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1 **Ecology of peatland testate amoebae in the Alaskan continuous permafrost**
2 **zone**

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4

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10

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12

13 **Highlights:**

- 14 • We examine testate amoebae distribution in continuous permafrost peatlands
15 • Electrical conductivity is the dominant control on testate amoebae distribution
16 • Water-table depth is a secondary control on testate amoebae distribution
17 • Two new transfer functions for reconstructing WTD and conductivity are
18 presented

19

20 **Abstract:** Arctic peatlands represent a major global carbon store, but rapid warming
21 poses a threat to their long-term stability. Testate amoebae are sensitive
22 hydrological indicators that offer insight into Holocene environmental change in
23 peatlands. However, in contrast to temperate peatlands, there have only been a few
24 studies into the ecology of testate amoeba and their efficacy as environmental
25 indicators in permafrost peatlands. We present the first study of testate amoeba
26 ecology from peatlands in the continuous permafrost zone, based on samples from
27 across the Alaskan North Slope. Multivariate statistical analyses show that pore
28 water electrical conductivity (EC), a proxy for nutrient status along the ombrotrophic-
29 minerotrophic gradient, is the dominant control on testate amoeba distribution.

30 Water-table depth (WTD) is also a significant control on testate amoeba distribution,
31 but is secondary to EC. We present two new testate amoeba-based transfer
32 functions to reconstruct both EC (TF_{EC}) and WTD (TF_{WTD}), the first for peatlands in
33 the continuous permafrost zone. The transfer functions are based on Weighted
34 Averaging Partial Least Squares (WAPLS) regression and were assessed using
35 leave-one-out (LOO) cross-validation. We find that both transfer functions have good
36 predictive power. TF_{WTD} is the best performing model ($R^2_{JACK} = 0.84$, $RMSEP_{JACK} =$
37 6.66 cm), but TF_{EC} also performs well ($R^2_{JACK} = 0.76$, $RMSEP_{JACK} = 146 \mu\text{S cm}^{-1}$).
38 Our findings are similar to those conducted in peatlands in discontinuous permafrost
39 regions. The new transfer functions open the opportunity for reconstructing the
40 Holocene dynamics of peatlands of the continuous permafrost zone in Alaska, which
41 represent rapidly changing ecosystems.

42

43 **Key Words:** Arctic, Ecology, Palaeohydrology, Ecohydrology, Transfer Function,
44 Trophic Gradient.

45

46 **1. Introduction**

47 Climate warming over the last century has been most rapid at high-latitudes (Stocker
48 et al., 2013). Permafrost temperatures in the Northern Hemisphere have increased
49 by as much as 2°C since 1850, with the continuous permafrost zone warming most
50 rapidly (Vaughan et al., 2013). Peatlands in permafrost areas are especially
51 vulnerable to rapid change and anthropogenic warming (Minayeva et al., 2016) and
52 there is evidence that they are thawing at an accelerating rate (Payette et al., 2004).
53 Arctic peatlands are a major global carbon store of ~ 277 PgC and occupy 18.9% of
54 Northern circumpolar permafrost area (Tarnocai et al., 2009). Concern exists that as
55 permafrost peatlands thaw, a large proportion of their carbon stock may become
56 unstable and return to the atmosphere (Routh et al., 2014; Schuur et al., 2009).
57 Alternatively, surface peat may insulate permafrost below and limit such degradation
58 (Mann et al., 2010). Palaeoecological approaches have been used to identify recent
59 hydrological changes in domed permafrost peatlands, including conversion to
60 inundated Arctic fen systems (Swindles et al., 2015a; Galka et al., 2017). The
61 associated changes in vegetation structure (Christensen et al., 2004) and hydrology

62 (Quinton et al., 2011), combined with continued warming, are likely to promote
63 elevated methane release from degrading permafrost peatlands, with feedbacks to
64 the global climate system.

65

66 Permafrost peatlands are predominantly found in Eurasia and Canada, but remain
67 relatively unstudied given their remoteness. In Alaska, peatlands cover at least
68 78,000 km² (Xu et al., 2018) and are found across the Pacific coast, Aleutian Arc and
69 North Slope. Alaskan peatlands hold around 1% of carbon stored in Arctic peatlands
70 (Tarnocai et al., 2009), but are rapidly warming owing to rising air temperatures. This
71 has caused a 1-2°C warming of surface permafrost in the Northern Brooks Foothills
72 since 1977 (Osterkamp, 2007; Osterkamp, 2005; Osterkamp and Romanovsky,
73 1999). Warming and degradation of Alaskan permafrost peatlands may be broadly
74 similar to changes observed in peatlands across the wider Arctic, yet reliable proxy
75 methods to reconstruct past changes are incomplete for continuous permafrost
76 regions. Indeed, no such contemporary proxy record to reconstruct palaeohydrology
77 exists in any continuous permafrost peatlands globally, despite their vital role in the
78 carbon cycle and the importance of hydrology in carbon accumulation (Charman et
79 al., 2013; Holden, 2005; Belyea and Malmer, 2004).

80

81 Testate amoebae are single-celled protists that have been used extensively to
82 reconstruct peatland palaeohydrology in many regions of the world (e.g. Wilmshurst
83 et al., 2003; Payne and Mitchell, 2007; Lamentowicz et al., 2008; Swindles et al.,
84 2014; Swindles et al., 2015a; Amesbury et al., 2016). Testate amoebae form hard
85 shells (tests) that are often well preserved in Holocene peats (Mitchell et al., 2008a).
86 Species-level associations with a limited range of environmental and hydrological
87 conditions (Charman and Warner, 1992) mean that subfossil testate amoeba
88 assemblages have been widely utilised in palaeoenvironmental reconstructions,
89 particularly for water-table depth (WTD). Although testate amoebae have been used
90 to reconstruct hydrological change in discontinuous permafrost peatlands across
91 Europe (Zhang et al., 2017; Swindles et al., 2015b) and Canada (Lamarre et al.,
92 2013), little is known about their ecology and effectiveness as ecological indicators in
93 continuous permafrost. Previous studies have reported the presence of testate

94 amoebae in both the contemporary and fossil record of continuous permafrost (e.g.
95 Müller et al., 2009; Mitchell, 2004). However, the potential to use testate amoebae as
96 part of a multi-proxy study in palaeohydrological reconstruction has not yet been fully
97 developed in the continuous permafrost zone.

98

99 Our aim is to conduct the first detailed study of testate amoeba ecology in
100 continuous permafrost peatlands. In this investigation, we:

- 101 i. Examine the ecology of testate amoebae in continuous permafrost peatlands
102 from the North Slope, Alaska;
- 103 ii. Produce transfer functions that can be used to reconstruct the most important
104 environmental driver(s) of testate amoeba distribution and;
- 105 iii. Test the hypothesis that WTD is the primary control on the distribution of
106 testate amoebae species in continuous permafrost peatland ecosystems.

