Successional change of testate amoeba assemblages along a space-for-time sequence of peatland development

Zhang, H. [1, 2*], Väliranta, M.[1, 2], Amesbury, M. J. [3], Charman, D. J.[3], Laine, A.[4], Tuittila, E-S[5]

[1] ECRU, Ecosystems and Environment Research Programme, Faculty of Biological and Environmental Sciences, P.O. Box 65, FI-00014 University of Helsinki, Finland

[2] Helsinki Institute of Sustainability Science (HELSUS), Finland

[3] Geography, College of Life and Environmental Sciences, University of Exeter, UK

[4] Department of Biology, University of Oulu, Finland

[5] Department of Forest Sciences, University of Eastern Finland, Finland

* Corresponding author: Email: hui.palaeo@gmail.com

Abstract

It is well established that in ombrotrophic bogs, water-table depth (WTD) is the primary environmental control on testate amoeba distribution. However, the environmental controls on testate amoebae in minerotrophic fens are less well known and successional change in their assemblages associated with fen-bog peatland development has been scarcely investigated. Here we investigate a peatland space-for-time sequence resulting from postglacial rebound on the west coast of Finland, to assess successional patterns in testate amoeba communities and their relationships with environmental variables during peatland development. Sample sites along a 10-km transect from coast to inland ranged from a recently emerged wet meadow to a mature bog. Environmental variables (e.g., peat thickness, carbon and nitrogen content, pH, WTD and vegetation) were measured alongside testate amoeba samples. Results showed that even though the distribution of testate amoeba was to some extent determined by the succession stage, many taxa had wide WTD and pH ranges. The primary environmental control for many taxa changed along the succession. In conclusion, the ecological constraints on testate amoebae in minerotrophic systems are more complex than in bogs. The detected patterns also complicate the use of testate amoeba as a primary proxy in palaeoecological reconstructions where fen-to-bog shifts occur.

Keywords: testate amoeba, fen environment, peatland succession, ecological constraints

Introduction

Testate amoebae are widely used to (semi)quantitatively reconstruct past environmental changes and, in particular, changes in hydrological conditions (Charman et al. 1999; Booth 2008;

Lamarre et al. 2013; Amesbury et al. 2016; Zhang et al. 2017). Understanding their ecology is important in the development and application of these techniques. A new pan-European dataset (Amesbury et al. 2016) as well as many other previous studies on bogs from Eurasia (e.g. Woodland et al. 1998; Bobrov et al. 1999; Väliranta et al. 2012; Qin et al. 2013), North America (e.g. Charman and Warner 1992; Booth 2008) and South America (e.g. Swindles et al. 2014; van Bellen et al. 2014) have indicated that peatland water-table depth is the most important factor determining testate amoeba community composition in ombrotrophic systems. The strong relationship between taxa composition and hydrology allows reconstructions of past changes in bog water-table depth conditions based on a transfer function approach where fossil assemblages are modelled against modern assemblages with known ecological constraints. To date, testate amoeba response to other environmental variables such as pH or trophic status has been less thoroughly investigated, with the exception of limited transfer function studies on pH (e.g., Lamentowicz and Mitchell 2005; Lamentowicz et al. 2008) or nutrient status (Dudová et al. 2013; Lamentowicz et al. 2013a), although many studies have referred to the likely importance of these factors (Tolonen et al. 1992; Woodland et al. 1998; Booth et al. 2008; Mitchell et al. 2008a; Kroupalova et al. 2013; Jassey et al. 2014). An increasing number of studies infer that pH is an important determining factor for testate amoeba composition in many peatland systems (Opravilová and Hájek 2006; Lamentowicz et al. 2007, 2011; Payne 2011).

The fen-bog transition, one of the key landscape changes of the Holocene (Hughes and Barber 2003; Väliranta et al. 2017), shows obvious pH and nutrient status succession and provides important opportunities to study the corresponding responses of various biological organisms. Successional changes in plant species composition over the fen-bog transition are well known based on palaeoecological as well as spatial chronosequence studies (Klinger and

70 Short 1996; Hughes and Barber 2003, 2004; Tuittila et al. 2013; Ronkainen et al. 2014; Väliranta
71 et al. 2017). However, successional change in other organisms during mire development is much
72 less well understood (see however Merilä et al. 2006; Larmola et al. 2014) with a relatively
73 limited amount of data concerning the succession of testate amoeba communities (Opravilová
74 and Hájek 2006; Lamentowicz et al. 2010, 2013; Jassey et al. 2011; Payne 2011; Galka et al.
75 2017).

76 Here, we aim to define successional changes in testate amoeba assemblages during
77 peatland development and link taxa distribution to different environmental variables. We
78 hypothesise that testate amoebae show a clear successional pattern in their community structure
79 during mire development. Unlike previous successional studies (e.g., Hughes and Barber 2003,
80 2004), which are based on downcore sediment analysis, in this study we use a space-for-time
81 approach. The space-for-time approach is justified because the changes in site type which occur
82 over our spatial transect relate closely to downcore changes in preserved vegetation remains seen
83 over the fen to bog transition in previous studies (Hughes and Barber 2003; Väliranta et al. 2017).
84 By using this approach, we: i) avoid the loss of decomposition-prone taxa, and ii) can directly
85 measure environmental variables. In addition, we aim to improve the level of understanding of
86 ecological constraints of testate amoeba in fen environments.

87

88 **Study area**

89 The study area is located on the western coast of Finland in Siikajoki (SJ) commune (64°45'N,
90 24°42'E) (Fig. 1). The region represents the middle boreal ecoclimate zone. The mean annual
91 temperature and precipitation are 2.6°C and 539 mm respectively and the length of the growing
92 season is 150 days (observation period 1979–2009; Revonlahti, Siikajoki, 64°41'N, 25°05'E, 48

93 m a.s.l, Finnish Meteorological Institute). Primary paludification is an ongoing process in the
94 area and postglacial land uplift has created a space-for-time sequence of peatlands.

95 Seven study sites (SJ0-SJ6; Table 1) form a 10-km long transect from the coast to inland.
96 They have been selected to represent different stages of mire development, with SJ0 being a
97 newly formed shoreline meadow (*ca*. 50 years) and SJ6 being a fully developed bog community
98 with an estimated age of *ca*. 3000 years (Table 1). In between there are young minerotrophic
99 meadows and fens a few kilometres from the shore.

100 The vegetation at site SJ0 was dominated by graminoids (e.g., *Festuca rubra*,
101 *Calamagrostis stricta*, *Carex glareosa* and *Juncus gerardii*), with very few bryophytes present.
102 Site SJ1 was a wet meadow with a patchy cover of brown mosses such as *Warnstorfia* spp. At
103 SJ2, bryophytes were more extensive and *Sphagnum* mosses occurred as patches among brown
104 mosses. Otherwise, both SJ1 and SJ2 were dominated by sedges and grasses such as *Carex nigra*
105 and *Agrostis canina* while the forbs *Comarum palustre* and *Lysimachia thyrsiflora* were also
106 common. Sites SJ3 and SJ4 were featured by mesotrophic and oligotrophic fen vegetation,
107 respectively. At both sites, the vegetation consisted mainly of sedges (e.g., *Carex chordorrhiza*,
108 *Carex rostrata* and *Carex limosa*), but dominant forbs at SJ3 and SJ4 were *C. palustre* and
109 *Menyanthes trifoliata* respectively. Hummock formation with very dense *Sphagnum* carpets was
110 evident at the edges of both SJ3 and SJ4. Site SJ5 was at the fen-bog transition stage with a
111 mosaic of clearly ombrotrophic hummock surfaces with *Rubus chamaemorus*, *Empetrum nigrum*,
112 *Vaccinium oxycoccos* and *Sphagnum fuscum* and wetter surfaces dominated by *Scheuchzeria*
113 *palustris*, *Carex livida*, *C. limosa* and *C. chordorhiza*. *Sphagnum* species accustomed to different
114 water-table depths formed a continuous moss layer. Site SJ6 was an ombrotrophic bog,
115 characterised by *S. fuscum*, *Sphagnum angustifolium* and dwarf shrubs such as *E. nigrum* and

116 *Rhododendron tomentosum* at the hummock surfaces and *Sphagnum balticum* and *Eriophorum*
117 *vaginatum* in wetter depressions. The modern spatial mire succession series realistically mimics
118 the historical (vertical) peatland development pattern where initial minerotrophic plant
119 communities are, over the course of time, replaced by bog plant communities (Tuittila et al.
120 2013).
121
122 **Materials and methods**
123 Sampling
124 To investigate changes in testate amoeba assemblages related to successional stage, in August
125 2007 we sampled surface soil from each study site along a transect of 9-12 sample plots that
126 covered the microtopographic variation characteristic of each site. In total, 61 samples were
127 collected from seven study sites (Table 1). Microtopographical variation was minimal in the
128 youngest sites SJ0 (pre-meadow on mineral soil), SJ1 and SJ2 (wet meadows), but in the fen
129 sites SJ3 (mesotrophic fen) and SJ4 (oligotropic fen) there was clear eco-hydrological variation
130 from drier hummock to wet flark level (Fig. A.1). Microtopographical variation was most
131 pronounced in SJ5 (fen-bog transition) where wet fen surfaces and drier ombrotrophic
132 hummocks formed a mosaic. In the oldest site SJ6 (bog), sampling covered variation from
133 *Sphagnum fuscum* hummocks to *S. balticum* lawns.
134 We collected two parallel sets of soil cores (one for microbiological and one for
135 physicochemical analyses) with a box sampler ($8 \times 8 \times 100$ cm^3) or with a cylinder sampler (Ø
136 4.5 cm with 50 cm length) from each site along the transect. The uppermost *ca*. 5-10 cm was
137 taken for testate amoeba analysis (Booth et al. 2010). In younger and/or more minerotrophic sites,
138 some samples may be older than modern, depending on sedimentation rate.

