
Summary information and performance statistics for regional transfer function models run using a WA-Tol (inv) model type and the reduced dataset after the removal of outlier samples (n = 1302).

Region	n	Mean WTD (cm) (range)	$\text{RMSEP}_{\text{LOO}}$	R^2 (LOO)	Average bias (LOO)	Maximum bias (LOO)
Atlantic	461	16.0 (-10–52)	8.06	0.60	0.03	−20.25
Scandinavia	341	12.7 (-5–50)	7.36	0.53	0.08	15.19
Continental	500	16.0 (-5–52)	6.94	0.67	0.05	14.30

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 (Fig. 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 (Fig. 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; Fig. 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; Fig. S4; range of 55 cm, compared to 57 cm and 62 cm for the Atlantic and Continental models respectively).

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

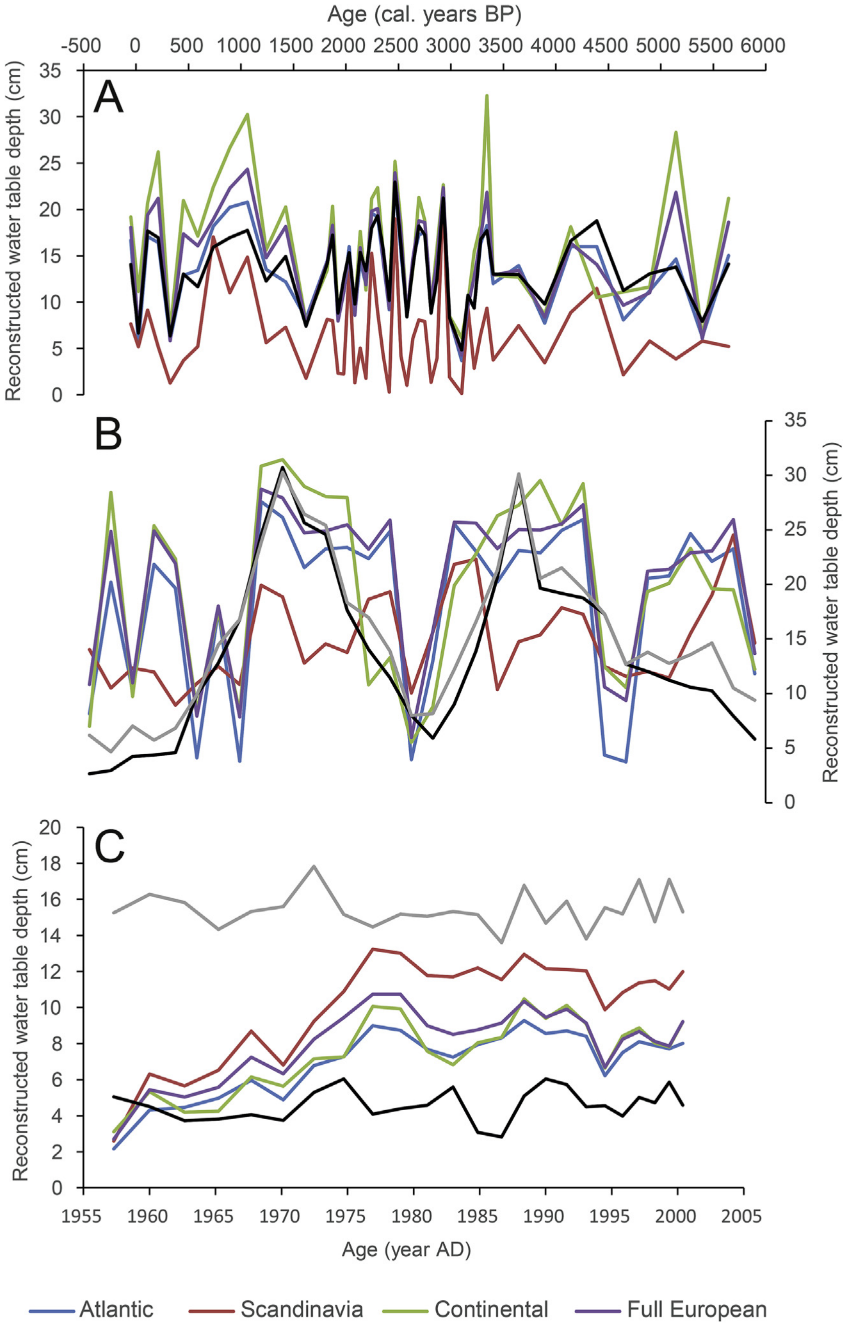


Fig. 7. Comparison of three regional transfer function reconstructions to the full European model for the same three independent test sets as Fig. 6. All reconstructions use the WA-Tol (inv) model type. A: Tor Royal Bog (black line uses the established ACCROTELM European transfer function of Charman 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).

cosmopolitan, or tended to have skewed distributions, favouring a particular region (Fig. 8; Fig. 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 (Fig. 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; Fig. 8).

