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# 1 Development of a new pan-European testate amoeba transfer 2 function for reconstructing peatland palaeohydrology

3  
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23  
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25 and wrote the manuscript. All other authors contributed data, actively discussed the direction of the  
26 research, developed the approach to taxonomic harmonisation and/or contributed to manuscript  
27 editing.

## 28 29 **Abstract**

30 In the decade since the first pan-European testate amoeba-based transfer function for peatland  
31 palaeohydrological reconstruction was published, a vast amount of additional data collection has  
32 been undertaken by the research community. Here, we expand the pan-European dataset from 128  
33 to 1799 samples, spanning 35° of latitude and 55° of longitude. After the development of a new  
34 taxonomic scheme to permit compilation of data from a wide range of contributors and the removal  
35 of samples with high pH values, we developed ecological transfer functions using a range of model  
36 types and a dataset of ~1300 samples. We rigorously tested the efficacy of these models using both  
37 statistical validation and independent test sets with associated instrumental data. Model  
38 performance measured by statistical indicators was comparable to other published models.  
39 Comparison to test sets showed that taxonomic resolution did not impair model performance and  
40 that the new pan-European model can therefore be used as an effective tool for palaeohydrological  
41 reconstruction. Our results question the efficacy of relying on statistical validation of transfer  
42 functions alone and support a multi-faceted approach to the assessment of new models. We  
43 substantiated recent advice that model outputs should be standardised and presented as residual

44 values in order to focus interpretation on secure directional shifts, avoiding potentially inaccurate  
45 conclusions relating to specific water-table depths. The extent and diversity of the dataset  
46 highlighted that, at the taxonomic resolution applied, a majority of taxa had broad geographic  
47 distributions, though some morphotypes appeared to have restricted ranges.

48

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

51

## 52 **Highlights**

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

58

## 59 **Introduction**

60 Testate amoebae are microscopic, unicellular shelled protozoa that are abundant in a range of  
61 wetlands, including peatlands (Mitchell et al., 2008). Early research demonstrated the close  
62 ecological coupling between testate amoebae and hydrological parameters such as water-table  
63 depth and moisture content in such environments (e.g. Jung, 1936; Schönborn, 1963). Quantitative  
64 ecological approaches demonstrated the strength of this relationship and used it to derive  
65 reconstructions of hydrological variability from fossil testate amoebae (Warner and Charman, 1994;  
66 Woodland et al., 1998). This approach has subsequently been thoroughly developed and extended  
67 geographically, using more advanced statistical techniques (e.g. Charman et al., 2007; Booth, 2008;  
68 Swindles et al., 2009, 2014, 2015a; Amesbury et al., 2013). Testate amoeba-based hydrological  
69 reconstructions are now frequently used as hydroclimate proxies in studies of Holocene climate  
70 change (e.g. Charman et al., 2006; Swindles et al., 2010; Elliott et al., 2012; Lamentowicz et al., 2015;  
71 Willis et al., 2015). Central to such research is typically the application of a transfer function. These  
72 statistical models apply the observed modern ecological preferences of amoebae via a range of  
73 mathematical approaches (Juggins and Birks, 2011) to fossil assemblages to quantitatively  
74 reconstruct environmental variables of interest, primarily water-table depth in ombrotrophic  
75 peatlands, but occasionally other parameters such as pH (Markel et al., 2010; Mitchell et al., 2013).  
76 Testate amoeba-based hydrological transfer functions have now been developed in a wide range of  
77 locations (e.g. Li et al., 2015; Swindles et al., 2015a, 2014; van Bellen et al., 2014) and wetland types,  
78 primarily in bogs, but also in fens (Payne, 2011; Lamentowicz et al., 2013a; Lamentowicz et al.,  
79 2013b). Recent debates in this field have focussed on 1) more rigorous analysis of transfer function  
80 results, whether via statistical testing (Telford and Birks, 2005, 2009, 2011a, 2011b, Payne et al.,  
81 2012, 2016; Amesbury et al., 2013), or by comparison with instrumental data (Swindles et al.,  
82 2015b); 2) the appropriateness of varying spatial scales for transfer function development (Turner et  
83 al., 2013); and 3) the validity of applying models outside of the geographic range over which they  
84 were developed (Turner et al., 2013; Willis et al., 2015), and hence the cosmopolitanism of testate  
85 amoeba ecological preferences (Booth and Zygmunt, 2005) across a range of geographical locations  
86 (Smith et al., 2008).

87

88 When transfer function models developed in one region are applied in a different region where no  
89 local model exists, results may theoretically be undermined by a number of factors. These include  
90 missing modern analogues, differences in testate amoeba ecology or biogeography between the two  
91 regions (Turner et al., 2013), the technique used to measure water-table depth in the calibration  
92 data sets (Markel et al., 2010; e.g. long-term mean versus one-off measurement), regionally diverse  
93 seasonal variability (Sullivan and Booth, 2011; Marcisz et al., 2014) or vertical zonation (van Bellen et  
94 al., 2014) of testate assemblages, or local-scale variability in the response of certain taxa, or even  
95 communities, of testate amoebae to environmental variables (e.g. Booth and Zygmunt, 2005).  
96 However, in practice, when transfer functions from one region are applied to fossil data from a  
97 separate region, even over distances of thousands of kilometres (Turner et al., 2013; Willis et al.,  
98 2015), or when regional- and continental-scale models are compared (e.g. Amesbury et al., 2008;  
99 Charman et al., 2007; Swindles et al., 2009; Turner et al., 2013), it is largely only the absolute values  
100 and magnitude of reconstructed water-table shifts that vary between models, with the timing and  
101 direction of change being generally consistent. Given that the absolute values and magnitude of  
102 transfer function-reconstructed change in water-table depth have recently been questioned by  
103 direct comparison of reconstructed and instrumental water-table depths (Swindles et al., 2015b), it  
104 could be argued that a) testate amoeba-based transfer function reconstructions should be viewed as  
105 semi-quantitative and interpretation should be based only on the timing and direction of change;  
106 and that b) the general ecological cosmopolitanism of testate amoebae (e.g. Mitchell et al., 2000;  
107 Booth and Zygmunt, 2005) when studied at coarse taxonomic level (i.e. morphotypes – but see  
108 Heger et al., 2013 for an example of cryptic diversity showing geographical patterns) means that  
109 regional transfer functions are widely applicable, at least at an intra-continental or even intra-  
110 hemispheric scale.