Portions of the soil samples were used for measuring pH (1:5 soil:water suspension) and the rest of the samples were frozen (-20°C) for testate amoeba analyses. The samples from parallel volumetric soil cores were used to determine bulk density, loss on ignition (LOI; 500°C, 4 h) and total carbon (C) and nitrogen (N; LECO CHN-2000 analyser). C and N contents were calculated on a volume basis based on the bulk density of the volumetric sample slices (g dm^{-3}).

We measured peat depth at each sampling point. Water-table depth (WTD), which indirectly represents peat surface moisture conditions, was measured weekly throughout the 2007 growing season (from May to September) from water wells located next to each sampling point and the minimum, maximum, mean and median WTD and the variation range were determined. Plant community composition was surveyed from sample plots (56 × 56 cm) located next to sampling points by estimating the proportion cover of each species (%). For further analysis, plant data were clustered into 12 plant functional types (PFTs): grasses, minerotrophic forbs, minerotrophic sedges, ombrotrophic forbs, ombrotrophic sedges, minerotrophic shrubs, ombrotrophic shrubs, brown mosses, hollow Sphagna, lawn Sphagna, hummock Sphagna and feather mosses, according to Tuittila et al. (2013) and references therein.

Testate amoeba analysis

Subsamples of known volume (1-5 cm^3 and generally 4 cm^3) were processed in the laboratory according to the protocol described in Charman et al. (2000). Samples were sieved with a 300-µm mesh and back-sieved with a 15-µm mesh (Payne, 2011). One *Lycopodium* tablet was added to each sample to calculate testate amoeba concentration (test cm^{-3}) by using the formula: testate amoeba concentration = testate amoeba counts * the total *Lycopodium* spores added to the sample /counted *Lycopodium* spores/ sample volume. A minimum of 150 tests was counted in

each sample (Payne and Mitchell 2009). Raw counts were converted to percentages for the data analysis. Eight subsamples with low testate amoeba concentrations were rejected from the data analysis because the number of tests counted was < 50 (Payne and Mitchell 2009) (Table 1). These samples represented minerogenic samples derived from the youngest end of the succession stages, where true peat formation had not yet occurred. Tests were identified and counted under a high-power light microscope using Charman et al. (2000) as a main key for identification, supplemented with online sources (e.g. Siemensma 2018). The Charman et al. (2000) taxonomic scheme has been widely applied in a range of recent testate amoeba ecological studies, including in fens (e.g. Payne 2011), but its conservative nature means that ecological inferences for some taxa (particularly *Corythion-Trinema* type) must be viewed with caution given the potential for individual taxa exhibiting variable ecological responses to be included in the same taxonomic grouping.

Data analysis

All ordination analyses were carried out using Canoco 5 (ter Braak and Šmilauer 2012). Taxa with < 5% occurrences were omitted from all ordination analysis to minimise the influence of rare taxa. This resulted in the removal of four taxa (i.e. *Heleopera sphagni*, *Nebela parvula*, *Nebela tubulosa*, *Nebela* sp.). In addition, the final analysed Corythion-Trinema type was a combination of three sub-types. We used Detrended Correspondence Analysis (DCA) to study testate amoeba community variation along the successional gradient. The gradient length of 3.8 standard deviations (SDs) indicated the use of both linear and unimodal models were reasonable (Lepš and Šmilauer 2003). We applied Canonical Correspondence Analysis (CCA) to relate the variation in testate amoeba assemblages to variations in environmental data. Although there have

been some criticisms of the use of the χ^2 distance in CCA in community ecology studies (e.g., Legendre and Gallagher 2001), it has been widely applied in previous studies of peatland testate amoebae (e.g., Amesbury et al. 2013; Lamarre et al. 2013; Zhang et al. 2017) as it provides robust results in the presence of clear environmental controls. We used different physico-chemical variables and plant functional types (PFT) as environmental variables. We ran a forward selection of the explanatory variables and removed non-significant variables and also redundant factors that have collinearity with other selected variables. A Monte Carlo permutation test was used to determine the statistical significance of the species-environment relationships. A series of partial CCAs was applied to investigate the relative contributions of the environmental variables. We calculated the Shannon diversity index (α diversity) for each sample and applied one-way ANOVA (with Tukey's HSD test) analysis to test the differences in testate amoeba assemblage diversity between the study sites. We also calculated the Whittaker diversity index (β diversity) for each site.

Results

Altogether 58 testate amoeba taxa were encountered. The most abundant taxa were *Centropyxis cassis* type, *Corythion-Trinema* type, *Assulina muscorum* and *Euglypha compressa*. Some taxa were abundant (*ca*. 50%) in only one or two samples, such as *Hyalosphenia papilio*, *Euglypha rotunda* type and *Valkanovia elegans* (Fig. A.1). Some rare taxa were only found in one sample, for example *Difflugia lanceolata*. Test concentration varied from 55,887 tests cm^{-3} in a young fen sample to < 50 tests cm^{-3} in a minerogenic sample without an organic soil layer.

Successional change of testate amoeba assemblages

In accordance to our hypothesis the main variation in the testate amoeba assemblage was related to the mire development gradient. The first two axes account for 15.24 % and 5.98% of the total variance respectively. The first DCA axis (eigenvalue = 0.578) spread the seven different SJ study sites from the oldest SJ6 (bog) and SJ5 (fen-bog transition) to the youngest SJ0 (pre-meadow stage) (Fig. 2a). The mire succession gradient is characterised by a change in several environmental properties, most importantly vegetation composition, thickness of organic layer, mineral nutrient level and pH. Oligotrophic fen SJ4 appeared to be transitional, overlapping the younger and older phases. The first axis separated taxa typical to ombrotrophic conditions such as *Hyalosphenia papilio* from the minerotrophic taxa such as *Sphenoderia fissirostris* and *S.lenta* (Fig. 2b). Sites SJ6, SJ5 and SJ3 (mesotrophic fen) are also scattered along the second axis (eigenvalue = 0.251) relating to variation in their within-site hydrological conditions. The wet – dry gradient shown in the second axis spread bog taxa between the wet cluster to the lower and dry cluster to the upper end of the gradient. The scattering of taxa was highest in the ombrotrophic end of the succession gradient (axis 1 in Fig. 2) and decreased towards sites with no microtopographical variations at the younger end.

CCA axes 1 (eigenvalue = 0.363) and 2 (eigenvalue = 0.179) explained 22.74% of the variance in the testate amoeba data ($p < 0.01$; Fig. 3). Seven variables were included in the analysis after the forward selection. Other variables like C and N content, other water table related variables (average, minimum, median and range), and other plant functional types (grasses, minerotrophic forbs, minerotrophic sedges, ombrotrophic forbs, minerotrophic shrubs, brown mosses, hollow Sphagna and feather mosses) were removed. A series of partial CCAs showed that variables related to peatland development had stronger explanatory power than variables related to vegetation and water level: peat thickness explained 12.7% of the variance in

231 the data (p = 0.002). Hummock Sphagna and lawn Sphagna explained 6.2% (p = 0.002) and 5.2%
232 (p = 0.002), respectively. WT maximum (3.5%, p = 0.002), ombrotrophic shrubs (3.2%, p =
233 0.004), pH (3.0%, p = 0.002) and ombrotropic sedges (2.7%, p = 0.006) explained less. Similarly
234 to the DCA, the main variation along the first CCA axis was related to the successional gradient,
235 where the highest pH characteristic to the young sites decreased with increasing peat layer
236 thickness and an increasing abundance of ombrotrophic sedges and lawn Sphagna (Fig. 3). Bog-
237 thriving testate amoeba taxa, such as *Nebela* spp. were plotted to the right while young stage
238 taxa, such as *Centropyxis cassis* that prefer higher pH and low organic content were plotted on
239 the left. The young stage communities were characterised by pioneering *Centropyxis spp*.,
240 accompanied by *Sphenoderia lenta*. Fen and meadow phase testate amoeba communities were
241 dominated by, for instance, *Difflugia* spp. Taxa such as *Assulina*/*Valkanovia* spp., *Nebela* spp.
242 and *Arcella catinus* were more common in the more mature mire phases. PFTs and WT variable
243 were correlated to the second CCA axis, which was indicated by the PFT composition: hummock
244 Sphagna and ombrotrophic shrubs decreased from the top to bottom along the second CCA axis
245 and lawn PFTs were found in the middle of the axis; water-table levels increased (dry to wet)
246 from the top to the bottom of the axis (Fig. 3).
247
248 Ecological constraints
249 *Testate amoeba taxa relationships with vegetation*
250 Most of the testate amoeba taxa were at least occasionally present in brown moss or vascular
251 plant-dominated samples. There appeared to be taxa that clearly preferred *Sphagnum* habitats
252 characteristic of well-developed mire sites. For instance *Heleopera rosea*, *Hyalosphenia elegans*,
253 *Nebela tincta* and *Planocarina carinata* only occurred in samples that contained at least small
254 amounts of Sphagna.

