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1 The performance of single- and multi-proxy transfer functions (testate amoebae,
2 bryophytes, vascular plants) for reconstructing mire surface wetness and pH

3

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25

26 **Abstract**

27 Peatlands are widely exploited archives of palaeoenvironmental change. We developed and
28 compared multiple transfer functions to infer peatland depth to the water table (DWT) and pH
29 based on testate amoeba (percentages or presence/absence), bryophyte presence/absence and
30 vascular plant presence/absence data from sub-alpine peatlands in the SE Swiss Alps in order
31 to compare the performance of single-proxy vs. multi-proxy models and assess the
32 performance of presence/absence models.

33 Bootstrap cross-validation showed the best-performing single-proxy transfer functions for
34 both DWT and pH were those based on bryophytes. The best-performing TF overall were
35 those based on combined testate amoebae %, bryophytes and vascular plants for DWT and on
36 testate amoebae and bryophytes for pH. The comparison of DWT and pH inferred from TA %
37 and presence/absence models showed that the general patterns were similar but the magnitude
38 and timing of some shifts were different.

39 These results show new directions for palaeoenvironmental research suggesting: 1) that it is
40 possible to build transfer functions which perform well using presence/absence average data,
41 although with some loss of accuracy, 2) supporting the idea that multi-proxy inference models
42 may improve palaeoecological reconstruction. The performance of multi-proxy and single-
43 proxy transfer functions should be further compared in palaeoecological data.

44

45 **Key words:** Peatlands; bryophytes; vascular plants; testate amoebae; transfer function;
46 quantitative palaeoecology; monitoring; water table depth; pH

47

48

49 **Introduction**

50 Testate amoebae (Protists) and plant macrofossils are the two most commonly used proxies
51 for reconstructing Holocene environmental change in peatlands (Booth, 2010; Hughes et al.,
52 2006; Mauquoy et al., 2004). These proxies primarily reflect surface wetness and pH and can
53 be used to study mire development, climate change, and human impacts (e.g. drainage,
54 grazing). The two proxies complement each other well in palaeoecological studies (Mauquoy
55 and van Geel, 2007; Mitchell et al., 2008) and also have a strong potential for use in the
56 biomonitoring and conservation management of peatlands (Lavoie et al., 2001). Most studies
57 on peatland testate amoeba ecology highlight the importance of surface wetness, and water
58 table depth or some related variable almost invariably emerges as the strongest environmental
59 variable explaining the testate amoeba community data (Booth, 2008; Charman et al., 2007;
60 Swindles et al., 2009). Relationships to water chemistry have also been documented but have
61 been much less studied, and focused mostly on pH (Lamentowicz et al., 2008; Mitchell et al.,
62 1999; Opravilova and Hajek, 2006; Tolonen et al., 1992). Despite the importance of wetland
63 plants in community ecology (Bridgham et al., 1996; Wheeler and Proctor, 2000), studies
64 providing quantitative inferences on the basis of sub-fossil plant remains are rare (Janssens,
65 1983; Kuhry et al., 1993; Välranta et al., 2007).

66 The use of multiple proxies in the same record is generally believed to lead to more
67 accurate and robust palaeoenvironmental reconstruction (Birks and Seppa, 2010; Caseldine
68 and Gearey, 2005; Charman et al., 1999; Long et al., 1996). The general rationale for this is
69 that while each proxy has its limitations a signal is more likely to be accurate if several
70 proxies show the same trend. Combining different proxies in a synthetic way (e.g. in a single
71 transfer function) is an alternative option. This option may be especially pertinent where
72 proxies differ slightly in their response (Lamentowicz et al., 2010b) or are useful for different
73 parts of a gradient (Gehrels et al., 2001). Previous studies have examined the combined use of
74 testate amoebae, diatoms, and foraminifera (Gehrels et al., 2001) and diatoms and
75 foraminifera (Kemp et al., 2009) for reconstructing past sea-level changes, and of
76 chironomids, diatoms, and chrysophytes for reconstructing alkalinity, DIC, altitude, pH and
77 dissolved CO₂ in mountain lakes (Thompson et al. (2008). This multi-proxy approach has not
78 been trialled for the peatland archive but may offer improved reconstructions.

79 This study is a continuation of earlier work on the same material, derived from sub-
80 alpine mires of SE Switzerland (Upper Engadine valley). We developed a testate-amoeba-
81 based transfer function for inferring depth to the water table (DWT) and applied it in a
82 palaeoecological study covering the instrumental period AD 1864–2003 (Lamentowicz et al.,
83 2010b) and the last millennium (van der Knaap et al., 2011). We also studied the relationships
84 among testate amoebae, bryophytes, vascular plants, and hydrochemical variables
85 (Lamentowicz et al., 2010a). One outcome of the latter study was that the three categories of
86 organisms had somewhat different, though overlapping responses to environmental gradients;
87 species-environment correlations were higher for testate amoebae than for bryophytes and
88 vascular plants and the individual environmental variables explained different proportions of
89 the variance. Transfer functions combining two or three proxies in a multi-proxy model would
90 therefore be ecologically justifiable and given the differing responses, might offer superior
91 performance. Here we therefore follow-up to assess if multi-proxy models (all possible
92 combinations of testate amoebae, bryophytes and vascular plants) would outperform single-
93 proxy models.