107

108 **2. Study Sites**

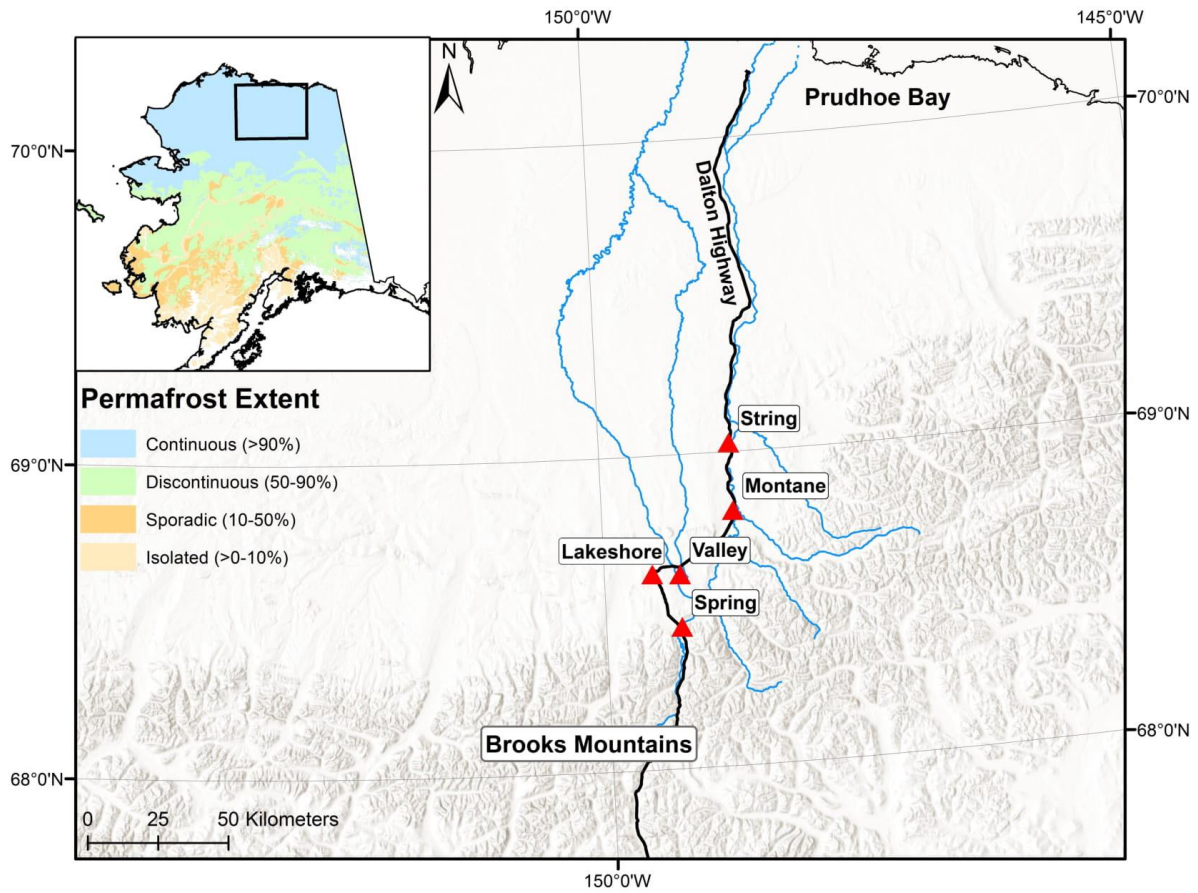
109 Our study comprises five sites across the Alaskan North Slope, within a 55 km radius
110 of Toolik Field Station (Figure 1; Table 1), and encompasses a range of ecological
111 and hydrological conditions. The five sites span a large trophic gradient, from
112 ombrotrophic bogs to minerotrophic fens, with electrical conductivity (EC) ranging
113 from 37 $\mu\text{S cm}^{-1}$ to 1176 $\mu\text{S cm}^{-1}$. The landscape is Arctic acidic tundra, with
114 thermokarst lakes and palaeoglaciological features remnant of the last ice age
115 (Gałka et al., 2018; Hinkel et al., 1987; Hamilton, 1986). Active layer (seasonally
116 thawed permafrost) thickness of the continuous permafrost at Toolik is between 40
117 and 50 cm (Brown, 1998). Air temperature is a key control on seasonal permafrost
118 thaw in the Alaskan North Slope, although topography can create local spatial
119 variability between sites (Hinkel and Nelson, 2003).

120

121

122

123



125

126 *Figure 1 – Map outlining the five sites studied on the Northern Brooks foothills, Alaska. All*
 127 *sites are peatlands within the continuous permafrost zone (Jorgenson et al., 2008).*

128

Site	Latitude (°N)	Longitude (°W)	Elevation (m)	WTD range (cm)	pH range	EC range ($\mu\text{S cm}^{-1}$)	Most common plant species (% abundance)
Montane	68.81367	148.841	451	16 – 56	5.82 – 6.50	224 – 509	<i>T. nitens</i> (70%)
Spring	68.45228	149.346	804	0 - 25	6.96 – 7.95	257 – 505	<i>T. nitens</i> (45%)
Valley	68.61953	149.338	864	4 - 53	5.41 – 6.66	595 – 1176	<i>S. terres/squarrosus</i> (85%)
String	69.02852	148.839	405	0 - 54	6.40 – 6.95	881 – 1124	<i>A. glaucophylla</i> / <i>S. scorpioides</i> (35%)
Lakeshore	68.62472	149.580	753	0 - 30	5.17 – 6.84	37 – 156	<i>S. cossoni</i> (45%)

129

130 *Table 1. Site overview and hydrological conditions. Full details of plant species are*
 131 *given in Appendix A.*

132

133 Peatlands around Toolik Lake initiated between 8 and 10 kyr in the Brooks foothills
134 (Reyes and Cooke, 2011; Jones and Yu, 2010) as a result of rapid warming (Mann
135 et al., 2010; Morris et al., 2018). Palaeoecological studies have used macrofossil and
136 pollen records to identify the vegetation succession in this region (Gałka et al.,
137 2018). Gałka et al. (2018) also used outline testate amoeba data to infer
138 palaeohydrological changes. However, no quantitative reconstruction of past
139 conditions was possible because no suitable transfer function existed at the time.

140

141 **3. Methods**

142 We collected 100 surface moss samples, 20 each from five peatlands across the
143 Alaskan North Slope, reflecting a range of hydrological conditions. A well was
144 augered at each sampling point and water level measured at regular intervals until it
145 equalised to determine depth to water table. pH and EC of pore water from each well
146 were measured using calibrated field meters. Approximately 5 g of each sample
147 were weighed, dried at 105°C overnight, re-weighed to determine gravimetric
148 moisture content (MC), and ignited in a muffle furnace at 550°C for at least 4 hours
149 to determine loss-on-ignition (LOI) (Chambers et al., 2011). We used the EC of pore
150 water as a proxy for peatland nutrient status (see Lamentowicz et al., 2013).

151

152 We isolated testate amoebae following Booth et al. (2010). Approximately half of
153 each moss sample was placed in boiling water for 15 minutes, shaken, passed
154 through a 300 µm sieve and back-sieved through a 15 µm mesh before being stored
155 in a 4°C cold store. Sub-samples were taken and used to prepare microscope slides
156 which were subsequently examined under a high-power transmitted light microscope
157 at 200 and 400 x magnification. We aimed to count 100 individuals per sample, in
158 addition to *Euglypha sp.*, *Trinema sp.* and *Tracheuglypha sp.*, as these species do
159 not preserve well in the subfossil peat record (Swindles and Roe, 2007a; Mitchell et
160 al., 2008b). Four samples had fewer than 100 individuals ($n = 97, 96, 88, 41$), but we
161 retained samples with counts 50-100 as they have been deemed statistically reliable
162 when diversity is low (Swindles et al., 2007b). Individuals were catalogued to species
163 level or 'type' (lowest division possible) using identification keys from Charman et al.
164 (2000), Booth and Sullivan (2007) and online guides (Siemensma, 2018).

165

166 Statistical analysis was performed in R version 3.4.1. (R Core Team, 2014), using
167 the *vegan* (Oksanen et al., 2017) and *analogue* (Simpson and Oksanen, 2016)
168 packages. Taxa were selected to isolate those that appear in abundance ($\geq 2\%$) in
169 any one sample to reduce the influence of rare taxa (following Swindles et al., 2009).
170 Detrended Correspondence Analysis (DCA) revealed that the data are characterised
171 by long axis gradient length, therefore Canonical Correspondence Analysis (CCA)
172 was subsequently performed on the 100 samples. Given the conflicting criticisms of
173 CCA (see Greenacre, 2013), we also performed ordination with non-metric
174 multidimensional scaling (NMDS) with the Bray-Curtis dissimilarity index and
175 redundancy analysis (RDA) with Hellinger transformed taxon data.