A relatively large number of taxa (12) were detected from samples that contained a considerable amount of mineral soil, these were: *Centropyxis aculeata* type, *Centropyxis cassis* type, *Centropyxis platystoma* type, *Corythion-Trinema* type, *Cyclopyxis arcelloides* type, *Difflugia pulex*, *Difflugia rubescens*, *Euglypha compressa*, *Euglypha rotunda* type, *Euglypha tuberculata* type, *Nebela tincta* and *Sphenoderia lenta*. Many of these, such as *Centropyxis spp*., *Cyclopyxis arcelloides*, *Corythion-Trinema* type, *Euglypha* spp. and *Sphenoderia lenta*, were also pioneering taxa, which colonized SJ0, the most recently emerged seashore pre-meadow habitat.

Testate amoeba taxa relationship with water-table depth

Sample plot water-table depth (WTD) varied between -13.5 and 54 cm (negative values indicate samples taken below surface water; Table 1 and Fig. A.1b). Typically, WTD increased from the seashore towards more developed mires in the inland. However, because of microtopographical variation, WTD also varied considerably within the sites, especially in the older ones. Many of the taxa seemed to be relatively tolerant in terms of WTD (often larger than 20 cm, Fig. 4 and Fig. A.1). The following taxa were abundant over a very wide WTD range (> 50 cm), especially for young sites (Fig. 4): *Arcella catinus* type, *Assulina muscorum*, *Cyclopyxis arcelloides*, *Corythion-Trinema* type, *Euglypha compressa*, *E.strigosa, E. tuberculata* type, *Nebela militaris* and *Nebela tincta*. In contrast to those WT generalists, most taxa had a much narrower WTD range over which they were abundant. Many of the taxa that seemed to have the narrowest WTD range were rare taxa such as *Pseudodifflugia spp*. Some taxa such as *Centropyxis cassis* type and *Hyalosphenia elegans* showed a pattern where the largest abundances occurred within a narrow WTD range but occasional individuals were also detected beyond this range.

278

279 *Testate amoeba taxa relationship with pH*

280 The pH of the sample plots varied between 3.92 and 6.72. Acidity increased along the succession
281 sequence from the seashore towards the bog stage (declined pH in Table 1 and Fig. A.1).
282 Considering the pH scale is logarithmic, most of the taxa had quite large pH ranges, wider than
283 1.5 pH units, and many taxa, such as *Euglypha* spp., had a range larger than 2 pH units (Fig. 4
284 and Fig. A.1c). However, some taxa clearly seemed to thrive in a narrow pH range, such as
285 *Difflugia pristis* type (~ 4.9-5.1), *N. militaris* (~ 4.5) and *Planocarina carinata* (~ 4.6-4.75).
286 Some taxa, for example, *A. seminulum*, *Valkanovia elegans* and *H. papilio* were more commonly
287 found in lower pH environments, while other taxa like *C. cassis* type were found more in high
288 pH conditions (Fig. A.1c).

289 When taxon distribution data were investigated in combination with WTD and pH data
290 (Fig. 4), it appeared that many taxa such as *Cyclopyxis arcelloides* type, *Assulina muscorum*, *A.*
291 *seminulum*, *Arcella catinus* type, most of the *Euglypha* taxa and *Valkanovia elegans* showed an
292 occurrence pattern where in more acidic environments they were detected from much drier
293 habitats than in the less acidic environment. This may also be influenced by the fact that there
294 were relatively few high pH locations with deep WTD (dry) (Fig. 4).

295

296 *Taxon diversity*

297 In SJ1-6 Shannon diversity index values fell between 1.5 and 2.5 while at SJ0 they were between
298 0.1 and 1.5 (Fig. 5). There was an increasing trend in diversity with successional stage up to the
299 fen stage (SJ4), after which there was a decline towards the ombrotrophic end stage (SJ6). The
300 Shannon diversity index value between individual plots ranged from 0.6 in SJ3-4 to 2.65 in SJ4-

11 and when the median plot values were combined to represent the seven SJ study sites, the diversity index varied between 1.2 (SJ0) and 2.2 (SJ5 and SJ4). One-way ANOVA analysis suggests that the variation of taxon diversity differed significantly between the study sites (Fig. 5) ($p = 0.003$). Tukey's HSD test implies that SJ0 was significantly different from site SJ2 to SJ6 ($p < 0.05$), while other paired combinations yielded no significant differences. There seemed to be more variation between the sample plots in the younger end of the space-for-time sequence than in more mature mire sites. The transitional mire phases, i.e. oligotrophic fen stage (SJ4) and fen-bog transition stage (SJ5), seemed to support slightly higher taxon diversity than the other mire phases. The high microtopographical variation between wet and dry habitat conditions, which characterized sites SJ6 and SJ5, did not seem to have similar effect on taxon diversity than the mixture of bog and fen habitats within the same site. The Whittaker diversity index results (Fig. 5) showed a continuous decreasing trend (i.e. an increasing number of total taxa) from SJ0 to SJ5, while the mature ombrotrophic site SJ6 had a higher value than SJ5.

Discussion

Test concentrations of young fen samples were comparable to the *ca*. 40,000 tests cm^{-3} found in surface peat in an ombrotrophic bog (Elliott et al. 2012), and within the large variations of test concentration found by Roe et al. (2017). However, the concentration of < 50 tests cm^{-3} of minerogenic samples without an organic soil layer were much lower than typically found in organic soils (e.g., Elliott et al., 2012; Roe et al., 2017).

In Siikajoki, sites SJ1-6 are relatively diverse (Shannon diversity index falls between 1.5 and 2.5) while SJ0 can be considered stressed with the index between 0.1 and 1.5 (Magurran 1988). The general species richness patterns resembled what has been reported recently for

several mire sites in the UK, with highest diversity in poor fen and transitional sites (Turner et al. 2013). Also, species richness was high in fen/young meadow sites and this corresponds with earlier observations (Opravilová and Hájek 2006; Lamentowicz et al. 2010; Lamentowicz et al. 2011), which showed that testate amoeba species richness did not correlate with nutrient or pH gradients. This suggests that physical vegetation composition or habitat structure properties, rather than chemical parameters define species richness. This is also supported by a recent study (Lizoňová and Horsák 2017), which found that brown moss habitats support larger species richness than *Sphagnum* habitats. In addition, moss types are suggested to drive the niche-size-structure of testate amoeba (the distribution of large and small taxa) across poor-rich fen gradient (Jassey et al. 2014), thus influencing diversity. Some species, especially *Difflugia* spp., require habitats where, for instance, diatoms and mineral particles are abundantly available for test construction (Lamentowicz et al. 2011).

Contrasting hydrological preferences of testate amoeba in Siikajoki

Even though ecological knowledge of testate amoebae has increased over recent years, the Siikajoki data set yielded valuable new ecological information that sometimes contrasts with prevailing perceptions. For example, *Difflugia pulex* has mainly been reported from lawn or dry habitats (Charman et al. 2007; Swindles et al. 2009; Lamarre et al. 2013), but in Siikajoki the taxon was present in wet habitats only and also in inundated plots (Fig. 4 and A.1b). Similarly, Lamentowicz et al. (2008) also observed that *Difflugia pulex* tended to occupy the wet end of the WTD gradient. In a recent study, *D. pulex* was specifically found in sedge-dominated minerotrophic habitats (Payne 2011). Our DCA ordination plotted the taxon clearly to the early end of the succession gradient (right in first DCA axis), suggesting a preference for

minerotrophic conditions and high pH (Fig. 2). We acknowledge that some of these differences may be due to taxon complexes with genuine ecological niche differences existing within taxonomic groupings, further microscopic and genetic work to better define the taxonomy in the future may help to elucidate that.