94

95 **Sites and methods**

96 Lamentowicz et al. (2010a) provided a full description of study sites, location map, and field
97 and laboratory methods. Summary information on the sites is given in Table 1. Field sampling
98 was done over a three-day period in August 2007 (97 plots) in the sub-alpine belt of the
99 Upper Engadin valley, SE Swiss Alps (average coordinates 46°27'00'' N; 9°46'30'' E;
100 elevation range 1810–1864 m a.s.l.). The sampled locations cover a wide range of surface
101 moisture, trophic states, and vegetation types. The largest mire studied is Mauntschas, a
102 Sphagnum mire at the valley bottom surrounded by natural conifer forest that includes
103 minerotrophic mire, sloping fen, Sphagnum fuscum hummocks, and ombrotrophic mountain-
104 pine bog. The peatlands near Maloja Pass lie on the side of the valley bottom and are
105 surrounded by non-natural Pinus mugo forest. They are poor fens dominated by Sphagnum
106 fallax, Carex rostrata, and Eriophorum angustifolium. Inn Fen is a peaty meadow along the
107 river Inn, dominated by sedge vegetation and with scattered Alnus incana and Salix trees; the
108 samples are mainly vascular-plant detritus. The mires of Lej da Staz, Lej Marsch, and Lej
109 Nair lie adjacent to small lakes close to the valley bottom and are surrounded by natural
110 conifer forest. Lej Marsch and Lej da Staz mires represent typical examples of

111 terrestrialisation with floating Sphagnum mats near the lakeshore and more stable peat closer
112 to the forest; sampling was done in a transect along this gradient. Lej Nair site is species-rich
113 calcareous sloping fen.

114 Four data sets were used for numerical analysis: testate amoeba (TA) percentages, TA
115 presence/absence, bryophyte presence/absence, and vascular plant presence/absence. Taxa
116 present in less than three samples were removed from the data sets. A limitation of our study
117 is that we did not have percentage data for bryophytes and vascular plants. There are practical
118 and theoretical reasons for this decision. Obtaining reliable percentage data for bryophytes
119 would have required sampling and identifying about 10'000 samples (ca. 100 per plot x ca.
120 100 plots). We felt that such an effort was not justified because bryophyte macrofossil data is
121 usually at best estimated on a semi-quantitative scale and there is no direct correspondence
122 between surface cover and macrofossil volume because the different moss species have
123 contrasting architecture and decay at different rates. Vascular plants, on the other hand, are
124 typically identified as presence-absence data in macrofossil analyses, and even for this the
125 amount of peat material needed often exceeds what is available, especially for studies aiming
126 at high temporal resolution.

127 Transfer functions were created separately for DWT (depth to the water table
128 measured at the time of sampling) and pH (measured on water extracted from the same moss
129 samples as those used to extract testate amoebae), using C2 (Juggins, 2003). Data filtering
130 (outlier sample removal), although criticised, is often used in palaeoecology (Booth et al.,
131 2008; Edwards et al., 2004; Wilmshurst et al., 2003; Woodland et al., 1998). The rationale for
132 this, besides improving model performance is that some sampled locations may correspond to
133 unusual situations (e.g. affected by a confounding factor such as plant faeces/urine) that are
134 impossible to model accurately. We filtered the data in a single step by removing outlier
135 samples with residuals higher than the standard deviation of the observed values. This
136 procedure was repeated a second time in three cases (as clear outliers remained): for 1)
137 bryophytes and vascular plants, 2) vascular plants, and 3) TA presence/absence. Transfer
138 functions were created for each of the four data sets separately and all appropriate
139 combinations of data sets (Table 2).

140 Combining presence/absence data with percentage data resulted in an imbalanced data
141 set in which the presence was interpreted as 1% cover. To assess how this affected the model
142 performance we compared three options. The first was the original presence/absence

143 (hereafter: 1/0) data. In the second case the data was multiplied by one hundred (hereafter:
144 100/0). In the third case the total percentage was adjusted to 100% by replacing each presence
145 by 100 divided by the number of species present in a sample [hereafter: (100/n)/0]. Note that
146 the resulting total “percentage” was therefore of 100% for one data set, 200% for two and
147 300% for the three sets. In this way, each data set was given equal weight in the overall
148 analysis.

149 The rationale for comparing TA 1/0 and % data sets was to assess how the
150 corresponding reduction in information affected model performance. In very few
151 palaeoecological studies are testate amoeba abundances too low to make calculations of
152 percentages meaningful and in such cases quantitative inference of DWT or other variables is
153 generally not performed (Wehrli et al., 2010). Percentage cover of plants does not directly
154 relate to volumetric percentages in palaeoecology. It should be recognised that apparent
155 presence/absence partly reflects count total for testate amoebae and quadrat size for plants.

156 Among the available transfer function models weighted averaging with classical
157 deshrinking was found to perform best in the majority of cases, so this was used to compare
158 the performance of the different combinations of proxies. Our goal here was not to find the
159 absolute best model for each combination of proxies but rather to assess in general how
160 different combinations perform. We assessed the performance of the different transfer
161 functions for DWT and pH on the basis of r^2 , root mean squared error of prediction (RMESP),
162 average bias and maximum bias all determined by both bootstrap and the recently-proposed
163 leave-one-site-out cross-validation (Payne et al., in press), using R (R Development Core
164 Team, 2010) and the rioja library (Juggins, 2011). We also compared DWT and pH
165 reconstructions from a 1000 years record from Maunstschas mire (REF XX) based on % or
166 1/0 testate amoebae filtered or raw models to assess what implications the observed
167 differences in model performance would have on palaeoenvironmental reconstruction.