176

177 Transfer functions were developed using C2 version 1.7.5 (Juggins, 2007). Weighted
178 Averaging (WA), Weighted Averaging Partial Least Squares (WAPLS) and Maximum
179 Likelihood (ML) transfer functions were developed and tested with the full data set to
180 identify the best performing method. R^2 , RMSEP, and Maximum bias values were
181 used as metrics of performance. ML was dismissed due to relatively poor
182 performance. WA and WAPLS were selected as the best performing models and
183 cross-validated with the 'leave-one-out' method and sites with residual values $\geq 20\%$
184 of the range (EC: $n = 228$; WTD: $n = 11$) removed. The $\geq 20\%$ threshold is used as
185 the standard cut-off in the development of testate amoebae based transfer functions
186 (e.g. Charman et al., 2007; Payne and Mitchell, 2007; Swindles et al., 2015b;
187 Amesbury et al., 2016). Tolerance and optima statistics for each taxa were
188 calculated through WA. We applied our transfer functions to a short core from the
189 Lakeshore peatland (Gałka et al., 2018). Transfer functions in peatlands from
190 discontinuous permafrost peatlands (Swindles et al., 2015b) are not suitable as there
191 are several non-analogue taxa. Common taxa that are found across Alaskan North
192 Slope peatlands that are not well-represented in the Swindles et al. (2015b) transfer
193 function include *Conicocassis pontigulasiformis*, *Diffflugia bryophila* and *Gibbocarina*
194 *galeata*.

195

196 We also explored how the host vegetation at each site was influenced by contrasting
197 environmental conditions in our peatlands. Additional sub-samples were suspended
198 in deionised water and the host vegetation was identified with light microscopy at
199 200 × magnification. Individuals were catalogued to species or ‘type’ level using
200 identification guides from Flora of North America North of Mexico (2007, 2014),
201 Hedenäs (2003) and Smith (2004). Nomenclature follows Walker et al. (1994) for
202 vascular plants and Flora of North America North of Mexico (2007, 2014) for
203 bryophytes.

204

205 **4. Results**

206 *4.1 Relationship between environmental variables and species distribution*

207 We identified 94 testate amoebae taxa from 29 genera and a total count of 15,723
208 individuals. The most abundant species were *Centropyxis aerophila*, *Euglypha*
209 degraded (individuals from the *Euglypha* genus that were not sufficiently well
210 preserved for species-level identification), *Cyclopyxis eurystoma*, *Phryganella*
211 *acropodia*, *Trinema lineare* and *Centropyxis ecornis*. NMDS shows that EC, LOI and
212 WTD are the most important variables in controlling the distribution of testate
213 amoebae species in these sites ($p < 0.001$) (Figure 2; Table 2). CCA supports this,
214 also identifying EC as the dominant control on testate amoebae distribution (Figure
215 3; Table 2). Partial CCAs show that EC explains 25.0% of data variance ($p < 0.001$),
216 WTD explains 16.5% ($p < 0.001$) and MC explains 13.3% ($p < 0.001$). RDA further
217 supports the premise that the trophic gradient (for which EC is a proxy) is controlling
218 species distribution. We also found a significant correlation between pH and EC ($r =$
219 0.499, $p < 0.1$), which is not unexpected as pH is also indicative of peatland trophic
220 status (Gorham et al., 1987).

221

222

223

224

225

234 (Loss-on-ignition), MC (Moisture Content) and pH. The species are broken down according
 235 to Table 4.

236

Variable	NMDS				pCCA	
	NMDS1	NDMS2	R ²	Significance	Variance explained	Significance
pH	0.261	0.966	0.289	$p < 0.001$	10.07%	$p = 0.005$
EC	0.902	-0.431	0.499	$p < 0.001$	24.98%	$p < 0.001$
WTD	-0.158	-0.988	0.339	$p < 0.001$	16.54%	$p < 0.001$
MC	-0.491	0.871	0.080	$p = 0.021$	13.26%	$p < 0.001$
LOI	-0.984	0.179	0.483	$p < 0.001$	8.93%	$p = 0.04$

237 Table 2. Ordination statistics of environmental variables.

238

239 The most abundant plant species at sampling sites included *Warnstorfia cf.*
 240 *exannulata*, *Andromedia glaucophylla*, *Campyllum stellatum*, *Cinclidium stygium*,
 241 *Scorpidium cossoni*, *Tomentypnum nitens*, *Sphagnum teres*, and *Andromedia*
 242 *glaucophylla* (Appendix A). Forty plant taxa were identified from a total of 27 genera.
 243 Partial CCAs show that all variables are highly significant ($p < 0.001$). The most
 244 important variables are wetness indicators, as MC explains 5.23% of data variance
 245 and WTD explains 3.02%. There is a significant correlation (Pearson's $r = 0.197$, $p =$
 246 0.0497 , $n = 100$) between the species richness of testate amoebae and plants.

247

248 Mean EC at each sampling location is strongly correlated ($p < 0.001$) with LOI, and
 249 WTD with MC ($p < 0.001$), emphasising two strong hydrological gradients of trophic
 250 status and wetness in our sites. We identified a statistically significant difference in
 251 testate amoebae communities (ANOSIM $r = 0.428$, $p < 0.001$; PERMANOVA $r =$
 252 0.303 , $p < 0.001$) and plant communities (ANOSIM $r = 0.730$, $p < 0.001$;
 253 PERMANOVA $r = 0.757$, $p < 0.001$) among sites (Appendix B).

254

255 4.2 Transfer function development

256 Transfer functions were developed for WTD (TF_{WTD}) and EC (TF_{EC}), because both
 257 variables were highly significant in ordination. R²_{JACK} and RMSEP_{JACK} values were
 258 used to identify the best performing models. For TF_{WTD}, WAPLS component 2
 259 performed better than WA.inv (Table 3) after removing large residuals (> 11 cm).

260 WA.inv did not perform well at either end of the WTD range, with high residuals at
 261 extreme wet and dry sites. We removed 31 samples to improve performance ($R^2_{\text{JACK}} = 0.842$,
 262 $\text{RMSEP}_{\text{JACK}} = 6.66$ cm, Maximum bias = 14.30 cm, $n = 69$). One species
 263 (*Arcella vulgaris*), present in one sample at 2.44% abundance, was also removed
 264 due to its high residual value. Dry indicator species include *Assulina muscorum*,
 265 *Nebela tinctoria*, *Corythion dubium* and *Euglypha* spp. Key wet indicator species
 266 include *Netzelia corona*, *Centropyxis declivistoma*, *Conicocassis pontigulasiformis*
 267 and *Diffflugia bryophila* (Figure 4). Optimum and tolerance statistics can be found in
 268 Figure 7.

269

270 TF_{EC} is also based on the second component of a WAPLS regression. ML appears
 271 to perform well prior to residual removal (Table 3). However, almost all of the large
 272 residuals that needed to be removed were in low-EC sites. Removal of the majority
 273 of low-EC sites would yield a transfer function with low skill in ombrotrophic
 274 conditions, biased towards minerotrophic sites. As a result, ML was not pursued
 275 further. WA and WAPLS represented the full gradient, with WAPLS outperforming
 276 WA. We removed 23 samples with large residuals (residuals $> 228 \mu\text{S cm}^{-1}$) from the
 277 transfer function to improve performance (after removal: $R^2_{\text{JACK}} = 0.756$, $\text{RMSEP}_{\text{JACK}}$
 278 $= 146 \mu\text{S cm}^{-1}$, maximum bias = $189 \mu\text{S cm}^{-1}$, $n = 77$). Minerotrophic habitat indicator
 279 species include *Cyclopyxis kahli*, *Centropyxis ecorinis*, *Phryganella acropodia* and
 280 *Diffflugia globulosa*. Key oligotrophic habitat indicator species include *Archerella*
 281 *flavum*, *Hyalosphenia papilio*, *Gibbocarina galeata* and *Centropyxis aculeata* (Figure
 282 5).