4. Discussion

4.1. 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 (Fig. 6). Particularly when records were standardised (Swindles et al., 2015b) to remove variability in specific reconstructed water-table depth values (Fig. 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.

4.2. 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 (Fig. 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; Fig. 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; Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 5). Given the similar mean values and water-table depth ranges of all regions, particularly Atlantic and Continental (Table 6; Fig. 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.

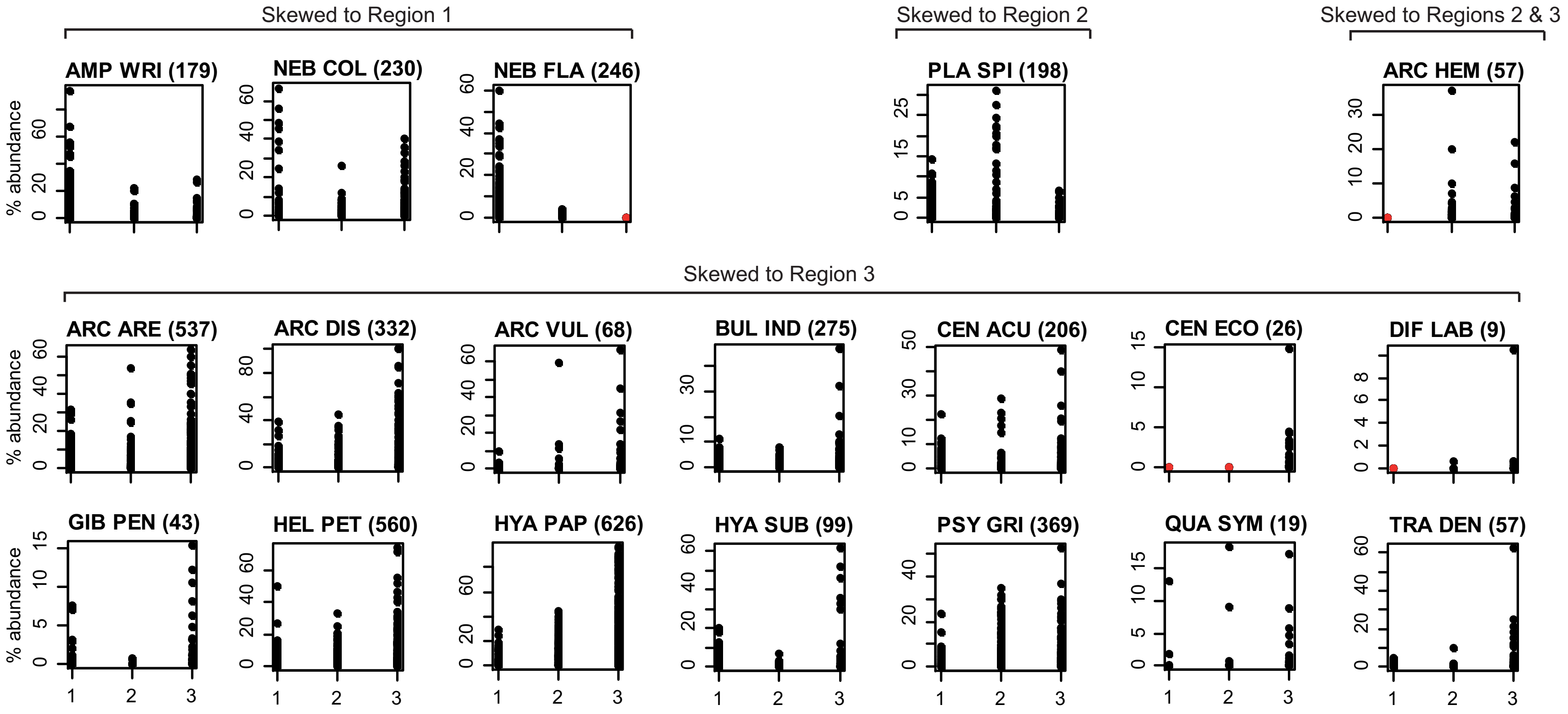


Fig. 8. Taxa with uneven distributions across the three regions (n = 19). Taxa with all occurrences <5% abundance have been excluded (n = 2; DIF GRA and PAR IRR). Region codes on x-axes: 1 = Atlantic, 2 = Scandinavia, 3 = continental Europe. For full taxa abbreviations, see Table 2. Number of occurrences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

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.

4.3. *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. Figs. 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 (Fig. 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 (Fig. 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 multifaceted 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 (Fig. 7). In addition, the similarity of reconstructions across model types and/or regions presented here (Figs. 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.

5. 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.

Author contributions

MJA and GTS conceived the work, compiled the data, conducted data analysis and wrote the manuscript. All other authors contributed data, actively discussed the direction of the research, developed the approach to taxonomic harmonisation and/or contributed to manuscript editing.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quascirev.2016.09.024.

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