168

169 **Results**

170 Among single proxy 1/0 models the best performance was found with bryophytes for DWT
171 and TA or bryophytes for pH (Table 2). The use of 1/0 compared to % reduced the
172 performance of TA transfer functions (e.g. for raw models r^2_{boot} respectively 0.53 versus 0.65

173 for DWT and 0.67 versus 0.73 for pH, Table 2). It is also noteworthy that the TA 1/0 model
174 failed to accurately predict water table depth below 20 cm (Supplementary Figures 3 & 4).

175 Our results provide support to the idea that multi-proxy transfer functions combining
176 TA and bryophytes or TA, bryophytes and vascular plants outperform single-proxy transfer
177 functions for both raw and filtered data. However, contrary to expectation and to results from
178 single-proxy models, the use of 1/0 TA data resulted in better multi-proxy models in two of
179 the three cases for DWT (unfiltered data, models including vascular plants) and one case for
180 pH (TA and vascular plants) (Table 2). Indeed the best performing DWT model overall with
181 unfiltered data combined TA 1/0, bryophytes and vascular plants. Thus for both DWT and pH
182 either better models could be produced using 1/0 data, or the use of % TA data only
183 marginally improved model performance.

184 Data filtering (outlier removal) strongly improved the performance of transfer
185 functions, especially for pH (Table 2, Supplementary Fig. 1–11). As for unfiltered data, TA
186 single proxy models performed better when based on % than on 1/0 data. Among the filtered
187 single-proxy models, bryophytes performed best for both DWT and pH. Among the multi-
188 proxy transfer functions, the DWT transfer function based on TA %, bryophytes, and vascular
189 plants performed best ($r^2 = 0.87$, RMSEP = 4.3 cm). For pH, the best multi-proxy model was
190 based on TA % and bryophytes ($r^2 = 0.94$, RMSEP = 0.27 pH units).

191 We next compared model performances based on three transformations: a) the original
192 presence/absence [1/0] data, b) the same data multiplied by one hundred [100/0], and c)
193 replacing each presence by 100 divided by the number of species present in a sample
194 [(100/n)/0] for unfiltered data. When combining TA % data and bryophyte and/or vascular
195 plant data, the use of 100/0 or (100/n)/0 data generally improved model performance. The
196 best overall model for DWT was for TA%, bryophytes and vascular plants 100/0. The best
197 model for pH was for TA 1/0 and bryophytes (100/n)/0 (Supplementary table 2). These
198 models are based on unfiltered (raw) data and could therefore be further improved through
199 filtering (but making direct comparison among models less meaningful).

200 Evaluating our models using the newly developed LOSO approach led to similar
201 results (Supplementary table 3). In all cases performance was marginally weaker with LOSO
202 than regular bootstrap validation. Models such as pH based on vascular plants with r^2 around

203 0.4 had no predictive power in this test (RMSEP>standard deviation), while the performance
204 of the best models was less affected.

205 Implications for palaeoenvironmental reconstruction

206 The DWT and pH reconstructions for Mauntschas mire for the last millennium using raw and
207 filtered % and 1/0 TA transfer functions illustrate how differences in model performance can
208 potentially affect palaeoenvironmental reconstruction (Figure 1 & 2, Supplementary Figure
209 12). The major phases are similar, but some quite important differences are also visible. For
210 example using the 1/0 model results in a ca. 2 cm lag (in sample depth) in the timing of the
211 dry shift occurring between 85 and 90cm depth. As this corresponds to a period of low peat
212 accumulation rate this translates into a 200-year difference in the timing of this shift.

213 Pairwise comparisons between models show that filtering leads to slight overestimation
214 of DWT for low values and underestimation of DWT for larger values (i.e. drier conditions are
215 inferred in wet phases and wetter conditions are inferred in dry phases) overall there is a
216 5.7cm average underestimation for the filtered model compared to the raw model. For pH the
217 effect is an almost constant overestimation of 0.08 pH units for the filtered model as
218 compared to the raw model. For both DWT and pH, 1/0 models yield results, which are in
219 some cases very different from the values produced by the % model (r^2 between values
220 inferred from raw % and 1/0 models = 0.773 and 0.619, for DWT and pH, respectively). The
221 DWT values inferred from the raw 1/0 model were in many cases lower than those from the
222 raw % model, especially for DWT >25 cm (Figure 2). Inferred pH values from the raw 1/0
223 model were on average 0.29 pH units lower than those obtained from the raw % model.

224

225 Discussion

226 Building models from presence/absence data

227 The first important result of our study is the good performance of transfer functions based on
228 presence/absence data. In these cases 'weighted averaging' is reduced to simple averaging
229 with the average environmental value of taxa occurrence used to represent its optima. This is
230 rarely done, maybe because it is mostly not considered useful although examples do exist, for
231 instance Mezquita et al. (2005), for freshwater ostracods. Given the computational simplicity

232 of this approach our results suggest that this method should be more widely investigated.
233 Presence/absence data may be quicker to obtain than relative abundance data but partly
234 reflects the count total used. The normal count totals for testate amoeba analysis (50-200
235 individuals) are insufficient to identify all taxa, so the recorded presence/absence of a taxon
236 reflects sampling intensity as well as real presence or absence (Payne and Mitchell 2009; Wall
237 et al. 2010). Future studies would need to assess the count total needed to achieve sufficiently
238 accurate presence/absence data before lower counts, and therefore quicker counting, could be
239 routinely implemented.

240 The comparison of inferred DWT and pH patterns from Mauntschas mire using % vs
241 1/0 models shows that it is possible to infer both variables using 1/0 models. The two types
242 of models however do not yield identical results. In some cases the interpretation could be
243 quite different, if not for the overall patterns at least for the precise timing and the magnitude
244 of changes. As both % TA models perform better than their corresponding 1/0 models these
245 difference suggest that inference from % models is more reliable than for 1/0 models.
246 Nevertheless as % models are not perfect 1/0 models could in some cases be more accurate.