The Siikajoki data confirmed earlier observations (Mitchell and Gilbert 2004; Opravilová and Hájek 2006; Lamentowicz et al. 2008) that *Arcella catinus* type has a broad ecological niche (Fig. 4) in contrast to older studies that have assumed it is exclusively a wet taxon (Paulson 1952-1953; Tolonen et al. 1992). However, the broader ecological niche may also due to the combination of taxa by some studies. Similarly, *Assulina muscorum*, which has been classified as a dry taxon (Tolonen et al. 1992), had a wide ecological prevalence in Siikajoki. The Siikajoki data show that *A. muscorum* occupies wetter habitats as pH increases, such that in poor fens it has a larger WTD niche than it has in bogs (Fig. 4). In Siikajoki, both *A. catinus* type and *A. muscorum* were also among those that had widest WTD ranges, > 50 cm (Fig. 4). Interestingly, comparison between Siikajoki and Poland (Lamentowicz et al. 2008) shows that those taxa that show the widest WTD ranges in Siikajoki did not have particularly wide ranges in the Polish data set; in fact, the pattern was sometimes quite contrasting. For instance, in Poland *Euglypha* taxa had small ranges in terms of WTD while in Siikajoki these taxa were among those with a maximum range, up to 65 cm (Fig. 4 and A.1b). However, the largest WTD range variations occurred in the mature end of the space-for-time sequence. Furthermore in Lamentowicz et al. (2008), *Heleopera rosea* had a wide WTD range, while in Siikajoki the range was only *ca*. 10 cm (Fig. 4 and A.1b).

Sphenoderia fissirostris is a relatively rare taxon and, for instance, in Charman et al. (2000) there is no taxon-specific ecological information available. *S. fissirostris* was also a rare taxon in

370 the Siikajoki samples. It had relatively narrow pH range < 1 pH units but in contrast a relatively
371 large WTD range, *ca*. 30 cm (Fig. 4). It was mainly detected in fen sites SJ3 and SJ4. In the
372 ordinations it was positioned at the minerotrophic high pH end of the succession gradient. This
373 roughly corresponds with few earlier observations where the taxon has been found in poor acid
374 fen habitats (Opravilová and Hájek 2006) and minerotrophic pools (Mitchell et al. 2000).

375 The Siikajoki data also revealed divergent habitat constraint patterns for several testate
376 amoeba species whose ecology has been reported to be well known (cf. Charman et al. 2000 and
377 references therein). For instance, *Hyalosphenia papilio* which has often been classified as a wet
378 species in ombrotrophic mires (Swindles et al. 2009), was often found in hummock plots with
379 WTD 30 cm or deeper. This agrees with observations of Opravilová and Hájek (2006) whose
380 data show that *Hyalosphenia papilio* can have a relatively large ecological range. In addition,
381 *Nebela militaris* and especially *Corythion-Trinem*a spp. that have been classified as dry taxa
382 (Tolonen et al. 1992; Warner and Charman 1994; Opravilová and Hájek 2006; Swindles et al.
383 2009; Lamentowicz et al. 2011) were abundantly present also in some relatively wet habitats in
384 Siikajoki (Fig. 4 and A.1b). In the case of *Nebela militaris*, pH seemed to be more important than
385 WTD; the highest abundances were detected in habitats with pH ~4.5, whereas the WTD range
386 was > 40 cm, and it was plotted within the medium wet bog species cluster (Fig. 2b). *Corythion-*
387 *Trinema* spp. occurred in drier habitats in more acidic conditions (Fig. 4 and A.1c), but in higher
388 pH conditions, it was present throughout wetter locations (Fig. 4 and A.1c). In the ordination
389 *Corythion-Trinema* spp. was associated with various types of sites from young meadows,
390 including plots dominated by vascular plants, to fen stage mires. In the bog sites SJ6 and SJ5
391 these species occurred in both dry and wet habitats. This agrees with the observation by Paulson
392 (1952-1953) whose data showed presence of *Corythion-Trinema* spp. in various different bog

habitats.

Challenges of using testate amoeba in minerotrophic environments

Many previous studies (from ombrotrophic bogs) have shown a primary relationship to hydrological variables with secondary variables (most often pH) rarely considered to be significantly explanatory (e.g. Amesbury et al. 2013; van Bellen et al., 2014). Some other ecological gradient studies have shown that the distribution of testate amoebae is related to more than one single environmental variable even though very often hydrology has been reported to be the strongest factor (Tolonen et al. 1994; Payne 2011). Most of the environmental variables are however interrelated. For example, ecohydrology (quantity and quality of water) influences WTD, pH and chemistry, that in turn regulates vegetation composition that provides a habitat for testate amoebae (cf. Mitchell et al. 2000, 2001; Lamentowicz et al. 2013b). The testate amoeba community is in turn controlled by the quality of litter regulated by vegetation composition (Straková et al. 2011). This is further complicated by the fact that most testate amoeba taxa seem to have relatively wide ecological ranges in respect of various environmental variables. For instance, pH ranges are typically 2-3 pH units (Paulson 1952-1953; Tolonen et al. 1992; Lamentowicz et al. 2008). Many taxa have been reported to thrive through a range of trophic levels, from brown moss-dominated rich fens to ombrotrophic bogs (Tolonen et al. 1992). There is increasing evidence that some testate amoeba taxa can also have a large WTD range (Tolonen et al. 1992; Bobrov et al. 1999; Lamentowicz and Mitchell 2005; Opravilová and Hájek 2006). Hummocks are normally occupied by taxa that have a narrow tolerance towards annual water-table fluctuations, while taxa with wider amplitude live in hollows (cf. Booth 2008). A previous study showed that for instance *Archerella flavum*, is relatively sensitive to seasonal-scale habitat

disturbance, while species such as *Difflugia pulex* and *Arcella discoides* can tolerate highly variable habitat conditions (Sullivan and Booth 2011).

The Siikajoki data showed that hydrological relationships can change (e.g., Kurina and Li 2018) or break down completely when nutrient and related factors influence the assemblages. In our results it was obvious that the successional gradient was stronger than the water table gradient alone when both were concurrently present. Consequently, it seems that large temporal ecological regime shifts critically hamper using a traditional transfer function approach to reconstruct any single environmental parameter (Juggins, 2013). Testate amoeba distribution in different mire types is determined by different environmental variables, which resembles previous studies (Opravilová and Hájek 2006; Lamentowicz et al. 2011; Payne 2011). The important succession elements, peat thickness and pH, form the first axis of variation in CCA plot (Fig. 3). At the more mature and more acidic end of the succession gradient the species are separated along the second axis of CCA (Fig. 3), which reflects variation in hydrological conditions, but at the younger end of the succession gradient and in higher pH conditions, this separation is not so evident, with 'ombrotrophic dry' taxa sometimes appearing in wetter locations under higher pH conditions. The Siikajoki data suggest that in minerotrophic environments, many taxa have relatively low ecological value in terms of palaeoecological applications, especially for hydrology (see also Payne 2011). To assess this issue more thoroughly, fen communities should be examined more extensively in order to identify those species that may have the highest indicative value in terms of hydrology or other specific ecological variables. It is especially important to include dry nutrient rich sites in new studies, as the Siikajoki data do not include such locations.

Conclusions

A surface peat sample data set from a mid-boreal Finnish mire succession transect provided new information and insight on the ecological constraints of testate amoeba assemblages and their relationships with mire succession and habitat change. Taxa changes along a successional gradient showed that a small number of early colonists occupied the earliest phases of wetland formation and as habitat diversity increased, so did testate amoeba diversity. Diversity declined in the mature ombrotrophic phase as the early colonists disappeared completely, and taxa were differentiated by local hydrological conditions. The data also indicated that ecological requirements are not yet fully understood for minerotrophic systems. Within ombrotrophic systems, the range of nutrient and pH variability is very narrow and therefore the single driving variable for testate amoebae is hydrology. However, when a full range of sites along a trophic gradient is included, the hydrological control becomes much less important and species niches are more closely defined by pH and nutrient availability. Moreover, excluding some promising exceptions, many taxa seemed to have relatively wide hydrological niches when a large pH range was considered. Further studies, especially including sampling in dry locations in fens, might help us to better assess the potential value of testate amoeba assemblages to reconstruct various environmental conditions in minerotrophic environments.

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462

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Table 1. Site and sampling information. Negative water-table depth (WTD) values indicate samples taken from below surface water. WTD values are median values of weekly measurements through the growing season in 2007. Figures in brackets show the number of samples excluded from the data analysis due to low testate amoeba concentration.