111

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

124

## 125 **Methods**

126

### 127 *Data compilation and taxonomy*

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

132

133 [INSERT FIGURE 1]

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

137

138

139 Although the potential risks of taxonomic inconsistency, especially in large data compilations with  
140 large numbers of analysts, are clear (Payne et al., 2011), the likely effect of using a low taxonomic  
141 resolution potentially decreased model performance (in statistical terms) rather than any effect on  
142 the timing or direction of major changes in wetness (Mitchell et al., 2014). Due to the high number  
143 of data contributors/analysts in this compilation and in order to ensure taxonomic consistency  
144 across the merged dataset, we adopted a low-resolution approach to defining an appropriate  
145 taxonomic scheme, merging morphologically similar taxa together into a series of newly defined  
146 groups. Initial examination of contributed datasets made it clear that different analysts had grouped  
147 (or 'lumped') or split taxa to varying extents, with many taxa only present in individual datasets. A  
148 low-resolution approach to taxonomy was therefore considered to be not only the most  
149 parsimonious, but also the only scientifically valid approach to the compilation of such a large  
150 dataset, despite genuine variation in water-table optima occurring between taxa within some new  
151 groupings (see Results). Individual analysts should not count new samples in line with the low-  
152 resolution taxonomic scheme applied here, but rather differentiate between readily identifiable taxa  
153 in line with current taxonomies and group taxa together only for statistical analysis. The majority of  
154 recently published papers on peatland testate amoebae use Charman et al. (2000) as a standard  
155 identification guide, with an increasing number of variations noted in recent years including, most  
156 prevalently, the reclassification of *Amphitrema flavum* as *Archerella flavum* (Loeblich and Tappan,  
157 1961), the splitting out of certain 'type' groupings into their constituent taxa (e.g. *Cyclopyxis*  
158 *arcelloides* type into *Cyclopyxis arcelloides sensu stricto*, *Phryganella acropodia* and *Diffflugia*  
159 *globulosa*; Turner et al., 2013) and more recent reclassifications based on phylogenetic studies (e.g.  
160 *Nebela* taxa moving to the genera *Longinebela*, *Planocarina* and *Gibbocarina*; Kosakyan et al., 2016).

161

162 Across all 1799 samples in the full dataset, a total of 186 individual taxa were identified, with the  
163 final taxonomic scheme containing a reduced 60 taxa, of which 41 were 'type' groupings (38 newly  
164 defined) that each contained between two and 11 taxa with similar morphological features (Table 2).  
165 These groups were defined with reference to a range of identification keys and source literature  
166 (Cash and Hopkinson, 1905, 1909, Cash et al., 1915, 1918; Ogden and Hedley, 1980; Meisterfeld,  
167 2000a, 2000b) as well as using the expertise and experience of the authors. Our treatment of the  
168 two *Euglypha* groups – *E. ciliata* type and *E. rotunda* type – provides an example of the low  
169 resolution approach we adopted. These groups contained 11 and eight individual taxa respectively  
170 that had been identified by individual analysts in the originally contributed datasets. However, the  
171 only morphological characteristic that we could identify as consistently applied across all datasets  
172 was size, with several datasets only defining *E. tuberculata* (i.e. larger type >45 µm) and *E. rotunda*  
173 (i.e. smaller type <45 µm). Since the presence/absence of spines (e.g. *E. strigosa* vs. *E. tuberculata*)  
174 may be biased by taphonomic processes (Payne et al., 2011), we therefore defaulted to a two-taxon  
175 system for this family.

176

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

187

188 [INSERT FIGURE 2]

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

191

192

### 193 *Statistics*

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

204

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

214

215 To test the applicability of the new model, we applied it to 1) downcore independent test data from  
216 a long-term (~6000 years) record from Tor Royal Bog (TRB) in Dartmoor, UK (Amesbury et al., 2008),  
217 2) a simulated palaeo dataset developed from surface samples with associated automated  
218 instrumental water-table depth measurements, ordered to 'create' two major shifts in water-table  
219 depth (Swindles et al., 2015b) and 3) downcore independent test data from a short-term record  
220 from Männikjärve Bog, Estonia with associated automated instrumental water-table data (Charman  
221 et al., 2004). For test sets 2 and 3, we used annual and summer (JJA) mean water-table depth values

222 in each case, calculated from multiple daily measurements (for full details see source publications).  
223 Sample-specific errors for the transfer function reconstructions were based on 1000 bootstrapping  
224 cycles. We compared our reconstructions with output from the previous European transfer function  
225 (Charman et al., 2007) and tested the significance of the new reconstructions using the 'randomTF'  
226 function in palaeoSig (Telford, 2015).