247 Single-proxy models

248 The second important, and surprising result is that single proxy DWT and pH models based
249 on TA, were out-performed by models based on bryophytes for filtered data (and also for raw
250 data in the case of DWT). This raises the question of a possible superior performance of
251 models based on percentage bryophyte data. This is however both difficult to achieve (and
252 was beyond the scope of the present study) and potentially of little practical use for several
253 reasons. First, obtaining reliable percentage data for bryophytes is not a simple task, as
254 precise identification requires microscopy analyses, in this case of thousands or tens of
255 thousands of samples for the full data set. Second, supposing that percentage data could be
256 generated for the modern data set these data would not be fully equivalent to the percentage
257 data obtained from macrofossil analyses (as estimated using e.g. the Quadrat and Leaf Count
258 method – Barber et al. 1994) because surface cover does not equate to volume. Further
259 limitations, are the possible differential preservation of bryophyte species and the variable
260 taxonomic resolution that can be achieved in the analysis of fossil material (Janssens, 1983).
261 Nevertheless, these results suggest that there is potential for quantitative reconstruction of
262 DWT and pH based on presence-absence bryophyte data.

263 Outlier removal

264 Filtering the data set by removing outlier samples with residuals higher than the standard
265 deviation of the observed values clearly improved the model performance in many cases.
266 However, these apparent improvements in model performance were often at the cost of a
267 considerable reduction in sample number; this number ranged from 2 to 33 depending on the
268 models (supplementary Table 2). While it can be understandable that a few samples represent
269 truly unusual conditions that do not justify inclusion in a transfer function, and their removal
270 from a data set can then be defensible, this cannot possibly be the case for 40% of the
271 samples as in the most extreme case (vascular plant pH model). This shows that outlier
272 removal should preferably not be based on “automatic”, apparently objective procedures but
273 rather on a cautious analysis of the community composition and ecological conditions of the
274 sampling location. Where these clearly indicate that the sample may not realistically be
275 modelled accurately by a transfer function this would warrant exclusion. In other cases it is
276 clearly advisable to keep the samples in the model, even if its apparent performance is not
277 ideal.

278 Applying the raw and filtered models to the palaeoecological record from Mauntschas
279 mire produced little change in reconstructions. The r^2 of inferred values between raw and
280 filtered models were high (0.95 - 0.996). This clearly shows, at least for the data set on which
281 this comparison is based, that model “optimisation” by data filtering has little effect on
282 palaeoenvironmental reconstruction.

283 Comparing single-proxy and multi-proxy models

284 We compared the performance of single-proxy and multi-proxy transfer functions for DWT
285 and pH using TA, bryophytes, and vascular plants. The expectation that multi-proxy models
286 out-perform single-proxy models was confirmed for both DWT and pH and for both raw and
287 filtered data.

288 Compared to other multi-proxy transfer function studies (e.g. Gehrels et al. 2001) the
289 three groups we compare here are very different in their morphology, ecology and life history.
290 Testate amoebae are mostly heterotrophic unicellular protists living in the upper accretelm;
291 bryophytes and vascular plants are autotrophic and multi-cellular, bryophytes being
292 dependent on the water available at the soil surface and vascular plants actively drawing water
293 from deeper-lying layers. This affects how the different groups respond to environmental

294 change. For example, fluctuations in surface moisture will directly affect testate amoebae and
295 the bryophytes, until the water table drops below ca. 30cm when a further lowering of the
296 water table is unlikely to produce further impacts (Mitchell et al. 1999). A further drop in the
297 water table may however significantly impact vascular plants, many of which extend their
298 root system several decimetres in the soil. This may make combined predictions based on all
299 groups sensitive to a broader hydrological gradient, but also makes interpretation of those
300 results more difficult. For instance while transfer functions for testate amoebae aim to
301 reconstruct water table depth, the transfer functions actually show the hydrological conditions
302 at quite different spatial scales. While amoebae respond to moisture conditions in their
303 immediate (μm^3 scale) vicinity (e.g. water film thickness on a Sphagnum leaf), in the case of
304 bryophytes the hydrological sensitivity is likely to be larger on the scale of cm^3 and for
305 vascular plants larger again, on the scale of dm^3 . This need not be a concern if hydrology at
306 all these scales is strongly correlated with water table depth, but this represents a source of
307 uncertainty in the results.

Comment [RP1]: Split as a long sentence

308 The difference in scale of environmental sensitivity also applies for pH. The pH was
309 measured on the bryophyte in which the testate amoebae live, whereas the rooting zone of
310 vascular plants may be influenced by water of a different pH due to vertical gradients in water
311 chemistry (Mitchell et al., 2000). In line with this, the best pH filtered model was the one
312 based on testate amoeba percentage and bryophyte data. The distribution of pH along the fen-
313 bog gradient is clearly bimodal and this results in generally poor model performance around
314 pH values of 5.0. A notable exception to this is the model combining testate amoebae
315 percentage data and vascular plants (Supplementary figure 10), which is precisely the best
316 performing raw model. This may be explained by the fact that surface pH values of 5.0
317 correspond to transitional mire where a clear vertical pH gradient develops (Tahvanainen,
318 2004). Thus under these conditions bryophytes and/or testate amoebae will indicate more
319 acidic conditions (and hence underestimate pH) while vascular plants will indicate less acidic
320 conditions (and hence overestimate pH). This should be especially important in habitats with
321 surface pH around 5.0, where calci-tolerant Sphagnum mosses (e.g. *S. contortum*, *S.*
322 *warnstorffii*, and *S. teres*) strongly acidify surface waters by releasing organic acids and hence
323 strengthen the vertical pH gradient (Andrus, 1986; Hajkova and Hajek, 2004).