	SJ0	**SJ1**	**SJ2**	**SJ3**	**SJ4**	**SJ5**	**SJ6**
Site type	Pre-meadow	Wet meadow	Wet meadow	Mesotrophic fen	Oligotrophic fen	Fen-bog	Oldest Bog
Peat depth (cm)	0	0-10	10	30-50	70-100	180-190	180-240
Site age (yr)	70	170-200	200-570	670-700	1070-1300	2410-2520	~3000
C:N ratio	9.4-13.5	11.7-19.5	14.5-23.4	17.5-38.7	15.9-47.0	25.0-52.5	42.3-65.8
WTD (cm)	3.5	-2 – 3.5	0.5 – 4.5	-13.5 – 30.0	-7.0 – 22.0	-0.5 – 27.8	8.0 – 54.0
pH	6.08 – 6.72	5.15 – 5.35	5.05 – 5.29	3.92 – 4.92	5.04 – 5.24	4.36 – 4.75	3.97 – 4.47
No. of samples	6 (1)	13 (7)	6	10	9	8	9

669 **Figure captions:**

670 **Fig. 1.** Location of the Siikajoki mire transect study sites in west coast of Finland. Seven
671 sampling sites (0-6) are along a 10-km transect from A (youngest newly-emerged shoreline
672 meadow) to B (mature bog). Base map was downloaded in November 2017 from the National
673 Land Survey of Finland Topographic Database under a CC 4.0 open source licence.

674

675 **Fig. 2.** a) DCA ordination of the study sites and plots along the mire succession gradient. b) DCA
676 ordination of the testate amoeba taxon distribution along the mire succession gradient. Full taxon
677 names see Table A.1.

678

679 **Fig. 3.** CCA ordination with physical parameters and plant functional types (after the forward
680 selection) used as explanatory environmental variables. Full environmental variable names see
681 Table A.1.

682

683 **Fig. 4.** Distribution of testate amoebae taxa along median water-table depth (WTD) and pH
684 gradients. The size of the circles in individual plots is scaled by taxon abundance with larger
685 circles representing higher % abundances and vice versa. Selected taxa are presented. Total
686 sample distribution along the WTD and pH gradients is presented in the top-left panel.

687

688 **Fig. 5.** Shannon diversity index box plot and Whittaker diversity index of testate amoebae taxon
689 diversity variations along the succession gradient. Median, minimum and maximum values are
690 indicated for the box plots.

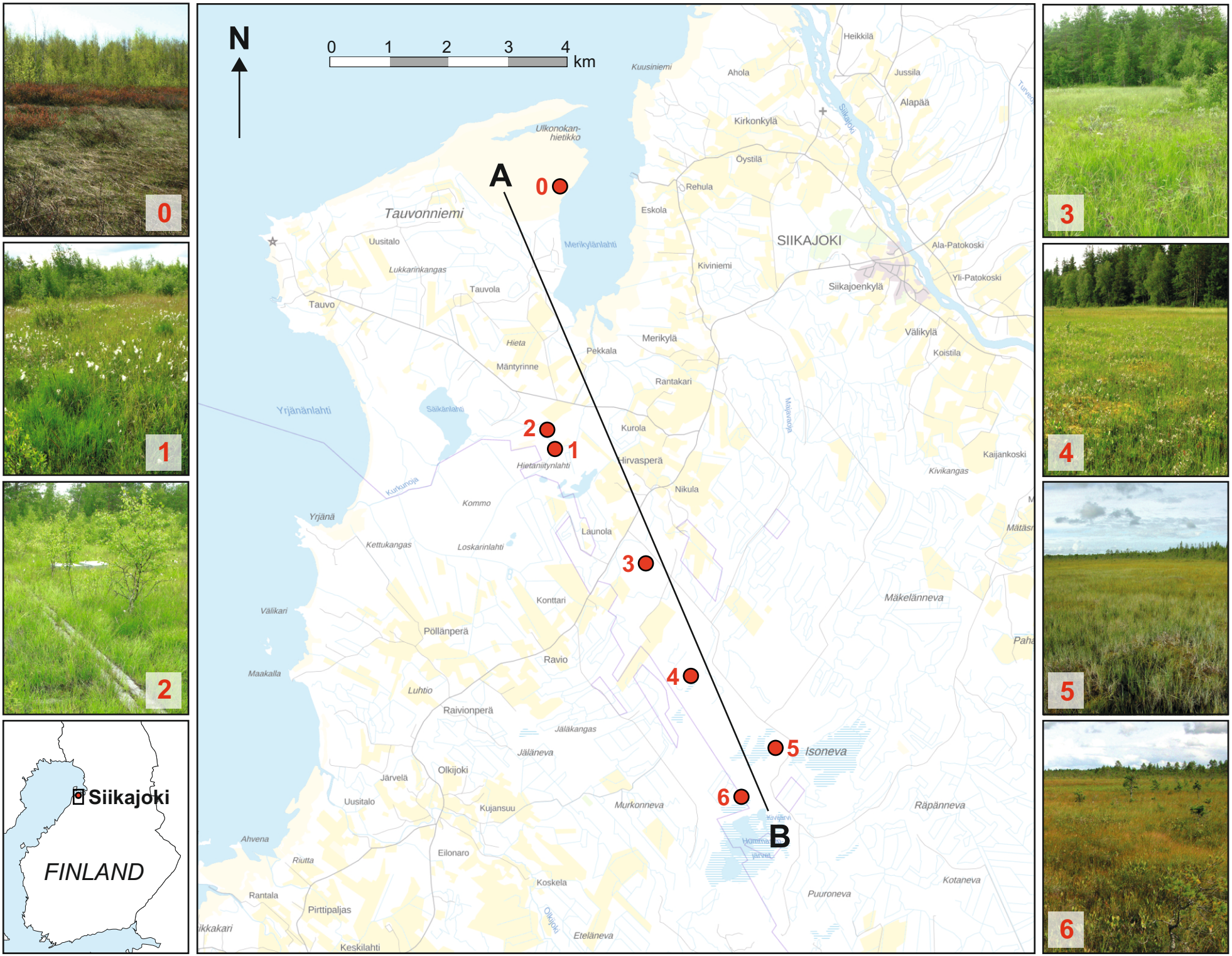
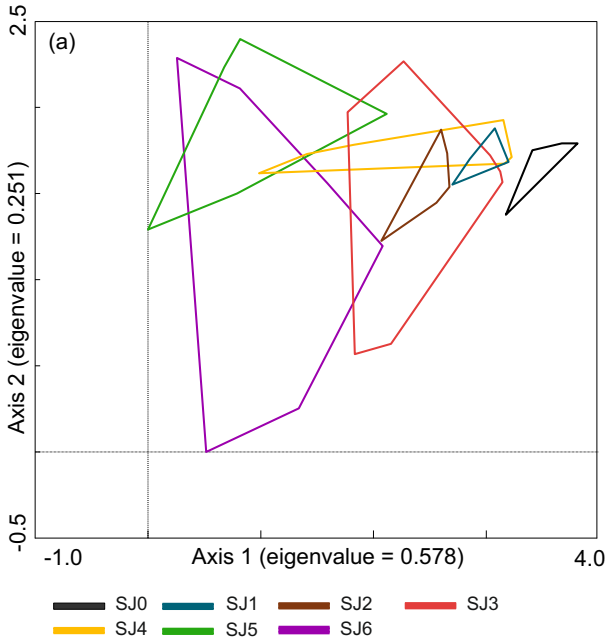

N
0 1 2 3 4 km
A
B
0
1
2
3
4
5
6
Tauvonniemi
Uusitalo
Lukkarinkangas
Tauvola
Tauvo
Merikylänlahti
Ulkonokan-
hietikko
Kuusiniemi
Heikkilä
Ahola
Jussila
Alapää
Kirkonkylä
Siikajoki
Öystilä
Rehula
Eskola
SIIKAJOKI
Ala-Patokoski
Kiviniemi
Yli-Patokoski
Siikajoenkylä
Välikylä
Koistila
Merikylä
Pekkala
Hieta
Mäntyrinne
Rantakari
Yrjänänlahti
Säikänlahti
Kurola
Hirvasperä
Hietäniitynlahti
Kaijankoski
Kivikangas
Kurkunoja
Nikula
Kommo
Yrjänä
Launola
Kettukangas
Loskarinlahti
Majavaoja
Mäkelänneva
Konttari
Välikari
Pöllänperä
Ravio
Maakalla
Luhtio
Raivionperä
Jäläkangas
Jäläneva
Isoneva
Olkijoki
Järvelä
Uusitalo
Kujansuu
Murkonneva
Räpänneva
Ahvena
Riutta
Eilonaro
Koskela
Kotaneva
Rantala
Pirttipaljas
Puuroneva
Olkijoki
Eteläneva
Keskilahti
Siikajoki
FINLAND