227

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

232

## 233 **Results**

### 234 *Ordination*

235 NMDS of the full dataset (n=1775; Figure 3A and B) showed that the primary environmental variable  
236 explaining species distribution along axis 1 was pH, as opposed to water-table depth, illustrating the  
237 influence of the peatland type gradient (i.e. ombrotrophic to minerotrophic). A distinct group of  
238 samples formed an outlying cluster with high NMDS axis 1 scores. To determine an appropriate pH  
239 cut-off to reduce the dataset to those containing nutrient poor, ombrotrophic peatlands, we used  
240 results of k-means cluster analysis, forcing the data into two clusters (Figure S1; i.e. lower pH values  
241 in Group 1, higher pH values in Group 2). 5.4 was the highest pH where the majority of samples fell  
242 in Group 1 and 5.5 was the lowest pH where the majority of samples fell in Group 2. We therefore  
243 removed all samples with pH $\geq$ 5.5. This division was supported by plotting NMDS scores against pH,  
244 which showed an abrupt jump to higher axis 1 values at this point in the pH range (Figure S1) and  
245 also by general peatland ecology: *Sphagnum* moss, the dominant peat-forming species in Northern  
246 Hemisphere ombrotrophic peatlands is known to actively acidify its environment (van Breemen,  
247 1995) and therefore ombrotrophic bogs are typically dominated by pH ranges of 3.0 – 4.5, with  
248 *Sphagnum*-dominated poor fens having marginally higher pH (4.5 – 5.5; Lamentowicz and Mitchell,  
249 2005). Using this cut-off resulted in the removal of 370 samples with pH values 5.5 – 8.1 and the  
250 removal of all samples from France, Greece and Israel. We re-ran NMDS ordination on the reduced,  
251 low-pH dataset (n=1405, including samples without a pH measurement (n=211); Figure 3C and D)  
252 which then showed that water-table depth was the primary environmental variable explaining  
253 species variation along axis 1 (p<0.001 using the 'envfit' function in vegan), providing a statistical  
254 foundation to proceed with transfer function development. Despite water-table depth being the  
255 primary explanatory variable after removal of high pH samples, there is still considerable variability  
256 along NMDS axis two (Figure 3D) that reflects previous axis one variability (Figure 3B), potentially  
257 driven by samples without pH values that may in reality be from sites with pH $\geq$ 5.5. In particular, a  
258 group of nine taxa (*Tracheleuglypha dentata* type, *Gibbocarina (Nebela) penardiana* type, *Arcella*  
259 *gibbosa* type, *Quadrulella symmetrica*, *Microclamys patella*, *Lesquereusia spiralis* type, *Cyphoderia*  
260 *ampulla* type, *Arcella dentata* type and *Pyxidicula operculata* type) fall outside of the main cluster of  
261 variability with more negative axis two scores (Figure 3D), potentially suggesting that these taxa may  
262 be less reliable water table indicators, associated more with nutrient enrichment (Payne, 2011;  
263 Lamentowicz et al., 2013a). Following the removal of high pH samples, all nine are relatively rare  
264 taxa, occurring in < 5% of the 1405 samples. Five of the taxa are defined as rare based on previously  
265 defined criteria (i.e. < 1% of the dataset, or n=14; *A. dentata* type, *C. ampulla* type, *L. spiralis* type,  
266 *M. patella*, *P. operculata* type) and so these were excluded from further analyses, reducing the

267 number of taxa in the dataset for transfer function development to 47. The number of samples in  
268 which the remaining four taxa (*A. gibbosa* type, *G. (N.) penardiana* type, *Q. symmetrica*, *T. dentata*  
269 type) were present was reduced by > 50% in all cases with the removal of high pH samples (e.g. by  
270 52% for *A. gibbosa* type but up to a reduction of > 80% for *Q. symmetrica*), so water-table depth  
271 reconstructions based on fossil assemblages containing significant proportions of these taxa should  
272 be treated with caution.

273

274 [INSERT FIGURE 3]

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

281

282

### 283 *Transfer function development and statistical assessment*

284 Before proceeding with transfer function development, we removed 12 further samples with  
285 extreme measured water table values (Table S1), resulting in a dataset for transfer function  
286 development of 1393 samples. These 12 samples fell below the 0.5th (i.e. representing deep surface  
287 ponding, n=2) and above the 99.5th (i.e. representing extreme deep water tables, n=10) percentiles  
288 of water-table depth and were removed to avoid the large increase in water-table depth range that  
289 would result from their inclusion and the subsequent effect on removal of samples with high  
290 residual values. In addition, the removal of extreme deep water-table depth samples is supported by  
291 Swindles et al., 2015b, who showed a disconnect between testate amoebae and water table in such  
292 circumstances. In keeping with standard practice, we then ran two iterations of models, the first  
293 using all samples and the second having removed samples with residual values greater than 20% of  
294 the range of water-table values in the dataset (min = -10 cm, max = 85 cm, range = 95 cm, 20% range  
295 = 19 cm) (e.g. Amesbury et al., 2013; Booth, 2008; Charman et al., 2007; Payne et al., 2006; Swindles  
296 et al., 2009). Residuals removed in the second iteration of model runs were specific to each model  
297 type and therefore varied in number (Table 5). The effect of removing residual samples is shown in  
298 Figure 4 for the best performing versions of the four model types under investigation (WA-Tol (inv) =  
299 weighted average tolerance downweighting with inverse deshrinking; WAPLS C2 = second  
300 component of weighted averaging partial least squares; WMAT K5 = weighted mean modern  
301 analogue technique with five nearest neighbours). Results for WAPLS C2 are included but fell  
302 marginally outside the recommended cut-off for acceptance (5% at  $p < 0.05$ ; Birks, 1998); the second  
303 component provided a 4.71% improvement ( $p = 0.001$ ) over the first component (i.e. simple weighted  
304 averaging). Residual error plots show that the majority of samples with high residual values fell at  
305 the 'dry' end of the water table gradient and that, in general, all models tended to under-predict at  
306 the dry end of the gradient (i.e. negative residual value) and over-predict at the wet end of the  
307 gradient (i.e. positive residual value). Biplots of observed and predicted water-table depths show  
308 that, particularly for both weighted average models and WMAT K5 but not so ML, models tended to  
309 reach a plateau of predicted values at around 40 – 50 cm regardless of the observed value. In  
310 contrast to previous studies (e.g. Amesbury et al., 2013) which found larger water table tolerances  
311 correlated with drier optima, tolerance ranges for the WA-Tol (inv) model were similar throughout



312 the water table gradient (Figure 5), potentially as a result of the ‘averaging out’ effect of taxonomic  
313 groupings, although a small group of hydrophilous taxa did have narrower tolerances. The ordering  
314 of taxa water table optima (Figure 5) reflected the positioning of taxa along NMDS axis one (Figure  
315 3).