324 Our results suggest that multiproxy transfer functions may be a useful new technique
325 for palaeoenvironmental reconstruction from peatlands but further work is necessary to

326 understand the sensitivity of these models. Conventional single proxy reconstructions allow
327 the comparison of different reconstructions: where these diverge an informed ecological
328 judgement can be made about which may be more reliable in each specific circumstance. By
329 combining very different groups of organisms in a single model no such judgement can be
330 reached and it is unclear how a combined model would react in a situation where different
331 groups of organisms indicate different conditions. We therefore recommend that both single
332 and multiproxy reconstructions be produced in parallel and multiproxy results treated with
333 caution where there is a divergence in the results of different proxies.

334 SUMMARY

335 Over the last 20 years the approach to macrofossil and testate amoeba-based
336 palaeoenvironmental reconstruction in peatlands has become increasingly standardised.
337 Testate amoeba percentage transfer functions are used to quantitatively reconstruct water table
338 (and less frequently pH) changes and ordination techniques used to summarise macrofossil
339 results in a single index assumed to primarily represent a, loosely defined, peatland surface
340 wetness. Our results show that alternative approaches can be applied and may present superior
341 performance. Bryophyte transfer functions can be produced from presence/absence data and
342 perform well in cross-validation. Presence/absence data might allow quicker testate amoeba
343 analysis, but with some loss of information. Multiproxy transfer functions based on more than
344 one group of organisms may out-perform single-proxy transfer functions. These new
345 approaches require further appraisal with palaeoecological data but offer exciting new options
346 that deserve exploration.

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359

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489 261-273.

490
491 **Tables**

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493 Note to editors. The tables were prepared in excel and are pasted as images (more readable).

494 Editable versions are provided separately

495

Table 1. Location and general characteristics of the studied sites

Site name	Coordinates		Altitude [m a.s.l.]	Depth to water table [cm]			pH			n *
	Latitude	Longitude		Min	Max	Average	Min	Max	Average	
Mauntschas	46°27'27"N	09°51'22"E	1818	-20	41	11.7	3.61	6.98	5.13	39 (3)
Lej da Staz	46°29'50"N	09°52'10"E	1810	0	76	18.4	4.51	6.15	5.24	11 (1)
Lej Marsch	46°28'31"N	09°49'11"E	1813	5	70	33.2	3.72	4.61	4.09	12 (2)
Lej Nair	46°28'13"N	09°49'12.5"E	1864	0.5	27.5	8.1	3.95	6.80	5.5	12 (2)
Inn Fen	46°24'28"N	09°42'10"E	1803	-4	17.5	5.35	5.77	7.12	6.51	13 (3)
Maloja mire	46°24'19"N	09°41'24.3"E	1850	0	22.5	11.38	3.67	4.22	3.86	8

* Number of samples taken; in brackets samples with incomplete measurements

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Table 2. Summary performance indicators of the transfer function models (classical weighted averaging) for depth to the water table (DWT) and pH in peatlands of the Eastern Swiss Alps (Engadine valley). For each group of models (singly proxy, combined with presence-absence data, combined including testate amoeba percentage data) the best values are indicated by undelined numbers both for raw and filtered models, the best of the two are bolded (taking into consideration exact values). The best models overall for DWT and pH are indicated in grey background. Raw: unfiltered models, filtered: filtered models (with removal of rare species and outlier samples, see text for details).

		DWT [cm]		pH	
		Raw	Filtered	Raw	Filtered
Models on individual groups					
Bryophytes presence/absence	r^2_{Boot}	<u>0.71</u>	0.79	0.67	0.89
	RMSEP	<u>9.0</u>	6.2	0.71	0.37
Vascular plants presence/absence	r^2_{Boot}	0.62	0.66	0.46	0.87
	RMSEP	11.5	7.4	1.08	0.45
Testate amoebae presence/absence	r^2_{Boot}	0.53	0.71	0.67	0.83
	RMSEP	13.2	8.0	0.70	0.46
Testate amoebae percentages	r^2_{Boot}	0.65	0.73	<u>0.73</u>	0.86
	RMSEP	12.2	6.8	<u>0.62</u>	0.43
Multi-group models with presence/absence data only					
Bryophytes & vascular plants	r^2_{Boot}	0.64	0.74	0.55	0.80
	RMSEP	10.8	6.3	0.90	0.53
Testate amoebae & bryophytes	r^2_{Boot}	0.62	0.83	0.68	0.82
	RMSEP	9.9	5.2	0.62	0.47
Testate amoebae & vascular plants	r^2_{Boot}	0.71	0.81	0.75	0.79
	RMSEP	9.3	5.3	0.58	0.52
Testate amoebae, bryophytes & vascular plants	r^2_{Boot}	0.73	0.81	0.70	0.75
	RMSEP	7.6	5.4	0.59	0.52
Multi-group models with testate amoeba percentages data					
Testate amoebae % & bryophytes	r^2_{Boot}	0.66	0.78	0.70	0.94
	RMSEP	9.1	5.3	0.61	0.27
Testate amoebae % & vascular plants	r^2_{Boot}	0.64	0.73	0.68	0.90
	RMSEP	11.0	5.7	0.70	0.37
Testate amoebae %, bryophytes & vascular plants	r^2_{Boot}	<u>0.68</u>	0.87	<u>0.71</u>	0.92
	RMSEP	<u>8.7</u>	4.3	<u>0.60</u>	0.30

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502 **Figures**

503

504 **Figure 1.** Reconstruction of depth to water table (DWT) and pH from a 1000 years record
505 from Mauntschas mire, Engadine, Switzerland (van de Knaap et al. 2011) using raw
506 (unfiltered) percentage (thick curves) and presence/absence (thin curves) testate amoeba-
507 based transfer functions. The data are plotted according to sampling depth so as to best show
508 the differences among models. The same data plotted against sample age and using additional
509 models are shown as supplementary material.