Fig. 1


(a)
2.5
-0.5
Axis 2 (eigenvalue = 0.251)
-1.0
Axis 1 (eigenvalue = 0.578)
4.0
SJ0
SJ1
SJ2
SJ3
SJ4
SJ5
SJ6

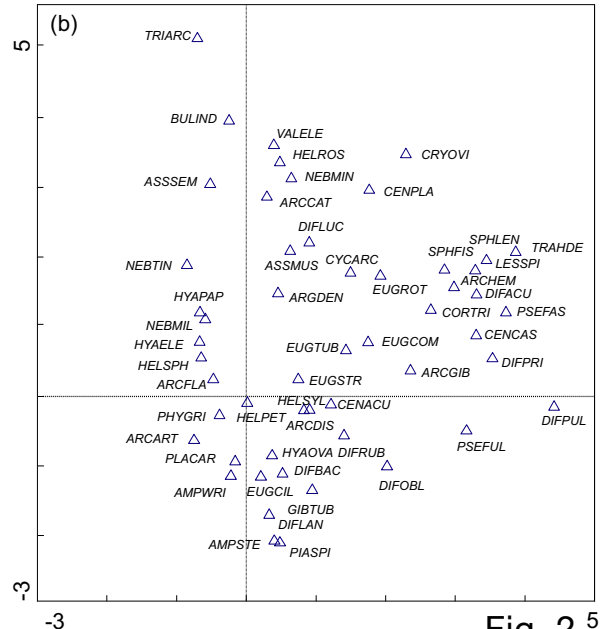

(b)
5
-3
-3
5
TRIARC
BULIND
ASSSEM
NEBTIN
HYAPAP
NEBMIL
HYAELE
HELSPH
ARCFLA
PHYGRI
ARCART
PLACAR
AMPWRI
AMPSTE
VALELE
HELROS
NEBMIN
ARCCAT
DIFLUC
ASSMUS
CYCARC
ARGDEN
EUGTUB
EUGSTR
HELSYL
CENACU
HELPET
ARCDIS
HYAOVA
DIFRUB
DIFBAC
EUGCIL
GIBTUB
DIFLAN
PIASPI
CRYOVI
CENPLA
EUGROT
EUGCOM
ARCGIB
PSEFUL
DIFOBL
SPHLEN
TRAHDE
SPHFIS
LESSPI
ARCHEM
DIFACU
CORTRI
PSEFAS
CENCAS
DIFPRI
DIFPUL

Fig. 2

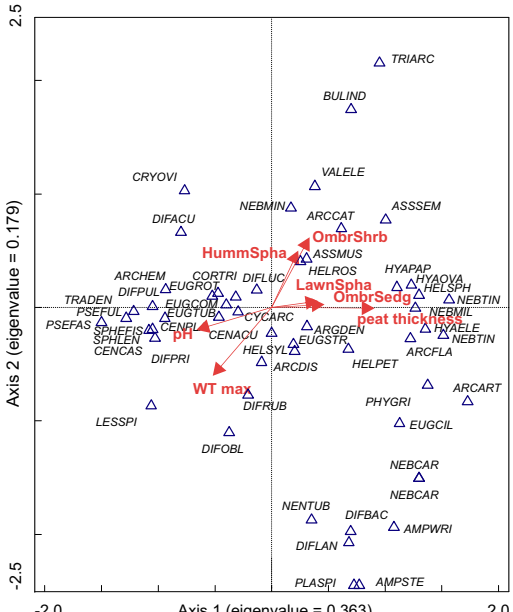

2.5
-2.5
Axis 2 (eigenvalue = 0.179)
-2.0
Axis 1 (eigenvalue = 0.363)
2.0
TRIARC
BULIND
VALELE
CRYOVI
NEBMIN
ARCCAT
ASSSEM
DIFACU
OmbrShrb
HummSpha
ASSMUS
HELROS
HYAPAP
ARCHEM
CORTRI
DIFLUC
HYAOVA
LawnSpha
DIFPUL
EUGROT
HELSPH
OmbrSedg
TRADEN
NEBTIN
EUGCOM
PSEFUL
NEBMIL
EUGTUB
peat thickness
CYCARC
PSEFAS
CENRUB
ARGDEN
HYAELE
SPHFIS
pH
CENACU
EUGSTR
NEBTIN
SPHLEN
CENCAS
HELSYL
ARCFLA
DIFPRI
ARCDIS
HELPET
WT max
ARCART
DIFRUB
PHYGRI
LESSPI
EUGCIL
DIFOBL
NEBCAR
NEBCAR
NENTUB
DIFBAC
AMPWRI
DIFLAN
PLASPI
AMPSTE

Fig. 3

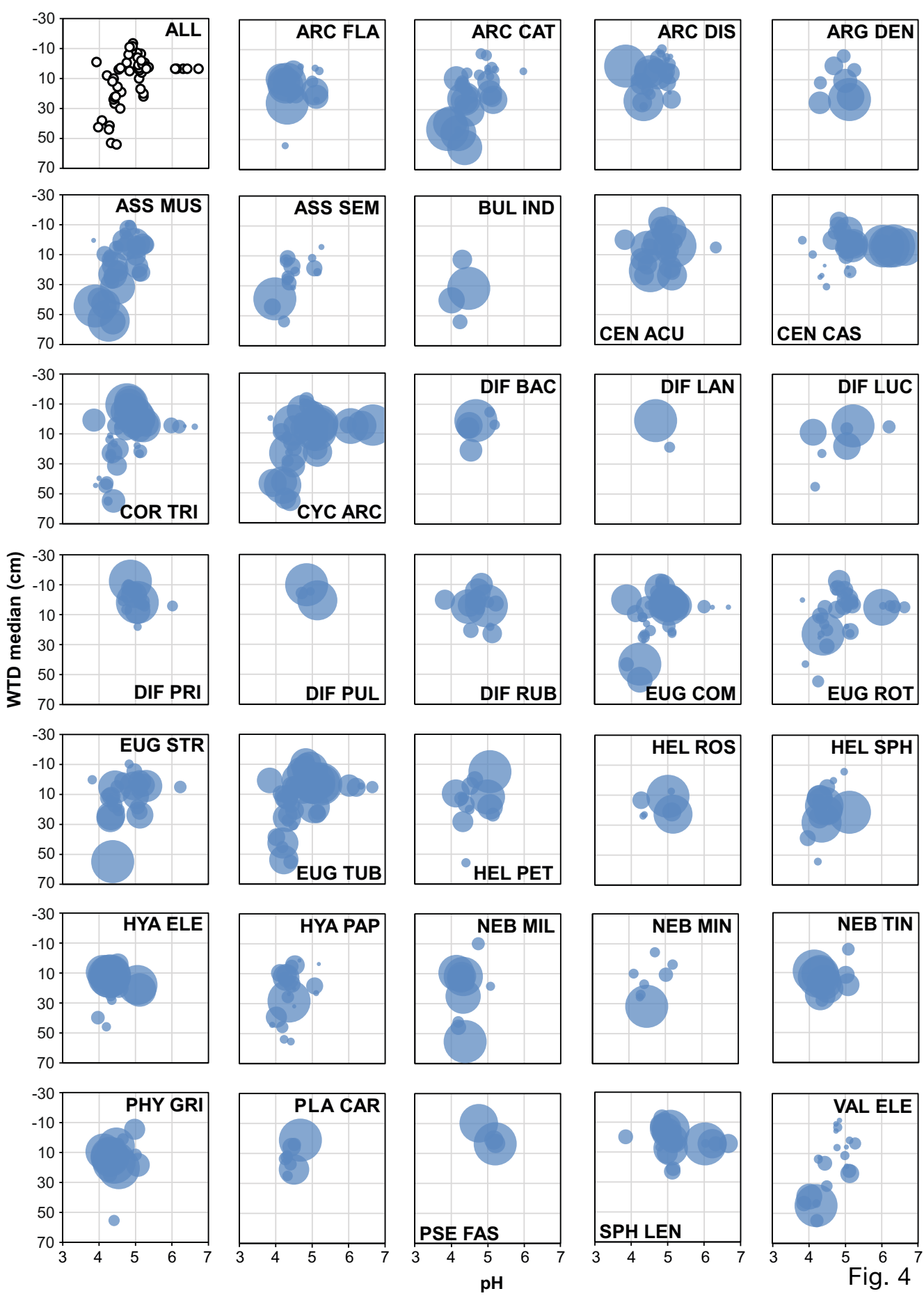

ALL
ARC FLA
ARC CAT
ARC DIS
ARG DEN
ASS MUS
ASS SEM
BUL IND
CEN ACU
CEN CAS
COR TRI
CYC ARC
DIF BAC
DIF LAN
DIF LUC
DIF PRI
DIF PUL
DIF RUB
EUG COM
EUG ROT
EUG STR
EUG TUB
HEL PET
HEL ROS
HEL SPH
HYA ELE
HYA PAP
NEB MIL
NEB MIN
NEB TIN
PHY GRI
PLA CAR
PSE FAS
SPH LEN
VAL ELE
WTD median (cm)
-30
-10
10
30
50
70
3
4
5
6
7
pH