Model	TF_{WTD}			TF_{EC}		
	R^2_{JACK}	$\text{RMSEP}_{\text{JACK}}$	Max Bias	R^2_{JACK}	$\text{RMSEP}_{\text{JACK}}$	Max Bias
<i>Initial transfer function performance</i>						
WAPLS	0.414	14.76	17.56	0.493	249.69	331.68
WA.inv	0.414	14.76	17.60	0.382	273.61	444.14
ML	0.471	16.20	19.38	0.452	269.63	478.26
<i>After removing high residual sites (< 20%)</i>						
WAPLS	0.842	6.66	14.30	0.756	146.04	188.82
WA.inv	0.734	9.36	14.18	0.680	151.52	606.32

283

284 *Table 3 – Transfer function performance metrics. WAPLS statistics are all reported*
 285 *from the second component, as this was the best performing.*

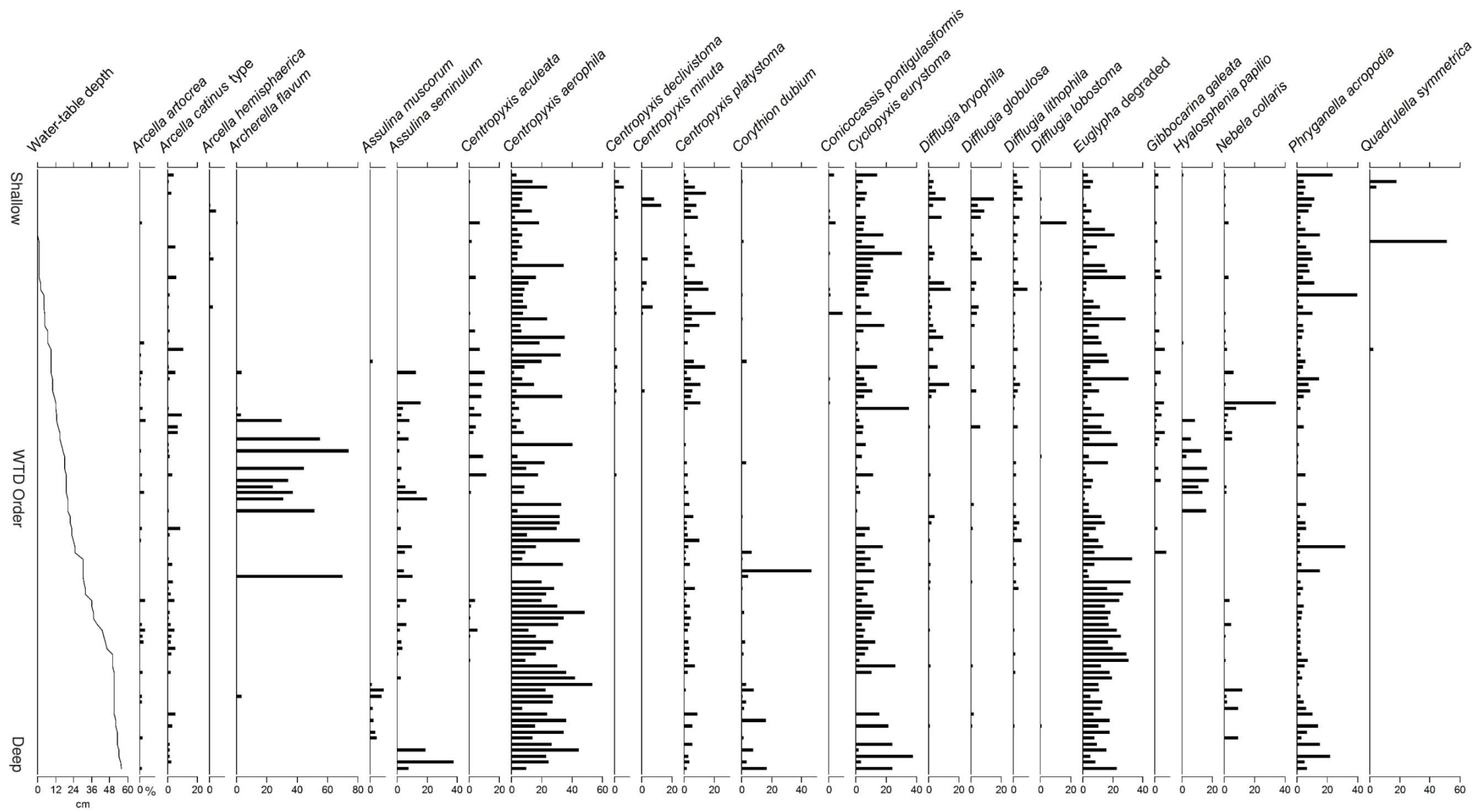


Figure 4) Percentage abundance of selected testate amoebae taxa that indicate a range of WTD conditions, ranked by observed WTD.