316

317 [INSERT FIGURES 4 AND 5]

318

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

327

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

330

331

332 Performance statistics (Table 5; principally, RMSEP and  $R^2$ ) before the removal of outlier samples  
333 were generally poor, though equivalent to some published models (e.g. Swindles et al., 2015a; van  
334 Bellen et al., 2014). After the removal of outlier samples with high residual values (Figure 4),  
335  $RMSEP_{LOO}$  values for the WA-Tol (inv), WAPLS C2 and WMAT K5 models fell in the range 7 – 8 cm,  
336 equivalent to that generally seen in other published transfer functions (Booth, 2008; Markel et al.,  
337 2010; Amesbury et al., 2013; Lamarre et al., 2013; Li et al., 2015; Swindles et al., 2015a) and,  
338 notably, similar to the ACCROTELM European model (Charman et al., 2007).  $RMSEP_{LOSO}$  values  
339 showed a mean relative decrease in performance of only 0.068 (mean of 0.036 without WMAT K5)  
340 compared to  $RMSEP_{LOO}$ , less than that in Payne et al. (2012; mean decrease in performance of  
341 0.141). Calculation of  $RMSEP_{SW}$  (Figure S2; single value for  $RMSEP_{SW}$  is a mean of all individual  
342 segment RMSEPs) resulted in a decrease in performance compared to  $RMSEP_{LOO}$  for all models with  
343 the exception of ML, which supports previous research that found ML outperformed MAT- and WA-  
344 based models on unevenly sampled gradients (Telford and Birks, 2011b). There was a prevalence of  
345 samples in the water-table depth range 0 – 35 cm, with water-table depths <0 cm and >35 cm less  
346 well represented (although it should be noted that due to the high overall number of samples in the  
347 dataset, even the lowest frequency segment, 45 – 49.5 cm still contained 15 – 18 samples,  
348 depending on model type). Individual segment RMSEP values generally increase where sampling  
349 frequency is lower, particularly at the ‘dry’ end of the water table gradient, in keeping with  
350 expectation (Telford and Birks, 2011b), except for ML, which shows more consistent RMSEP values  
351 across all segments, driving the observed relative improvement in  $RMSEP_{SW}$  against other model  
352 types. In all cases, RMSEP values, however calculated, remained lower than the standard deviation  
353 of all water table measurements (Table 5), suggesting all models have a degree of predictive ability  
354 (*cf.* Amesbury et al., 2013; Mitchell et al., 2013). All models display a degree of spatial  
355 autocorrelation (Figure S3), given that  $r^2$  values decline more steeply when geographically proximal,  
356 as opposed to random, samples are removed (Telford and Birks, 2009). For all models to some

357 extent, but for WMAT K5 in particular, the decline in  $r^2$  over the first 100 km is similar to the decline  
358 for the most environmentally similar samples, indicating that geographically proximal samples are  
359 also the most environmentally similar across the dataset. Coupled to the general similarity of  $R^2$   
360 from 100 – 1000 km, this reflects the spatial structure of the data whereby each individual data  
361 contribution (Table 1) tended to include multiple sites/samples, with individual study locations being  
362 widely distributed across Europe (Figure 1).

363

#### 364 *Testing model efficacy*

365 In addition to statistical assessment of model performance, we used three independent data sets,  
366 two with associated instrumental water table measurements, to test the new models. Broadly  
367 speaking, reconstructions using the four different model types under consideration (WA-Tol (inv),  
368 WAPLS-C2, ML, WMAT-K5) showed similar patterns of change to either alternative published  
369 transfer function reconstructions or instrumentally recorded water table fluctuations, although  
370 water-table depth ranges were more variable (Figure 6). For the Tor Royal Bog test set, all model  
371 types reconstructed generally drier conditions and ranges of reconstructed water-table depths were  
372 much higher for all model types when compared to a published reconstruction (Figure 6A; Amesbury  
373 et al., 2008), particularly for ML. However, when viewed as residual plots (Figure 6B; Swindles et al.,  
374 2015b), all models show extremely similar patterns of change over the ~6000 year record. For the  
375 simulated shifts in water-table depth (Figure 6C and D; Swindles et al., 2015b), all models again  
376 produced comparable reconstructions with the exception of ML. All models reconstructed the  
377 simulated shifts in water table with the correct frequency and direction of change, but reconstructed  
378 shifts were more abrupt, occurring over 2 – 3 samples, with simulated shifts more gradual, occurring  
379 over 6 – 10 samples. Whereas the wet and dry ends of the simulated shifts were single point  
380 extremes, modelled reconstructions exhibited more rapid, threshold-type switches in water table  
381 interspersed with plateaux of more consistently wet or dry conditions. Reconstructions of monitored  
382 water-table depth at Männikjärve fell between the annual and summer mean values for water-table  
383 depth (Figure 6E), but when viewed as residual values (Figure 6F), differences were evident in the  
384 patterns of change over the c. 50 year record, with the comparatively smooth reconstructions  
385 suggesting a broadly drier period during the 1970s and 1985 – 1995, with wetter conditions before  
386 and after, whereas instrumental data show that water table varied over shorter time scales  
387 throughout the period of monitoring.

388

389 [INSERT FIGURE 6]

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

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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  $RMSEP_{LOO}$  and  $RMSEP_{LOSO}$  values (Table 5). Performance statistics ( $RMSEP_{LOO}$ ,  $R^2$ ) 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]

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

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

467

468 [INSERT FIGURE 8]

469 **Figure 8:** Taxa with uneven distributions across the three regions ( $n = 19$ ). Taxa with all occurrences  
470  $< 5\%$  abundance have been excluded ( $n = 2$ ; DIF GRA and PAR IRR). Region codes on x-axes: 1 =  
471 Atlantic, 2 = Scandinavia, 3 = continental Europe. For full taxa abbreviations, see Table 2. Number of  
472 occurrences in 1302 samples shown in brackets after taxon code. Red dots indicate complete  
473 absence from a particular region. For reference to colour, readers are referred to the online version  
474 of this article.

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476

## 477 Discussion

### 478 Data compilation

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

492 resolution in reality given the demonstrated existence of high cryptic and pseudo-cryptic diversity  
493 (e.g. Oliverio et al., 2014; Kosakyan et al., 2016), so some degree of taxonomic parsimony will always  
494 be necessary.

495

#### 496 *Spatial scales and regional variability*

497 The debate surrounding the degree of cosmopolitanism exhibited in free-living microorganisms, of  
498 which testate amoebae provide a good model group, is well established and on-going (e.g. Heger et  
499 al., 2009). Conflicting views assume universal ubiquity (Finlay and Clarke, 1999; Finlay et al., 1999,  
500 2001) or (occasional) limited geographical distribution of microorganisms (i.e. the 'moderate  
501 endemism model'; Foissner, 2008, 2006, 1999). An increasing number of studies focussing on the  
502 distribution of testate amoebae have observed taxa which do not appear to support the theory of  
503 ubiquity (Smith and Wilkinson, 2007; Smith et al., 2008; Heger et al., 2009, 2011; Yang et al., 2010;  
504 Turner et al., 2013; Fournier et al., 2015; Lara et al., 2016).