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511 **Figure 2.** Correlation biplots and r^2 comparing reconstructed depth to water table (DWT – A
512 and B) and pH (C and D) from Mauntschas mire, Engadine, Switzerland (van de Knaap et al.
513 2011), using raw percentage testate amoeba data transfer functions vs. filtered models (outlier
514 removal) (A and C), and raw percentage testate amoeba data transfer functions vs. raw
515 presence/absence models (B and D). The data in biplots B and D corresponds to the curves
516 shown in Figure 1.

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519 **Supplementary online material**

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521 **3 tables and 12 figures**

Supplementary table 1. Summary performance indicators of the transfer function models (classical weighted averaging) for depth to the water table (DWT) and pH in peatlands of the Eastern Swiss Alps (Engadine valley). For each group of models (singly proxy, combined with presence-absence data, combined including testate amoeba percentage data) and for either raw or filtered models, the best values for r^2_{boot} , Maximum Bias_{boot} and RMSEP are indicated by underlined numbers both for raw and filtered models, the best of the two are bolded (taking into consideration exact values). The best models overall for DWT and pH are indicated in grey background. Raw: unfiltered models, filtered: filtered models (with removal of rare species and outlier samples, see text for details).

		DWT [cm]		pH	
		Raw	Filtered	Raw	Filtered
Models on individual groups					
Bryophytes presence/absence	r^2_{boot}	<u>0.71</u>	<u>0.79</u>	0.67	<u>0.89</u>
	Maximum Bias _{boot}	35.6	<u>16.2</u>	0.56	0.75
	RMSEP	<u>9.0</u>	<u>6.2</u>	0.71	<u>0.37</u>
	Number of samples	65	62	65	58
	Number of species	12	12	12	11
Vascular plants presence/absence	r^2_{boot}	<u>0.62</u>	0.66	0.46	<u>0.87</u>
	Maximum Bias _{boot}	26.6	17.8	1.20	0.28
	RMSEP	11.5	7.4	1.08	0.45
	Number of samples	82	77	82	49
	Number of species	30	30	30	30
Testate amoebae presence/absence	r^2_{boot}	0.53	0.71	0.67	0.83
	Maximum Bias _{boot}	36.1	34.8	0.96	0.60
	RMSEP	13.2	8.0	0.70	0.46
	Number of samples	93	74	93	71
	Number of species	69	69	69	69
Testate amoebae percentages	r^2	0.65	0.73	<u>0.73</u>	0.86
	Maximum Bias	26.5	19.4	1.01	0.80
	RMSEP	12.2	6.8	<u>0.62</u>	0.43
	Number of samples	93	81	93	78
	Number of species	69	68	69	69
Multi-group models with presence/absence data only *					
Bryophytes & vascular plants	r^2_{boot}	0.64	0.74	0.55	0.80
	Maximum Bias _{boot}	27.7	14.8	0.77	0.92
	RMSEP	10.8	6.3	0.90	0.53
	Number of samples	82	78	82	65
	Number of species	42	42	42	42
Testate amoebae & bryophytes	r^2_{boot}	0.62	<u>0.83</u>	0.68	<u>0.82</u>
	Maximum Bias _{boot}	43.1	<u>12.2</u>	<u>0.65</u>	0.75
	RMSEP	9.9	<u>5.2</u>	0.62	0.47
	Number of samples	65	57	65	54
	Number of species	83	83	83	83
Testate amoebae & vascular plants	r^2_{boot}	0.71	0.81	0.75	0.79
	Maximum Bias _{boot}	<u>30.5</u>	19.6	0.84	<u>0.71</u>
	RMSEP	9.3	5.3	0.58	<u>0.52</u>
	Number of samples	82	75	82	76
	Number of species	106	106	106	106
Testate amoebae, bryophytes & vascular plants	r^2_{boot}	<u>0.73</u>	0.81	0.70	0.75
	Maximum Bias _{boot}	36.5	12.7	0.71	0.73
	RMSEP	<u>7.6</u>	5.4	0.59	0.52
	Number of samples	65	63	65	62
	Number of species	105	105	105	103
Multi-group models with testate amoeba percentages data *					
Testate amoebae % & bryophytes (100/0)	r^2_{boot}	0.66	0.78	0.70	0.94
	Maximum Bias _{boot}	39.2	<u>11.0</u>	0.66	0.69
	RMSEP	9.1	5.3	0.61	0.27
	Number of samples	65	52	65	45
	Number of species	81	81	81	75
Testate amoebae % & vascular plants (100/0)	r^2_{boot}	0.64	0.73	0.68	0.90
	Maximum Bias _{boot}	<u>29.6</u>	19.4	1.03	0.76
	RMSEP	11.0	5.7	0.70	0.37
	Number of samples	82	64	82	63
	Number of species	106	102	106	102
Testate amoebae %, bryophytes & vascular plants (100/0)	r^2_{boot}	<u>0.68</u>	0.87	<u>0.71</u>	0.92
	Maximum Bias _{boot}	38.4	15.3	0.68	<u>0.67</u>
	RMSEP	<u>8.7</u>	4.3	<u>0.60</u>	0.30
	Number of samples	65	50	65	46
	Number of species	105	105	105	103

* For models based on presence-absence data, using 0/1 or 0/100 coding does not modify the model performance. In models combining percentage and binary data 0/100 coding of binary data is used.