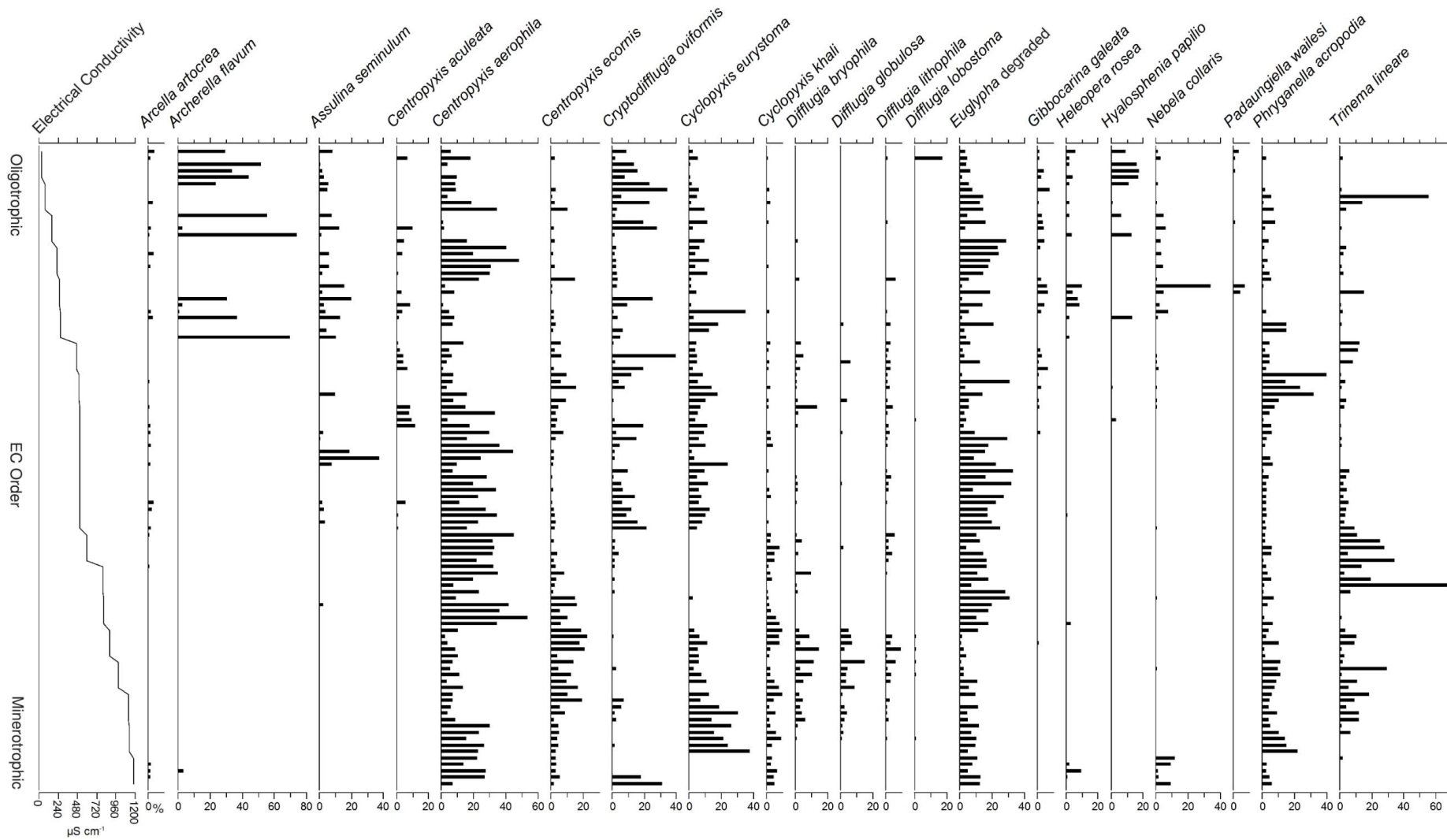


Figure 5) Percentage abundance of selected testate amoebae taxa that indicate a range of EC conditions, ranked by observed conductivity values.

288 *4.3 Removing high conductivity sites*

289 We removed the 35 samples with the greatest (top 50%) EC values ($\geq 600 \mu\text{S cm}^{-1}$)
290 from the full dataset. This allows us to test whether the control of trophic status on
291 the distribution of testate amoebae species was being affected by sites that are
292 unusually nutrient rich. NMDS continued to show EC as the primary control on
293 species distribution, while MC, LOI, and WTD also remained highly significant ($p <$
294 0.001). CCA showed WTD as the primary control, with MC, EC and organic matter
295 content also highly significant ($p < 0.001$).

296

297 *4.4 Transfer function performance*

298 Both transfer functions perform well in terms of performance statistics (Table 3,
299 Figure 6), so we applied them to a short core (from Gałka et al., 2018) from a
300 peatland adjacent to Toolik Lake (Appendix C). Gałka et al. (2018) found that no
301 existing transfer function was suitable for reconstructing WTD in their core, instead
302 opting to use the percentage of wet indicators as a semi-quantitative index of
303 wetness. Our WTD transfer function offers a significant improvement over this
304 approach and shows that the peatland has remained moderately dry throughout the
305 core. Wetness gradually increases from the bottom of the core and peaks at 12.5 cm
306 depth. TF_{WTD} offers extra insight to the wetness indicators, by quantifying the dry
307 periods either side of this peak. This shows that the peatland has gradually
308 increased in wetness and transitioned recently to a state that is notably drier than
309 any period in its past, reaching a WTD of 85.0 cm at the surface. In addition, TF_{EC}
310 shows that this transition to dryness at the top of the core is accompanied by a shift
311 towards minerotrophic conditions.

312

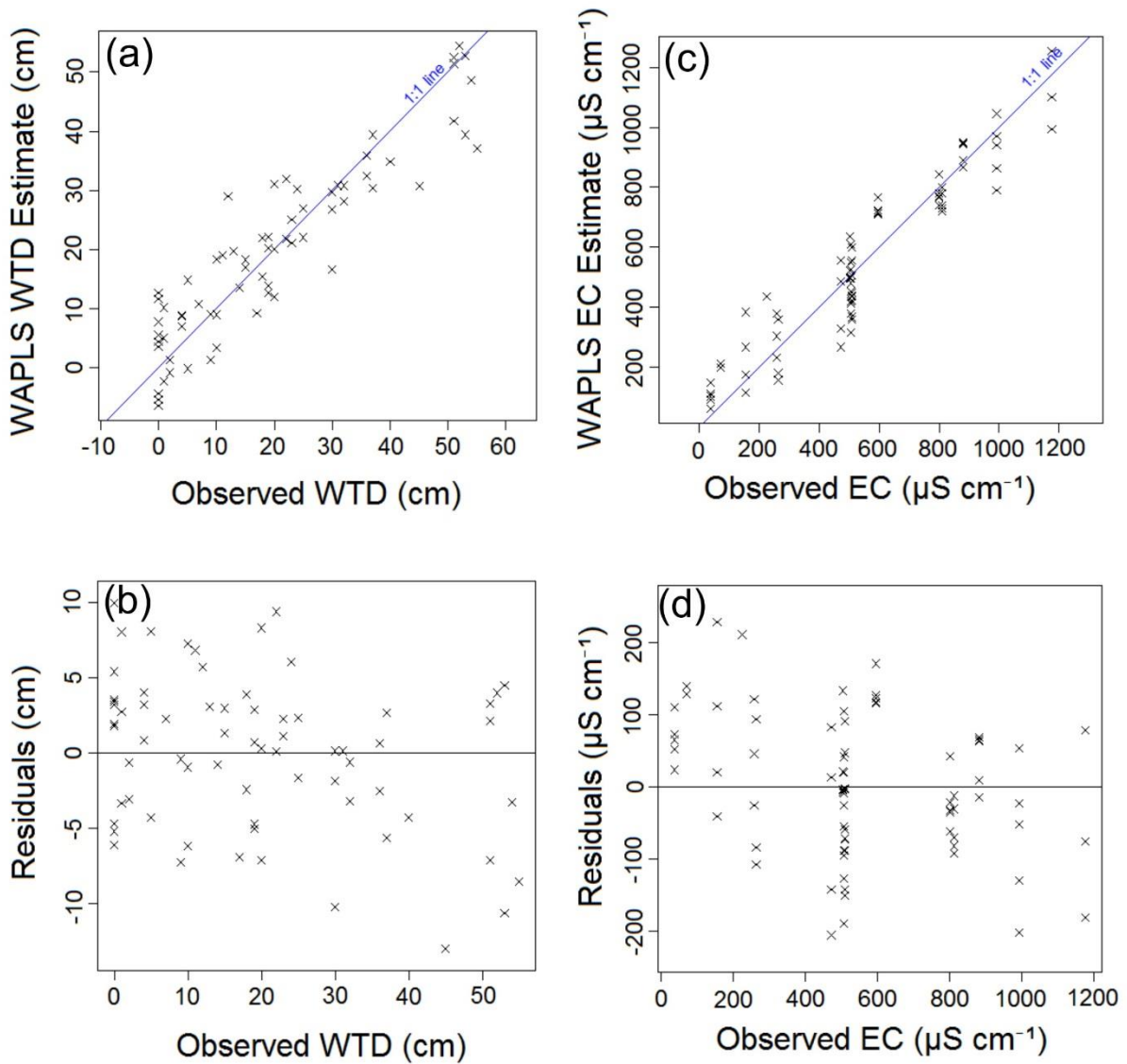
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319 *Figure 6) Transfer function performance. (a) TF_{WTD} estimates of each site against*
 320 *observations and (b) residuals of each site against observed WTD. (c) TF_{EC} estimates of*
 321 *each site against observations and (d) residuals of each site against observed conductivity.*