505

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

512

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

536 from such site types were included in the model as a result of their lacking associated pH  
537 measurements.

538

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

557

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

566

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

568 Due to the complexity of peatland water table and testate amoeba ecological responses, both  
569 moderated by a range of differing factors, it is becoming clear that transfer function reconstructions  
570 should not be seen as simple metrics of past climate (Turner et al., 2013). In addition, the apparent  
571 inaccuracy of reconstructed water-table depth values, particularly towards the 'dry' end of the  
572 gradient where both methodological and ecological problems are exacerbated, suggests that  
573 reconstructions should be displayed as residuals or standardised values and interpreted primarily as  
574 metrics of directional shifts between wetter and drier conditions, an approach in which context  
575 reconstructions from a range of models have shown to be robust (Swindles et al., 2015b). Our own  
576 data (e.g. Figures 6 and 7) show that, with some exceptions, our models are relatively consistent in  
577 performance for reconstructed water-table depth values across the full gradient. This is likely as a  
578 result of the high number of samples characterising all water table segments (Figure S2); although  
579 there are relatively fewer samples in drier water table segments, a common problem identified in  
580 other studies (Amesbury et al., 2013; Swindles et al., 2015a). Segment-specific n values are still high

581 (lowest is  $n=25$  for 45 – 49.5 cm, mean for all water-table depth segments  $>20$  cm is  $n=70$ ),  
582 highlighting the value of the large compiled dataset. However, the variability present between both  
583 model types and regions, despite an unprecedented training set size, argues that reconstructions  
584 should be standardised and presented as residual values (*sensu* Swindles et al., 2015b) in order to  
585 focus interpretation on secure directional shifts, avoiding potentially inaccurate conclusions relating  
586 to specific water-table depths.

587

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

612

### 613 **Conclusions and guidelines for the application of the transfer function**

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

626 applied, a majority of taxa had broad geographic distributions, though some morphotypes appeared  
627 to have restricted ranges.

628

629 To facilitate future research, we provide the full compiled dataset, along with R code to allow the  
630 free application of our transfer function to fossil data by individual users, as supplementary online  
631 material. The R code facilitates the application of the WA-Tol (inv) model and conversion of  
632 reconstructed water-table depth values to standardised residual z-scores. The WA-Tol (inv) model  
633 type has been commonly applied in other studies (e.g. Amesbury et al., 2013; Swindles et al., 2015a,  
634 2009) and compared favourably to other model types in terms of reported performance statistics in  
635 this study. Users are free to alter the R code as appropriate to apply other model types, but should  
636 justify these changes in their work.

637

638

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655

### 656 **Figure captions:**

657

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

661

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

664

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



671

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

680

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

683

684 **Figure 6:** Comparison of transfer function reconstructions from four model types (WA-Tol (inv),  
685 WAPLS-C2, ML, WMAT-K5) with independent test sets. A, C & E are raw water-table depth values; B,  
686 D & F are residual z-scores. A and B: reconstructions from Tor Royal Bog, Dartmoor, UK (Amesbury et  
687 al., 2008) compared (panel A only) with a published reconstruction using a European transfer  
688 function (black line; Charman et al., 2007). C and D: reconstructions of simulated wet and dry shifts  
689 derived from reordered surface samples with associated instrumental water table measurements  
690 (black line = annual mean water-table depth, grey line = summer (JJA) mean water-table depth;  
691 Swindles et al., 2015b). Y-axis (not shown) is randomly ordered surface sample codes. E and F:  
692 reconstructions of near-surface fossil data from Männikjärve Bog, Estonia with associated long-term  
693 instrumental water table measurements (black line = annual mean water-table depth, grey line =  
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696

697 **Figure 7:** Comparison of three regional transfer function reconstructions to the full European model  
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700 Charman et al., 2007); B: simulated changes in water-table depth (black (annual) and grey (summer)  
701 lines are instrumental water table measurements); C: Männikjärve Bog, Estonia (black (annual) and  
702 grey (summer) lines are instrumental water table measurements). For reference to colour, readers  
703 are referred to the online version of this article.

704

705 **Figure 8:** Taxa with uneven distributions across the three regions (n = 19). Taxa with all occurrences  
706 < 5% abundance have been excluded (n = 2; DIF GRA and PAR IRR). Region codes on x-axes: Atl. =  
707 Atlantic, Scan. = Scandinavia, Cont. = continental Europe. For full taxa abbreviations, see Table 2.  
708 Number of occurrences in 1302 samples shown in brackets after taxon code. Red dots indicate  
709 complete absence from a particular region. For reference to colour, readers are referred to the  
710 online version of this article.

711

712

713 **Supplementary Figure captions:**

714

715 **Figure S1:** NMDS axis 1 scores plotted against pH for all samples (n = 1775). Inset: pH of two k-means

716 cluster analysis groups. Red line in both plots shows cut-off applied of  $pH \geq 5.5$ .