Fig. 4

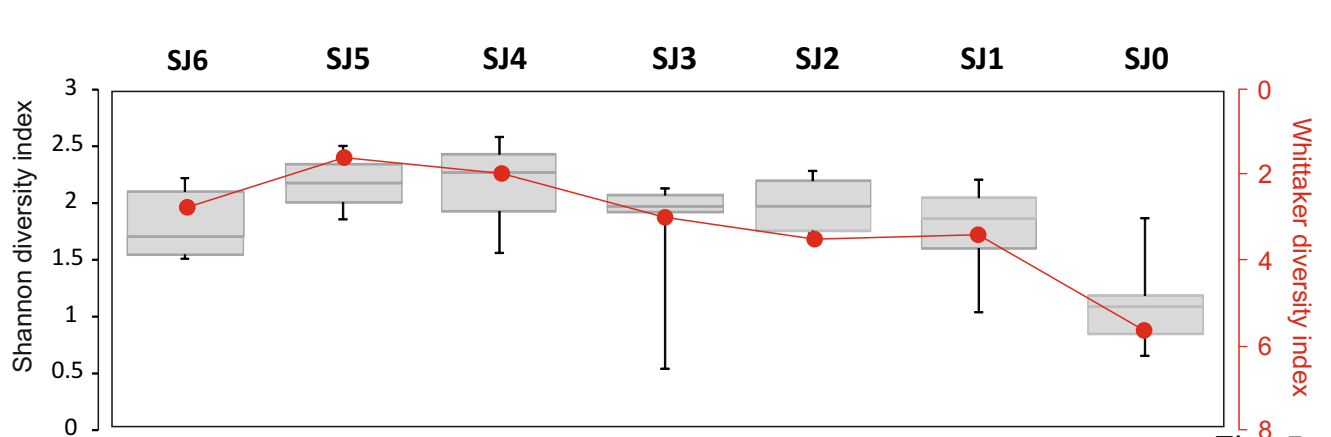

SJ6
SJ5
SJ4
SJ3
SJ2
SJ1
SJ0
Shannon diversity index
3
2.5
2
1.5
1
0.5
0
Whittaker diversity index
0
2
4
6
8

Fig. 5

Electronic supplement materials

Successional change of testate amoeba assemblages along a space-for-time sequence of peatland development

Zhang, H. [1, 2*], Väliranta, M.[1, 2], Amesbury, M. J. [3], Charman, D. J.[3], Laine, A.[4], Tuittila, E-S[5]

[1] ECRU, Ecosystems and Environment Research Programme, Faculty of Biological and Environmental Sciences, P.O. Box 65, FI-00014 University of Helsinki, Finland

[2] Helsinki Institute of Sustainability Science (HELSUS), Finland

[3] Geography, College of Life and Environmental Sciences, University of Exeter, UK

[4] Department of Biology, University of Oulu, Finland

[5] Department of Forest Sciences, University of Eastern Finland, Finland

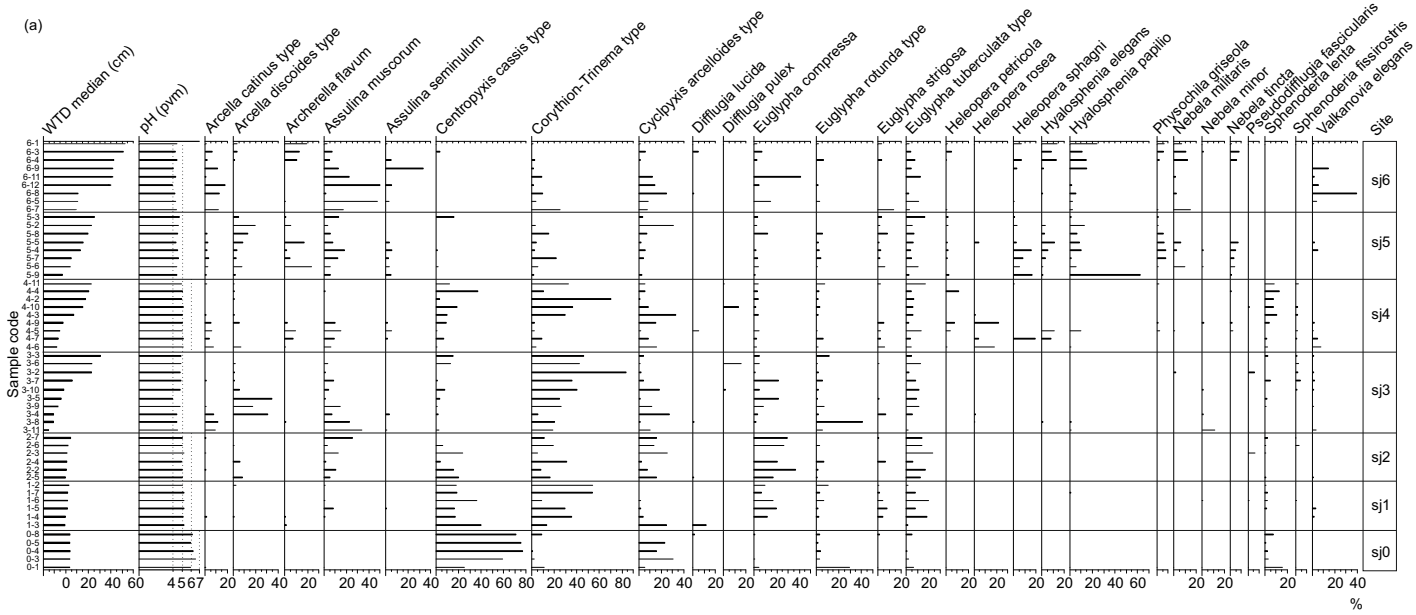
* Corresponding author: Email: hui.palaeo@gmail.com

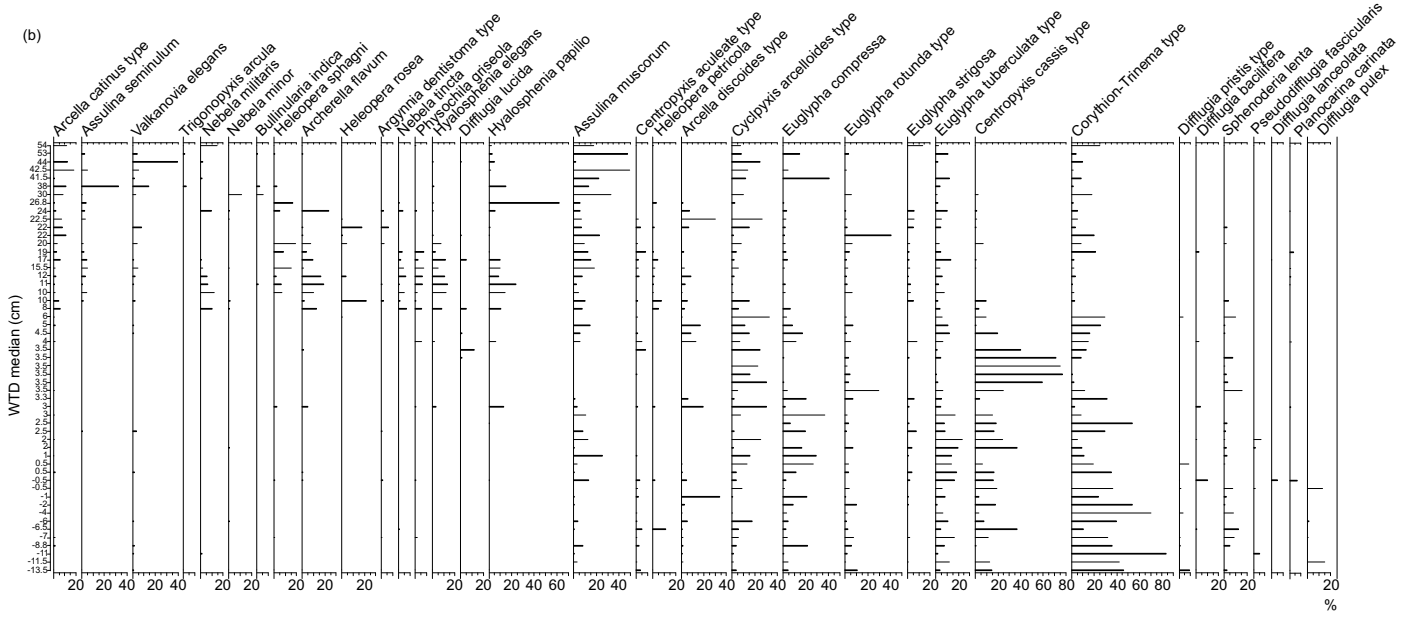
Table A.1. Full names of taxa and environmental variables used in DCA and CCA analysis.