Code	Taxa name	In n samples	Maximum abundance (%)
ARAT	<i>Arcella artocrea</i>	33	4.2
ARCA	<i>Arcella catinus</i> type	56	10.6
ARHE	<i>Arcella hemisphaerica</i>	5	4.5
ARFL	<i>Archerella flavum</i>	20	74.1
ASMU	<i>Assulina muscorum</i>	12	9.4
ASSC	<i>Assulina scandinavica</i>	40	10.9
ASSE	<i>Assulina seminulum</i>	35	37.7
BUIN	<i>Bullinularia indica</i>	37	6.4
CEAC	<i>Centropyxis aculeata</i>	30	11.8
CEAE	<i>Centropyxis aerophila</i>	96	53.8
CECO	<i>Centropyxis constricta</i>	3	7.1
CEDE	<i>Centropyxis declivistoma</i>	23	6.3

CEEC	<i>Centropyxis ecornis</i>	92	22.6
CEHE	<i>Centropyxis hemisphaerica</i>	13	3.3
CEMI	<i>Centropyxis minuta</i>	8	13.1
CEOR	<i>Centropyxis orbicularis</i>	48	6.7
CEPL	<i>Centropyxis platystoma</i>	75	20.8
CESY	<i>Centropyxis sylvatica</i>	11	2.9
CODU	<i>Corythion dubium</i>	39	47.0
CRCO	<i>Cryptodiffugia compressa</i>	24	55.7
CROV	<i>Cryptodiffugia oviformis</i>	77	40.0
COPO	<i>Coniocassis pontigulasiformis</i>	14	9.6
CYEU	<i>Cyclopyxis eurystoma</i>	81	37.7
CYKH	<i>Cyclopyxis kahli</i>	73	9.8
CYAM	<i>Cyphoderia ampulla</i>	10	2.5
DIBR	<i>Diffugia bryophila</i>	49	14.5
DIGE	<i>Diffugia geosphaira</i>	2	2.7
DIGL	<i>Diffugia globulosa</i>	3	15.5
DILI	<i>Diffugia lithophila</i>	58	9.7
DILO	<i>Diffugia lobostoma</i>	1	0.5
DIPY	<i>Diffugia pyriformis</i>	15	6.8
EUDE	<i>Euglypha degraded</i>	99	33.3
EURO	<i>Euglypha rotunda</i>	51	20.7
EUST	<i>Euglypha strigosa</i>	47	8.1
EUTU	<i>Euglypha tuberculata</i>	30	4.7
GIGA	<i>Gibbocarina galeata</i>	40	8.1
HERO	<i>Heleopera rosea</i>	30	10.3
HESY	<i>Heleopera sylvatica</i>	61	10.4
HYEL	<i>Hyalosphenia elegans</i>	21	4.4
HYIN	<i>Hyalosphenia insecta</i>	7	3.8
HYOV	<i>Hyalosphenia ovalis</i>	1	5.4
HYP A	<i>Hyalosphenia papilio</i>	17	18.1
HYPL	<i>Hyalosphenia platystoma</i>	27	4.7
NECO	<i>Nebela collaris</i>	48	34.1
NEMI	<i>Nebela militaris</i>	4	2.4
NETI	<i>Nebela tincta</i>	17	33.3
NECR	<i>Netzelia corona</i>	4	4.5
NEWA	<i>Netzelia wailesi</i>	8	5.2
PALA	<i>Padaungiella lageniformis</i>	20	4.0
PAWA	<i>Padaungiella wailesi</i>	16	7.1
PHAC	<i>Phryganella acropodia</i>	88	39.8
PLSP	<i>Placocista spinosa</i>	1	2.8
PLMA	<i>Planocarina marginata</i>	2	3.2
QASY	<i>Quadrullella symmetrica</i>	5	51.7
TRDE	<i>Tracheleuglypha dentata</i>	31	9.5
TRCO	<i>Trinema complanatum</i>	60	10.5
TREN	<i>Trinema enchelys</i>	2	53.5
TRGR	<i>Trinema grandis</i>	11	2.1
TRLI	<i>Trinema lineare</i>	73	67.1

322

323 *Table 4. Overview of testate amoebae identified in abundances greater than 2% in*
324 *any one sample.*

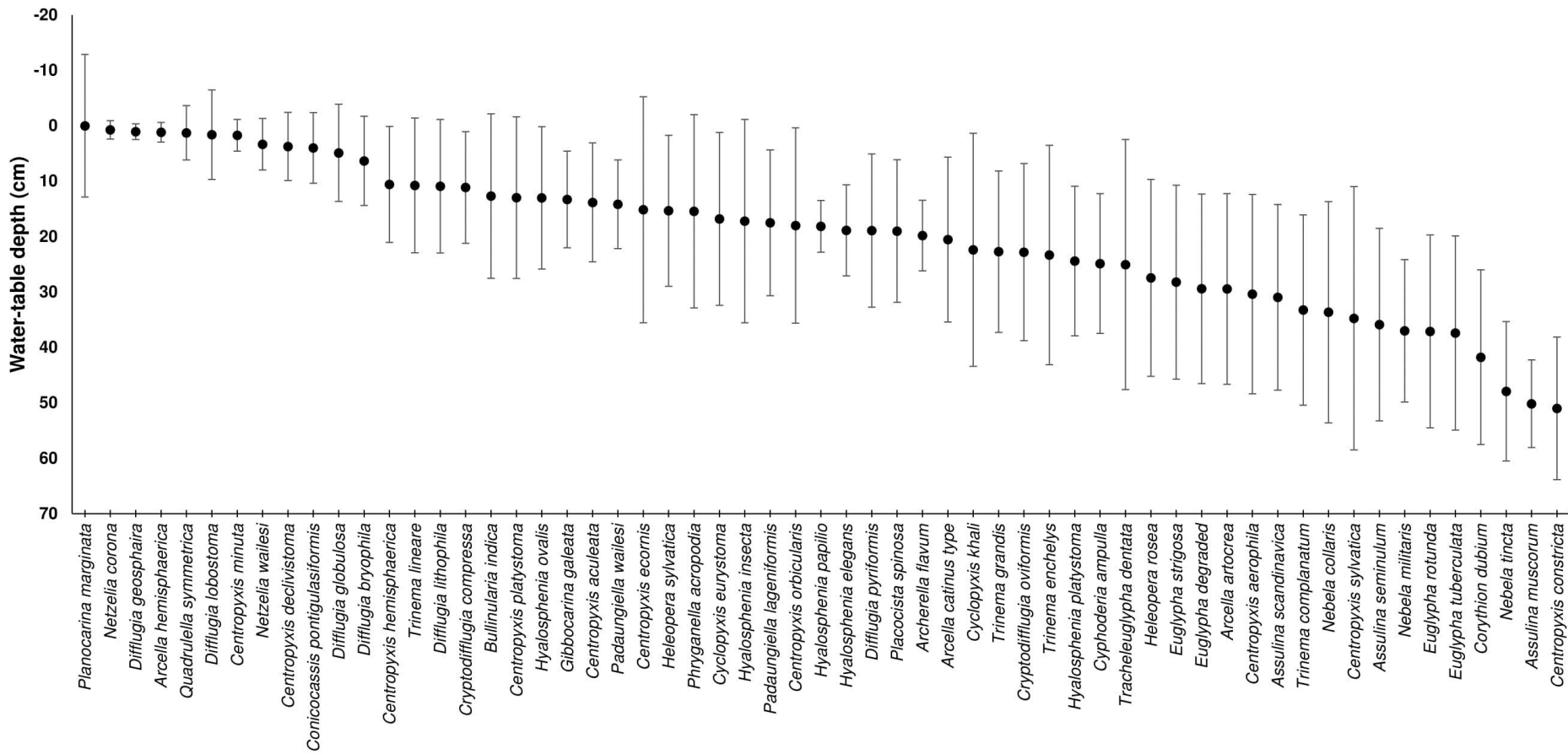


Figure 7 – WTD tolerance and optima statistics for testate amoebae calculated through Weighted Averaging.