717

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

721

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

727

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

729

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

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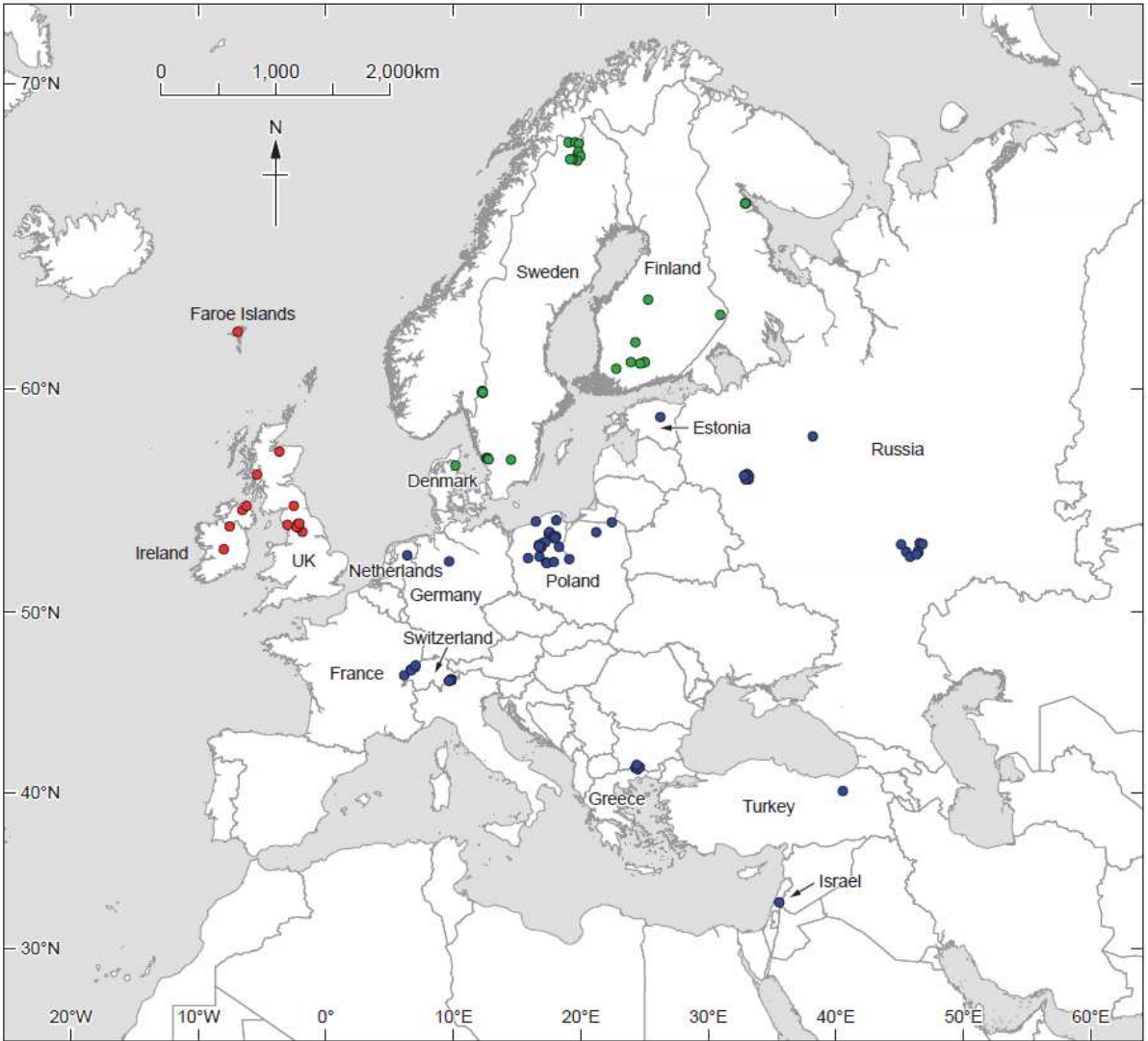
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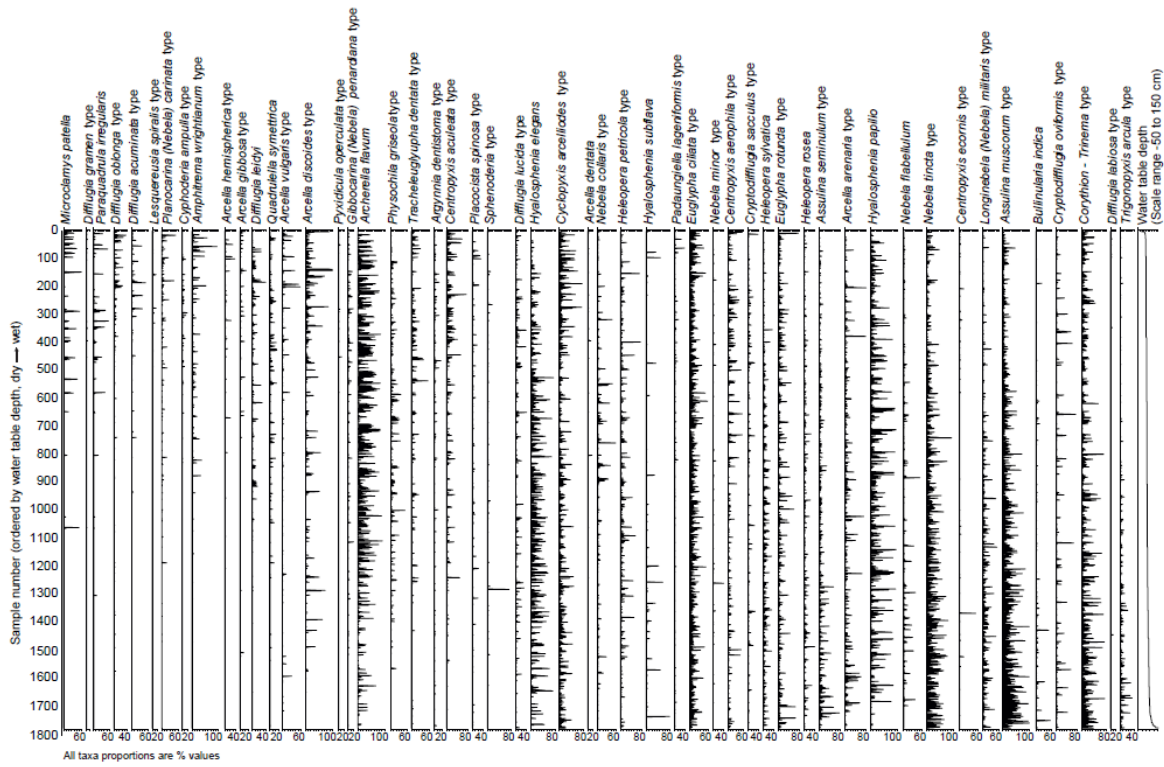
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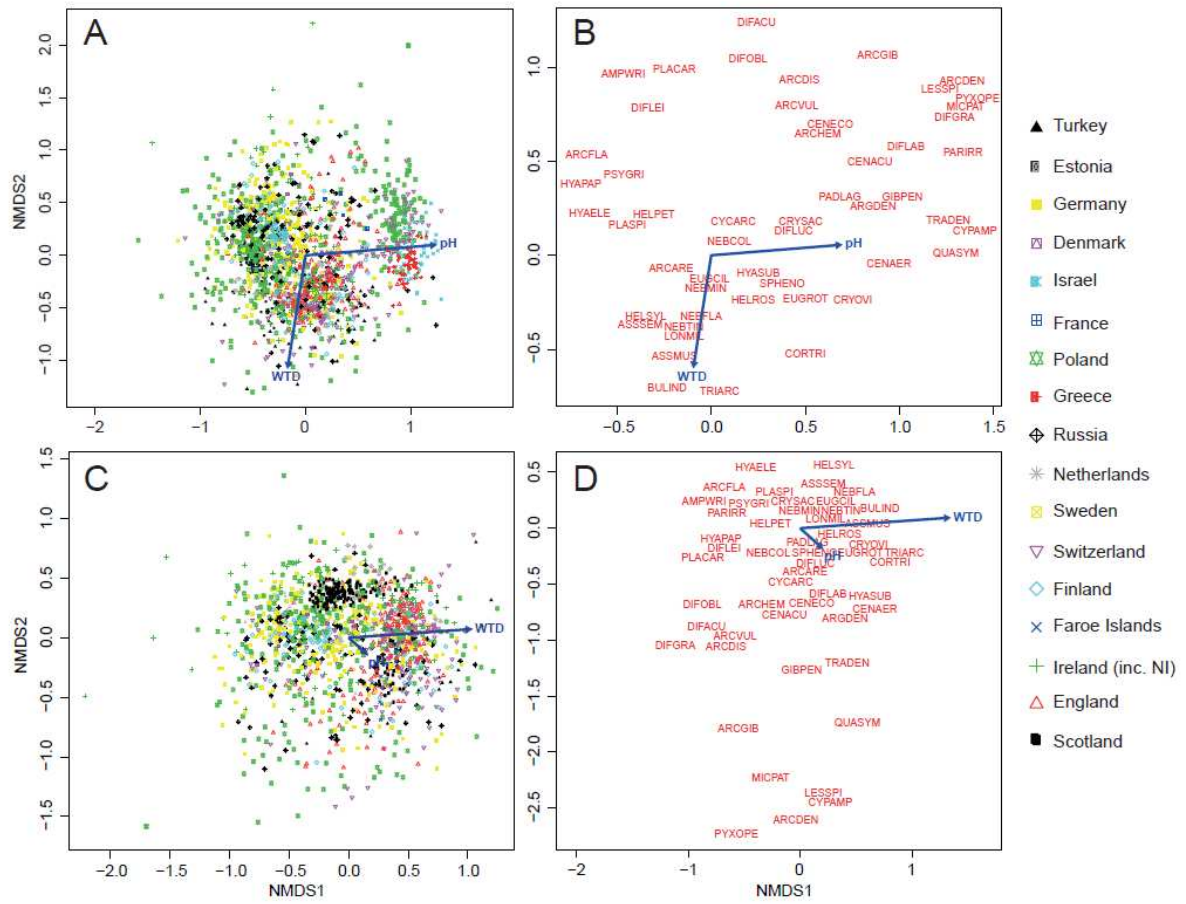
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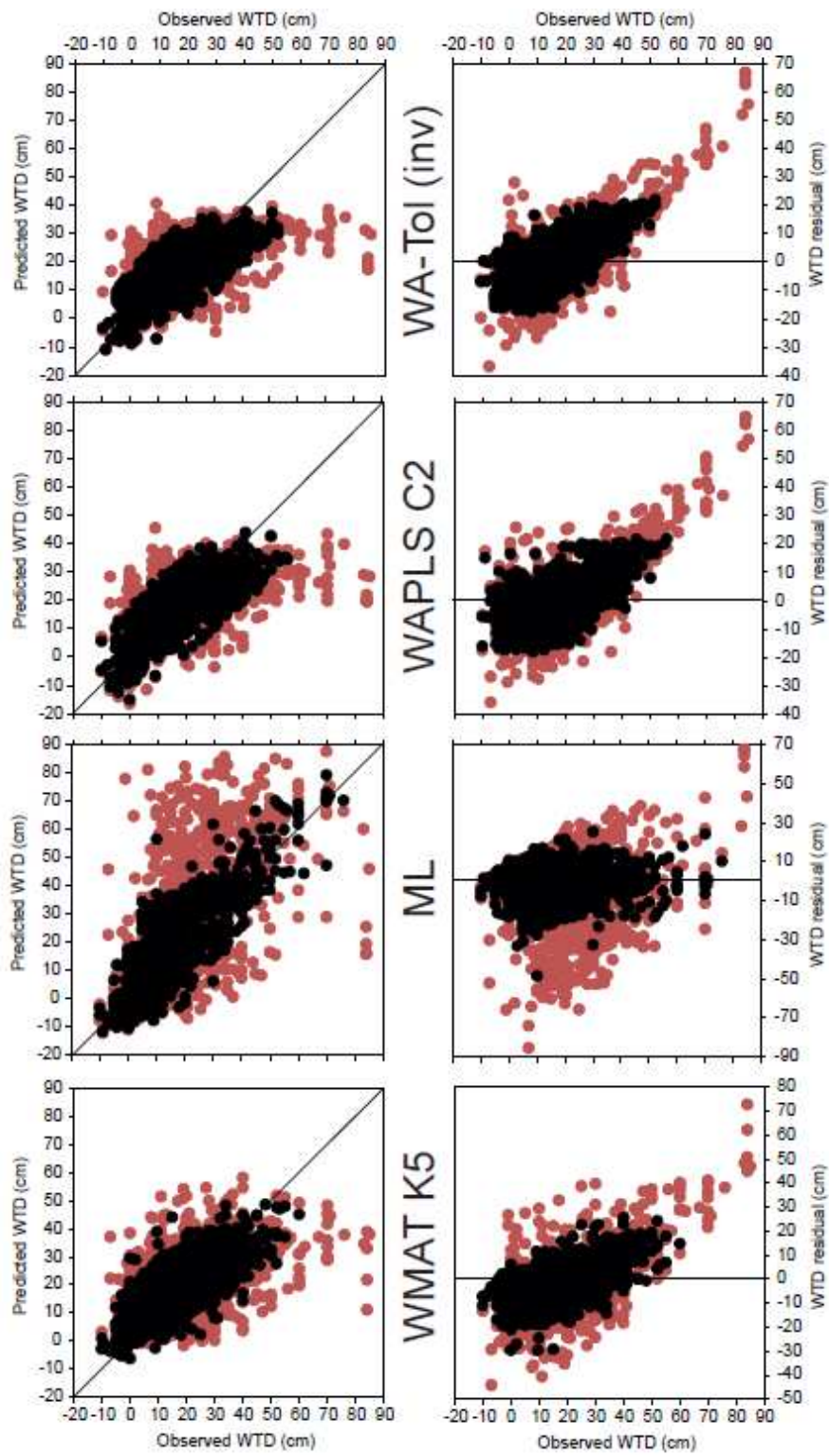