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Supplementary table 2. Comparison of transfer function models (classical weighted averaging) performance indicators of raw (unfiltered) models for depth to the water table (DWT) based on testate amoeba (TA), bryophyte (Bryo), vascular plant (Vasc) data, and combinations of these from peatlands of the Eastern Swiss Alps (Engadine valley). The best models for each category are underlined, best models per transformation of binary data option are indicated in grey shading and best models overall are in bold.

		DWT [cm]				pH			
		Transformation of binary data				Transformation of binary data			
		1/0	(100/n)/0	100/0 #	%	1/0	(100/n)/0	100/0 #	%
Models on individual groups									
Bryo	r^2 _(boot)	0.71	<u>0.71</u>	0.71	n.a.	0.67	0.68	0.66	
	Max Bias _(boot)	35.6	35.5	35.9	n.a.	0.56	0.60	0.69	
	RMSEP	9.0	<u>8.7</u>	8.7	n.a.	0.71	0.69	0.73	
Vasc	r^2 _(boot)	0.62	0.61	0.62	n.a.	0.46	0.38	0.43	
	Max Bias _(boot)	26.6	21.9	25.8	n.a.	1.20	1.37	1.35	
	RMSEP	11.5	12.0	11.7	n.a.	1.08	1.18	1.14	
TA	r^2 _(boot)	0.53	0.61	0.54	0.65	0.67	0.72	0.68	<u>0.73</u>
	Max Bias _(boot)	36.1	35.8	36.3	26.5	0.96	1.02	0.96	<u>1.01</u>
	RMSEP	13.2	11.0	12.9	12.2	0.70	<u>0.62</u>	0.68	0.62
Multi-group models without percentage data									
Bryo & Vasc	r^2 _(boot)	0.64	0.64	0.64		0.55	0.59	0.54	
	Max Bias _(boot)	<u>27.7</u>	28.4	28.3		0.77	0.88	0.79	
	RMSEP	10.8	11.0	10.9		0.90	0.83	0.93	
TA & Bryo	r^2 _(boot)	0.62	<u>0.72</u>	0.61		0.68	0.75	0.68	
	Max Bias _(boot)	43.1	38.9	43.6		0.65	<u>0.64</u>	<u>0.64</u>	
	RMSEP	9.9	8.0	9.7		0.62	0.56	0.62	
TA & Vasc	r^2 _(boot)	0.71	0.72	0.72		0.75	0.66	0.72	
	Max Bias _(boot)	30.5	28.2	29.0		0.84	0.97	1.13	
	RMSEP	9.3	9.3	9.2		0.58	0.71	0.69	
TA, Bryo & Vasc	r^2 _(boot)	0.73	0.69	<u>0.73</u>		0.70	0.69	0.70	
	Max Bias _(boot)	36.5	31.5	37.2		0.71	0.97	0.72	
	RMSEP	7.6	9.8	7.7		0.59	0.68	0.58	
Multi-group models with TA percentages data									
TA % & Bryo	r^2 _(boot)	0.66	0.72	0.74		0.70	<u>0.75</u>	0.66	
	Max Bias _(boot)	39.2	38.8	37.2		0.66	0.64	0.60	
	RMSEP	9.1	<u>8.0</u>	7.9		0.61	<u>0.56</u>	0.78	
TA % & Vasc	r^2 _(boot)	0.64	0.72	0.69		0.68	0.66	0.58	
	Max Bias _(boot)	29.6	28.8	<u>26.8</u>		1.03	0.96	0.49	
	RMSEP	11.0	8.7	10.1		0.70	0.66	0.92	
TA %, Bryo & Vasc	r^2 _(boot)	0.68	0.68	0.77		0.71	0.68	0.57	
	Max Bias _(boot)	38.4	31.8	32.3		0.68	0.97	0.48	
	RMSEP	8.7	9.9	7.1		0.60	0.68	0.87	

#: 0/1 and 0/100 models are identical except when combined with TA % data

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Supplementary table 3. Summary performance indicators of the LOSO (Leave One Site Out) validation of raw (unfiltered) transfer function models (classical weighted averaging) for depth to the water table (DWT) and pH in peatlands of the Eastern Swiss Alps (Engadine valley). The best models for each category are underlined, best models per transformation of binary data option are indicated in grey shading and best models overall are in bold. Note that WA is not necessarily the best-performing transfer function technique and other models with the same data may have more predictive power.