326

327 **5. Discussion**

328 Our new transfer functions can be used for palaeoenvironmental reconstruction in
329 permafrost peatlands to understand both long-term and recent changes in wetness
330 and hydrochemistry. This is the first study examining testate amoebae as
331 environmental indicators in continuous permafrost peatlands. This study supports
332 existing research that suggests testate amoebae are a useful way to reconstruct
333 palaeohydrology in permafrost peatlands (Swindles et al., 2015b; Lamarre et al.,
334 2012, 2013; Bunbury et al., 2012; Gałka et al., 2017). Testate amoebae based
335 reconstructions can form part of a multi-proxy toolkit to better understand the
336 changing nature of peatlands in the continuous permafrost zone through the
337 Holocene. The ecology of testate amoebae in continuous permafrost is similar to
338 those found in discontinuous permafrost, although the key hydrological control on
339 species distribution is different. EC plays a more important role in continuous
340 permafrost, suggesting a strong ombrotrophic-minerotrophic gradient may dominate
341 Alaskan North Slope peatlands. Our results also show that the peatlands in this
342 region are a mixture of both ombrotrophic and minerotrophic systems, and contain
343 variability between these categories within-site. Therefore, a transfer function
344 encompassing the entire nutrient-status gradient is more appropriate than splitting
345 the model into individual ombrotrophic and minerotrophic models.

346

347 *5.1 Species diversity*

348 Testate amoeba diversity is high (96 taxa identified from 15,723 classified individuals
349 across 5 sites), which gives us confidence that they are robust hydrological
350 indicators as they are found across the permafrost zone. Most testate amoebae
351 studies are performed exclusively in either oligotrophic or eutrophic environments,
352 rather than across a trophic gradient. Studies of testate amoebae and vegetation
353 diversity across trophic gradients produce conflicting results. The study carried out
354 by Lamentowicz et al. (2010) in temperate zone in sub-alpine peatlands of the Upper
355 Engadine (Swiss Alps) identified a weak correlation in the fen-bog gradient between
356 testate amoebae and moss diversity, while research by Opravilova and Hajek
357 (2006), in a mountain peatland located in the Western Carpathians, found no

358 correlation. We find that WTD is the only significant ($p < 0.001$) variable influencing
359 the species richness of both surface plant species and testate amoebae. EC shows
360 a very weak ($r = -0.177$, $p = 0.078$) correlation. MC is the most important influence
361 on testate amoebae species diversity.

362

363 5.2 Nutrient level as the dominant factor

364 This study is one of the first where the ombrotrophic-minerotrophic gradient is the
365 primary controlling factor on testate amoebae distribution, across a full range of
366 peatlands from bogs to fens. This accurately represents our observations of
367 peatlands in this region, as varying significantly in their trophic status. As a result,
368 this has allowed us to produce a transfer function to reconstruct EC throughout the
369 Holocene, which can allow future studies to better identify the timing of fen-bog
370 transitions or the change of a peatlands' nutrient status. This can directly influence
371 the net ecosystem productivity and subsequent carbon sequestration of Arctic
372 peatlands (Bubier et al., 1999). This distribution of testate amoebae along the
373 oligotrophic-eutrophic gradient has also been observed in aquatic ecosystems (e.g.
374 Beyens et al., 1986; Qin et al., 2009; Ju et al., 2014). The observed trophic gradient
375 does not affect surface vegetation though, as proxies for moisture (WTD, MC)
376 controlled their distribution. As WTD is a statistically significant second-order control
377 on species distribution from sites across a long trophic gradient, our transfer
378 functions can be applied to reconstruct ecosystem change across the Holocene
379 (such as a fen-bog transition), which contrasts findings from Payne (2011) from the
380 Mediterranean.

381

382 The response of particular testate amoebae species to the trophic gradient closely
383 matches an existing study from a peatland adjacent to Toolik Lake (Mitchell, 2004),
384 where species diversity of testate amoebae was examined in response to increased
385 nitrogen and phosphorus levels. We confirm results from Mitchell (2004) that
386 *Archerella flavum* and *Hyalosphenia papilio* are indicators of nutrient-poor peatlands,
387 while *Centropyxis aerophila* and *Phryganella acropodia* indicate minerotrophic
388 conditions. However, unlike Mitchell (2004), we found that *Assulina muscorum* was
389 indicative of minerotrophy in our dataset. We identified *Centropyxis aerophila* as a

390 dominant species, which has also been observed in Arctic lakes (Beyens et al.,
391 1986). However, Beyens et al. (1986) describe *Centropyxis aerophila* as a low-
392 conductivity indicator, whereas we find this species across the trophic gradient and
393 in greater abundance in higher EC sites.

394

395 *5.3 Reconstructing water-table depth*

396 We also present the first testate amoebae based transfer function to reconstruct
397 WTD in continuous permafrost peatlands. This increases the global extent of testate
398 amoebae as palaeohydrological indicators and opens opportunities to better
399 understand how high-latitude ecosystems have responded to a changing climate
400 throughout the Holocene. Individual taxa behave broadly as expected, comparing
401 results to other studies in discontinuous permafrost (Amesbury et al., 2013; Swindles
402 et al., 2015b; Zhang et al., 2017). Our largest anomaly was *Archerella flavum*, which
403 we observe to be an intermediate indicator with an optimum WTD of around 19 cm.
404 While the presence of *A. flavum* at this WTD has been observed in the compared
405 studies, it is generally an indicator of much wetter conditions. Conversely, we do not
406 observe *A. flavum* in abundance ($\geq 2\%$) drier than 9 cm WTD. This could be because
407 of the observed strong control of low nutrient status on this taxon.

408

409 *5.4 Future applications*

410 Testate amoebae can be used to investigate environmental change across Arctic
411 peatlands, as they respond to changes in climate throughout the Holocene. This, in
412 combination with other testate amoeba records from the Arctic (e.g. Müller et al.,
413 2009), expands the potential for using testate amoebae as palaeoenvironmental
414 indicators around the world. We applied both transfer functions to a short core from a
415 peatland in the Alaskan Arctic. We found that the site has been moderately dry in the
416 past, with gradually increasing wetness from the bottom of the core to a peak WTD
417 of 9.4 cm at 12.5 cm depth, but has entered a state of rapid transition towards
418 dryness from 12.5 cm depth to a WTD of 85.0 cm at the surface. There is now the
419 opportunity to apply our transfer functions to a fossil record from the continuous

420 permafrost zone to investigate WTD and ombrotrophic-minerotrophic transitions
421 since peatlands first began to develop in this area.

422

423 **6. Conclusion**

- 424 1. We present the first testate amoeba based transfer functions for
425 reconstruction of water-table depth and electrical conductivity in peatlands
426 from the Alaskan continuous permafrost zone.
- 427 2. Testate amoebae are valuable environmental indicators in continuous
428 permafrost peatlands.
- 429 3. Pore water electrical conductivity is the primary control on the distribution of
430 testate amoeba species in these sites. Electrical conductivity is a proxy for the
431 nutrient status of peatlands, suggesting that testate amoebae can be used as
432 reliable indicators of trophic status in peatlands of the North Brooks foothills,
433 Alaska.
- 434 4. The species richness of contemporary plants and testate amoebae taxa are
435 significantly correlated to each other, and independently to water-table depth.
- 436 5. Our new transfer functions may be valuable components of multi-proxy
437 investigations into the responses of Arctic permafrost peatlands to climate
438 change over the Holocene and in recent centuries.

439

440

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450

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710 **Appendix A** - Details of plant species identified at each of the five sampling sites in
 711 the Alaskan North Slope.