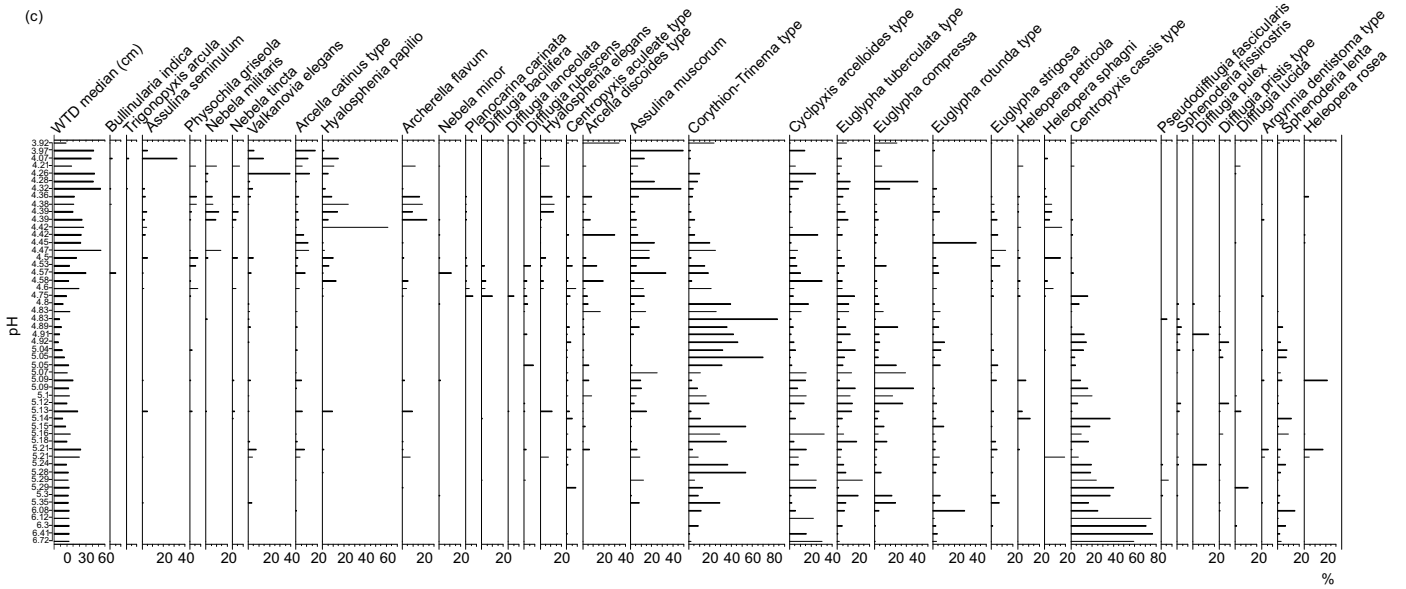
Code	Full name	Code	Full name
AMPSTE	*Amphitrema stenostoma*	EUGSTR	*Euglypha strigosa*
AMPWRI	*Amphitrema wrightianum*	EUGTUB	*Euglypha tuberculata* type
ARCCAT	*Arcella catinus* type	GIBTUB	*Gibbocarina tubulosa*
ARCDIS	*Arcella discoides* type	HELPET	*Heleopera petricola*
ARCGIB	*Arcella gibbosa*	HELROS	*Heleopera rosea*
ARCHEM	*Arcella hemisphaerica*	HELSYL	*Heleopera sylvatica*
ARCART	*Arcella artocrea*	HYAELE	*Hyalosphenia elegans*
ARCFLA	*Archerella flavum*	HYAOVA	*Hyalosphenia ovalis*
ARGDEN	*Argynnia dentistoma* type	HYAPAP	*Hyalosphenia papilio*
ASSMUS	*Assulina muscorum*	LESSPI	*Lesquereusia spiralis*
ASSSEM	*Assulina seminulum*	NEBMIL	*Nebela militaris*
BULIND	*Bullinularia indica*	NEBMIN	*Nebela minor*
CENACU	*Centropyxis aculeata* type	NEBTIN	*Nebela tincta*
CENCAS	*Centropyxis cassis* type	PHYGRI	*Physochila griseola*
CENPLA	*Centropyxis platystoma* type	PLACAR	*Planocarina carinata*
CORTRI	*Corythion-Trinema* type	PLASPI	*Plagiopyxis spinosa*
CRYOVI	*Cryptodifflugia oviformis*	PSEFAS	*Pseudodifflugia fascicularis*
CYCARC	*Cyclopyxis arcelloides* type	PSEFUL	*Pseudodifflugia fulva* type
DIFACU	*Difflugia acuminata*	SPHFIS	*Sphenoderia fissirostris*
DIFBAC	*Difflugia bacilifera*	SPHLEN	*Sphenoderia lenta*
DIFLAN	*Difflugia lanceolata*	TRADEN	*Tracheleuglypha dentata*
DIFLUC	*Difflugia lucida*	TRIARC	*Trigonopyxis arcula*
DIFOBL	*Difflugia oblonga*	VALELE	*Valkanovia elegans*
DIFPUL	*Difflugia pulex*	WT max	maximum WTD
DIFPRI	*Difflugia pristis* type	OmbrSedg	ombrotrophic sedges
DIFRUB	*Difflugia rubescens*	OmbrShrb	ombrotrophic shrubs
EUGCIL	*Euglypha ciliata*	LawnSpha	lawn Sphagna
EUGCOM	*Euglypha compressa*	HummSpha	hummock Sphagna
EUGROT	*Euglypha rotunda* type		

Figure caption:

Fig. A.1. Testate amoeba percentages (selected taxa). a) Site-specific median water-table depth (WTD) in descending order. Taxa are presented in alphabetical order. pH is also indicated for each sample; b) Taxa ordered according to their relationship to the median WTD; c) Taxa ordered according to their relationship to pH, with median WTD also shown.


(a)
WTD median (cm)
pH (pvm)
Arcella catinus type
Arcella discoides type
Archerella flavum
Assulina muscorum
Assulina seminulum
Centropyxis cassis type
Corythion-Trinema type
Cyclopyxis arcelloides type
Difflugia lucida
Difflugia pulex
Euglypha compressa
Euglypha rotunda type
Euglypha strigosa
Euglypha tuberculata type
Heleopera petricola
Heleopera rosea
Heleopera sphagni
Hyalosphenia elegans
Hyalosphenia papilio
Physochila griseola
Nebela militaris
Nebela minor
Nebela tincta
Pseudodifflugia fascicularis
Sphenoderia lenta
Sphenoderia fissirostris
Valkanovia elegans
Site
Sample code
sj6
sj5
sj4
sj3
sj2
sj1
sj0
%


(b)
Arcella catinus type
Assulina seminulum
Valkanovia elegans
Trigonopyxis arcula
Nebela militaris
Nebela minor
Bullinularia indica
Heleopera sphagni
Archerella flavum
Heleopera rosea
Argynnia dentistoma type
Nebela tincta
Physochila griseola
Hyalosphenia elegans
Difflugia lucida
Hyalosphenia papilio
Assulina muscorum
Centropyxis aculeate type
Heleopera petricola
Arcella discoides type
Cyclopyxis arcelloides type
Euglypha compressa
Euglypha rotunda type
Euglypha strigosa
Euglypha tuberculata type
Centropyxis cassis type
Corythion-Trinema type
Difflugia pristis type
Difflugia bacillifera
Sphenoderia lenta
Pseudodifflugia fascicularis
Difflugia lanceolata
Planocarina carinata
Difflugia pulex
WTD median (cm)
%


(c)
WTD median (cm)
Bullinularia indica
Trigonopyxis arcula
Assulina seminulum
Physochila griseola
Nebela militaris
Nebela tincta
Valkanovia elegans
Arcella catinus type
Hyalosphenia papilio
Archerella flavum
Nebela minor
Planocarina carinata
Difflugia bacillifera
Difflugia lanceolata
Difflugia tubescens
Hyalosphenia elegans
Centropyxis aculeate type
Arcella discoides type
Assulina muscorum
Corythion-Trinema type
Cyclopyxis arcelloides type
Euglypha tuberculata type
Euglypha compressa
Euglypha rotunda type
Euglypha strigosa
Heleopera petricola
Heleopera sphagni
Centropyxis cassis type
Pseudodifflugia fascicularis
Sphenoderia fissirostris
Difflugia pulex
Difflugia pristis type
Difflugia lucida
Argynnia dentistoma type
Sphenoderia lenta
Heleopera rosea
pH
%