		DWT [cm]				pH			
		Transformation of binary data				Transformation of binary data			
		1/0	(100/n)/0	100/0 #	%	1/0	(100/n)/0	100/0 #	%
Models on individual groups									
Bryophytes	r^2	<u>0.64</u>	0.63			0.53	0.54		
	Maximum Bias	35.2	35.4			0.55	0.57		
	RMSEP	<u>11.0</u>	11.3			0.84	0.83		
Vascular plants	r^2	0.41	<u>0.45</u>			0.00	0.00		
	Maximum Bias	25.0	<u>24.2</u>			2.39	<u>2.16</u>		
	RMSEP	15.2	<u>14.2</u>			1.66	<u>1.62</u>		
Testate amoebae	r^2	0.52	<u>0.56</u>	0.59		<u>0.66</u>	0.66		<u>0.65</u>
	Maximum Bias	37.8	<u>37.6</u>	29.2		<u>1.05</u>	1.13		<u>1.17</u>
	RMSEP	13.8	<u>11.8</u>	12.18		0.67	0.67		<u>0.69</u>
Multi-group models without percentage data									
Bryophytes & vascular plants	r^2	0.50	<u>0.53</u>			0.12	<u>0.24</u>		
	Maximum Bias	<u>27.9</u>	30.7			2.00	<u>1.67</u>		
	RMSEP	<u>13.2</u>	13.4			1.22	<u>1.10</u>		
Testate amoebae & bryophytes	r^2	0.62	<u>0.66</u>			<u>0.69</u>	0.65		
	Maximum Bias	41.6	<u>38.5</u>			0.67	<u>0.61</u>		
	RMSEP	10.1	<u>9.7</u>			0.60	0.64		
Testate amoebae & vascular plants	r^2	0.65	0.64			0.66	0.40		
	Maximum Bias	32.5	<u>31.7</u>			1.17	1.44		
	RMSEP	<u>9.9</u>	10.0			<u>0.64</u>	0.87		
Testate amoebae, bryophytes & vascular plants	r^2	0.64	0.59			0.65	0.47		
	Maximum Bias	<u>33.0</u>	34.1			1.16	1.37		
	RMSEP	<u>10.2</u>	11.6			<u>0.65</u>	0.83		
Multi-group models with testate amoeba percentages data									
Testate amoebae % & bryophytes	r^2	0.62	0.68	<u>0.69</u>		0.68	0.69	0.66	
	Maximum Bias	37.0	<u>36.0</u>	36.0		0.65	0.59	<u>0.58</u>	
	RMSEP	10.2	9.6	<u>9.5</u>		0.64	<u>0.61</u>	0.65	
Testate amoebae % & vascular plants	r^2	0.60	0.70	0.58		0.67	0.53	0.25	
	Maximum Bias	29.2	<u>27.5</u>	26.7		1.16	1.35	1.77	
	RMSEP	11.9	<u>9.2</u>	11.4		<u>0.66</u>	0.75	1.02	
Testate amoebae %, bryophytes & vascular plants	r^2	0.60	<u>0.65</u>	0.61		<u>0.67</u>	0.55	0.34	
	Maximum Bias	29.2	30.1	<u>28.6</u>		1.16	1.31	1.66	
	RMSEP	11.8	<u>10.7</u>	11.1		<u>0.66</u>	0.75	0.93	

#: 0/1 and 0/100 models are identical except when combined with TA % data

531 **Supplementary Figures: captions**

532

533 **Supplementary Figure 1.** Observed versus model-predicted values (left) and observed versus
534 residuals (right) of transfer function models of bryophytes for depth to the water table (DWT)
535 and pH.

536

537 **Supplementary Figure 2.** Observed versus model-predicted values (left) and observed versus
538 residuals (right) of transfer function models of vascular plants for depth to the water table
539 (DWT) and pH.

540

541 **Supplementary Figure 3.** Observed versus model-predicted values (left) and observed versus
542 residuals (right) of transfer function models of testate amoeba presence/absence data for depth
543 to the water table (DWT) and pH.

544

545 **Supplementary Figure 4.** Observed versus model-predicted values (left) and observed versus
546 residuals (right) of transfer function models of testate amoeba percentages for depth to the
547 water table (DWT) and pH.

548

549 **Supplementary Figure 5.** Observed versus model-predicted values (left) and observed versus
550 residuals (right) of transfer function models of bryophytes and vascular plants for depth to the
551 water table (DWT) and pH.

552

553 **Supplementary Figure 6.** Observed versus model-predicted values (left) and observed versus
554 residuals (right) of transfer function models of testate amoebae presence/absence data and
555 bryophytes for depth to the water table (DWT) and pH.

556

557 **Supplementary Figure 7.** Observed versus model-predicted values (left) and observed versus
558 residuals (right) of transfer function models of testate amoeba presence/absence data and
559 vascular plants for depth to the water table (DWT) and pH.

560

561 **Supplementary Figure 8.** Observed versus model-predicted values (left) and observed versus
562 residuals (right) of transfer function models of testate amoeba presence/absence data,
563 bryophytes and vascular plants for depth to the water table (DWT) and pH.

564

565 **Supplementary Figure 9.** Observed versus model-predicted values (left) and observed versus
566 residuals (right) of transfer function models of testate amoeba percentages and bryophytes for
567 depth to the water table (DWT) and pH.

568

569 **Supplementary Figure 10.** Observed versus model-predicted values (left) and observed
570 versus residuals (right) of transfer function models of testate amoeba percentages and vascular
571 plants for depth to the water table (DWT) and pH.

572

573 **Supplementary Figure 11.** Observed versus model-predicted values (left) and observed
574 versus residuals (right) of transfer function models of testate amoeba percentages, bryophytes
575 and vascular plants for depth to water table (DWT) and pH.

576

577 **Supplementary Figure 12.** Reconstruction of depth to water table (DWT, A and C) and pH
578 (B and D) from a 1000 years record from Mauntschas mire, Engadine, Switzerland (van de
579 Knaap et al. 2011), plotted against sample depth (A and B) and age (C and D), using raw and
580 filtered percentage (%) and presence/absence (1/0) testate amoeba-based transfer functions.

581 Black lines = raw %, red = filtered %, blue = raw 1/0, green = filtered 1/0.