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Taxa name	In <i>n</i> samples	Percentage of samples taxa is present in				
		Montane	Spring	Valley	String	Lakeshore
<i>Andromeda glaucophylla</i>	23	50	5	5	35	20
<i>Aulacomnium palustre</i>	6	0	15	10	5	0
<i>Aulacomnium turgidum</i>	7	5	5	0	0	25
<i>Betula nana</i>	7	20	0	5	0	10
<i>Brachythecium mildeanum</i>	1	0	5	0	0	0
<i>Bryum sp.</i>	14	15	20	0	30	5
<i>Calliergon cordifolium</i>	2	0	0	0	0	10
<i>Calliergon richardsonii</i>	2	0	0	0	0	10
<i>Campylium cf. laxifolium</i>	1	0	5	0	0	0
<i>Campylium stellatum</i>	22	40	20	0	30	20
<i>Cinclidium stygium</i>	17	20	40	0	15	10
<i>Cirriphyllum piliferum/cirrosom</i>	1	0	0	0	0	5
<i>Dicranella sp.</i>	8	5	10	0	15	10
<i>Dryas integrifolia</i>	6	5	25	0	0	0
<i>Fissidens sp.</i>	4	20	0	0	0	0
<i>Hylocomnium splendens</i>	6	10	5	0	0	15
<i>Hypnum pratense</i>	2	0	10	0	0	0
<i>Loeskygnum badium</i>	1	0	0	0	0	5
<i>Meesia triquetra</i>	13	0	20	0	30	15
<i>Paludella squarrosa</i>	5	0	5	0	0	20
<i>Pohlia sp.</i>	2	0	0	10	0	0
<i>Polytrichum cf. juniperinum</i>	2	0	10	0	0	0
<i>Polytrichum commune</i>	12	0	0	60	0	0
<i>Polytrichum juniperinum</i>	4	0	0	20	0	0
<i>Pseudocalliergon sp.</i>	3	15	0	0	0	0
<i>Salix reticulata</i>	2	10	0	0	0	0
<i>Salix sp.</i>	5	5	20	0	0	0
<i>Sarmentypnum sarmentosum</i>	1	0	0	0	5	0
<i>Scorpidium cossoni</i>	14	0	0	0	25	45
<i>Scorpidium cossoni/revolvens</i>	16	55	25	0	0	0
<i>Scorpidium scorpioides</i>	9	0	10	0	35	0
<i>Sparganium sp.</i>	1	0	0	5	0	0
<i>Sphagnum capillifolium</i>	4	0	0	0	0	20
<i>Sphagnum contortum</i>	1	0	0	0	0	5
<i>Sphagnum teres</i>	3	0	0	0	0	15

<i>Sphagnum teres/squarrosum</i>	17	0	0	85	0	0
<i>Sphagnum warnstorffii</i>	7	0	15	0	0	20
<i>Straminergon stramineum</i>	9	0	0	30	0	15
<i>Tomentypnum nitens</i>	33	70	45	0	20	30
<i>Warnstorfia cf. exannulata</i>	7	0	0	35	0	0

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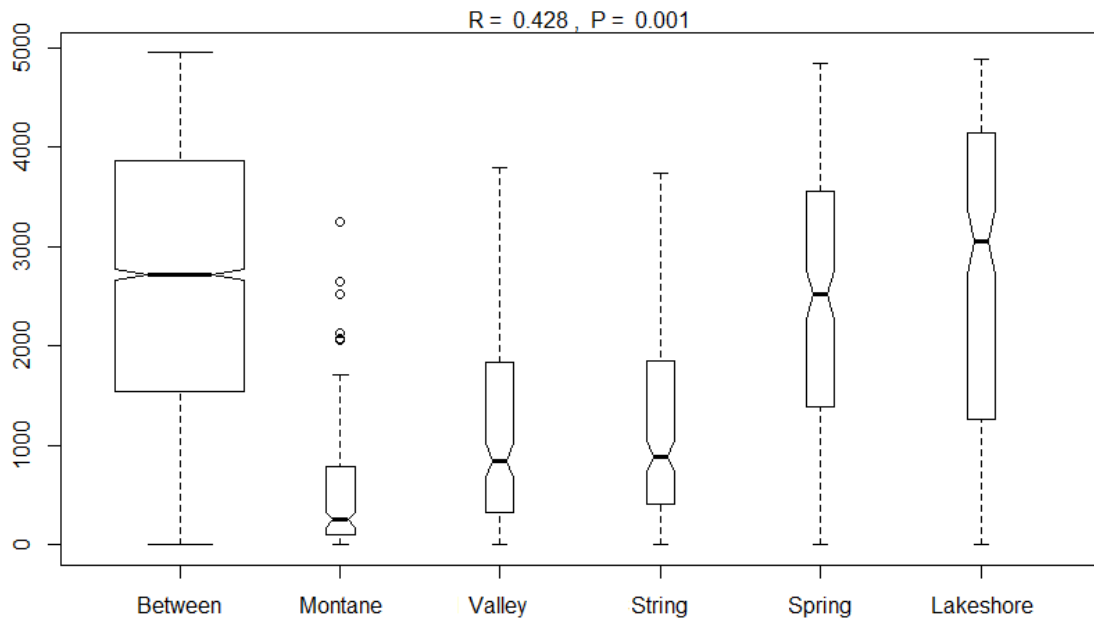
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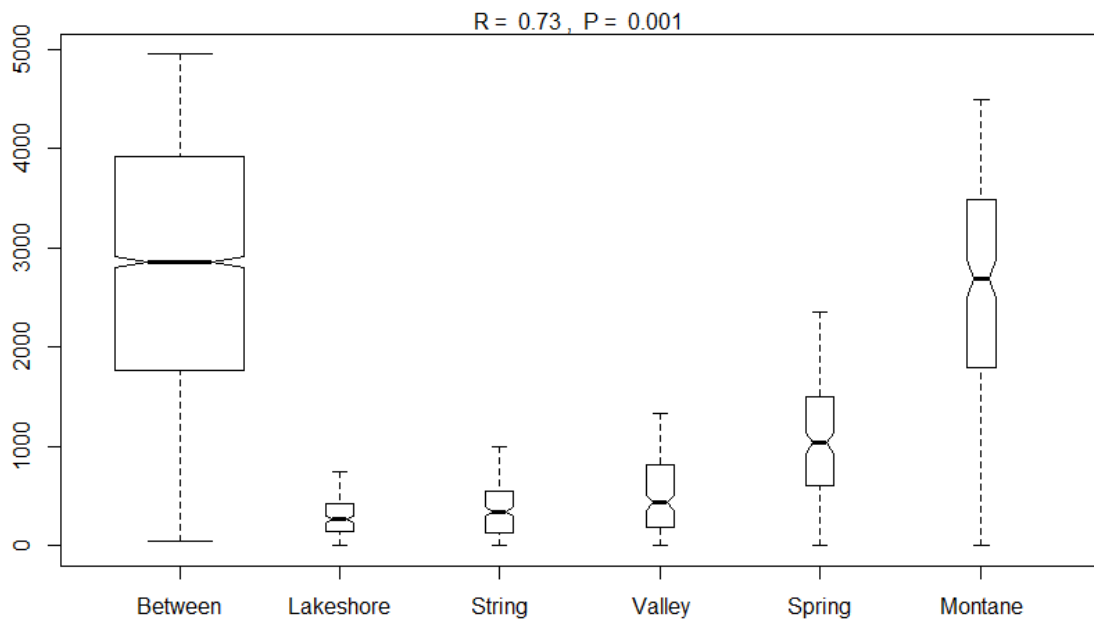
734 **Appendix B – ANOSIM analysis of testate amoebae and plant distribution between**
735 **our five sites.**



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737 *ANOSIM analysis of testate amoebae distribution between sites.*

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740 *ANOSIM analysis of contemporary plant species distribution between sites.*

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