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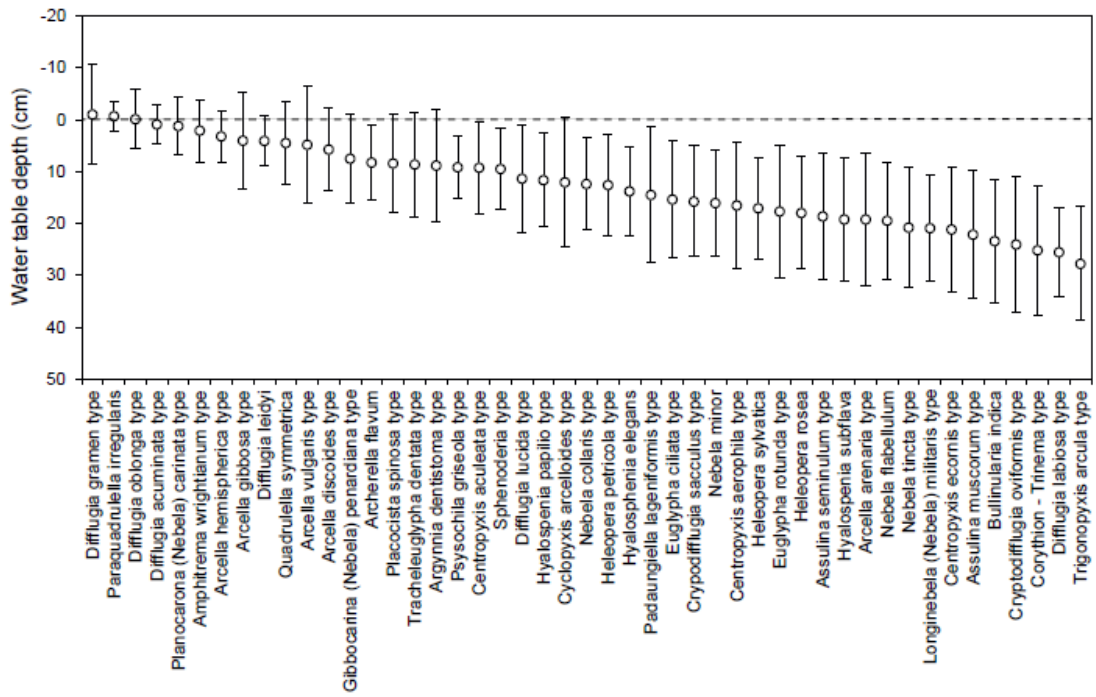
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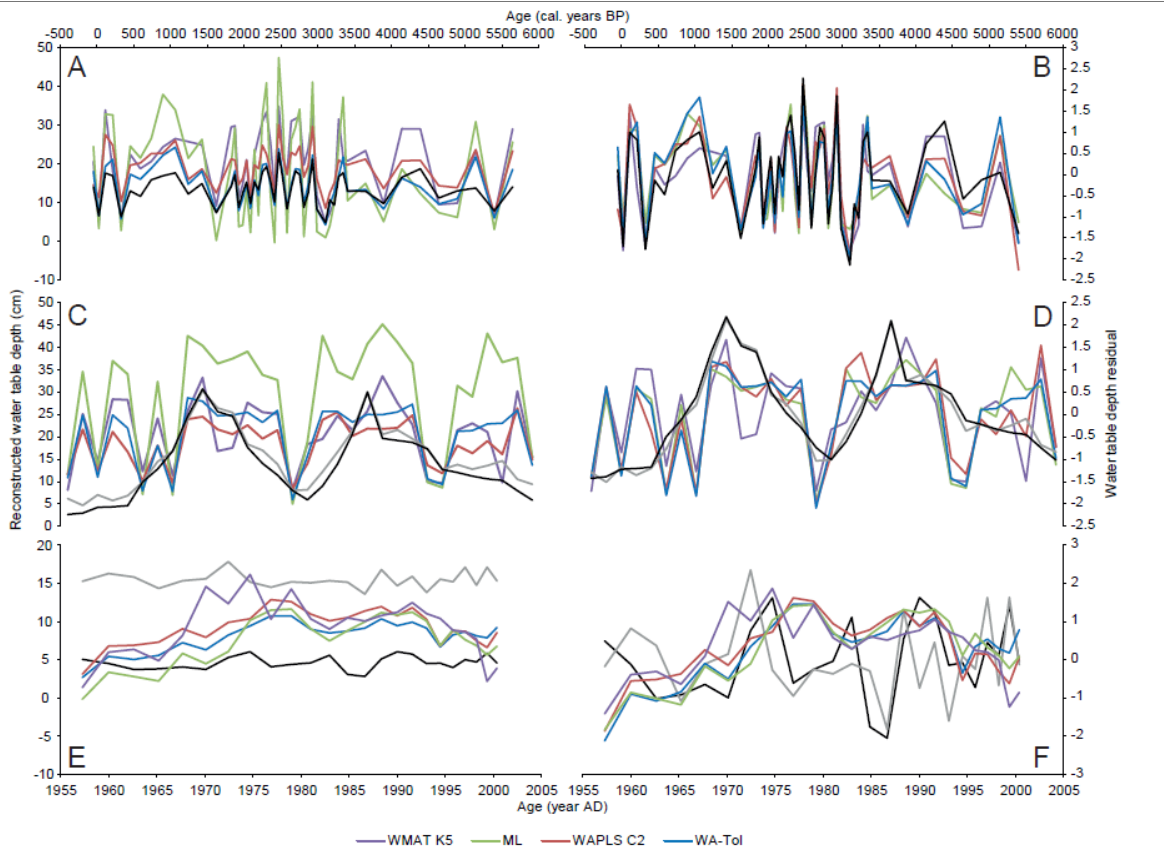








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