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

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

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

**Study area**

The study area is located on the western coast of Finland in Siikajoki (SJ) commune (64°45’N, 24°42’E) (Fig. 1). The region represents the middle boreal ecoclimate zone. The mean annual temperature and precipitation are 2.6°C and 539 mm respectively and the length of the growing season is 150 days (observation period 1979–2009; Revonlahti, Siikajoki, 64°41’N, 25°05’E, 48
m a.s.l, Finnish Meteorological Institute). Primary paludification is an ongoing process in the area and postglacial land uplift has created a space-for-time sequence of peatlands.

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

The vegetation at site SJ0 was dominated by graminoids (e.g., Festuca rubra, Calamagrostis stricta, Carex glareosa and Juncus gerardii), with very few bryophytes present. Site SJ1 was a wet meadow with a patchy cover of brown mosses such as Warnstorfia spp. At SJ2, bryophytes were more extensive and Sphagnum mosses occurred as patches among brown mosses. Otherwise, both SJ1 and SJ2 were dominated by sedges and grasses such as Carex nigra and Agrostis canina while the forbs Comarum palustre and Lysimachia thyrsiflora were also common. Sites SJ3 and SJ4 were featured by mesotrophic and oligotrophic fen vegetation, respectively. At both sites, the vegetation consisted mainly of sedges (e.g., Carex chordorrhiza, Carex rostrata and Carex limosa), but dominant forbs at SJ3 and SJ4 were C. palustre and Menyanthes trifoliata respectively. Hummock formation with very dense Sphagnum carpets was evident at the edges of both SJ3 and SJ4. Site SJ5 was at the fen-bog transition stage with a mosaic of clearly ombrotrophic hummock surfaces with Rubus chamaemorus, Empetrum nigrum, Vaccinium oxycoccos and Sphagnum fuscum and wetter surfaces dominated by Scheuchzeria palustris, Carex livida, C. limosa and C. chordorrhiza. Sphagnum species accustomed to different water-table depths formed a continuous moss layer. Site SJ6 was an ombrotrophic bog, characterised by S. fuscum, Sphagnum angustifolium and dwarf shrubs such as E. nigrum and
Rhododendron tomentosum at the hummock surfaces and Sphagnum balticum and Eriophorum vaginatum in wetter depressions. The modern spatial mire succession series realistically mimics the historical (vertical) peatland development pattern where initial minerotrophic plant communities are, over the course of time, replaced by bog plant communities (Tuittila et al. 2013).

Materials and methods

Sampling

To investigate changes in testate amoeba assemblages related to successional stage, in August 2007 we sampled surface soil from each study site along a transect of 9-12 sample plots that covered the microtopographic variation characteristic of each site. In total, 61 samples were collected from seven study sites (Table 1). Microtopographical variation was minimal in the youngest sites SJ0 (pre-meadow on mineral soil), SJ1 and SJ2 (wet meadows), but in the fen sites SJ3 (mesotrophic fen) and SJ4 (oligotrophic fen) there was clear eco-hydrological variation from drier hummock to wet flark level (Fig. A.1). Microtopographical variation was most pronounced in SJ5 (fen-bog transition) where wet fen surfaces and drier ombrotrophic hummocks formed a mosaic. In the oldest site SJ6 (bog), sampling covered variation from Sphagnum fuscum hummocks to S. balticum lawns.

We collected two parallel sets of soil cores (one for microbiological and one for physicochemical analyses) with a box sampler (8 × 8 × 100 cm$^3$) or with a cylinder sampler (Ø 4.5 cm with 50 cm length) from each site along the transect. The uppermost ca. 5-10 cm was taken for testate amoeba analysis (Booth et al. 2010). In younger and/or more minerotrophic sites, 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 $\chi^2$ 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 ($\alpha$ 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 ($\beta$ 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
the data ($p = 0.002$). Hummock Sphagna and lawn Sphagna explained 6.2% ($p = 0.002$) and 5.2% ($p = 0.002$), respectively. WT maximum (3.5%, $p = 0.002$), ombrotrophic shrubs (3.2%, $p = 0.004$), pH (3.0%, $p = 0.002$) and ombrotropic sedges (2.7%, $p = 0.006$) explained less. Similarly to the DCA, the main variation along the first CCA axis was related to the successional gradient, where the highest pH characteristic to the young sites decreased with increasing peat layer thickness and an increasing abundance of ombrotrophic sedges and lawn Sphagna (Fig. 3). Bog-thriving testate amoeba taxa, such as *Nebela* spp. were plotted to the right while young stage taxa, such as *Centropyxis cassis* that prefer higher pH and low organic content were plotted on the left. The young stage communities were characterised by pioneering *Centropyxis* spp., accompanied by *Sphenoderia lenta*. Fen and meadow phase testate amoeba communities were dominated by, for instance, *Difflugia* spp. Taxa such as *Assulina/Valkanovia* spp., *Nebela* spp. and *Arcella catinus* were more common in the more mature mire phases. PFTs and WT variable were correlated to the second CCA axis, which was indicated by the PFT composition: hummock Sphagna and ombrotrophic shrubs decreased from the top to bottom along the second CCA axis and lawn PFTs were found in the middle of the axis; water-table levels increased (dry to wet) from the top to the bottom of the axis (Fig. 3).

**Ecological constraints**

*Testate amoeba taxa relationships with vegetation*

Most of the testate amoeba taxa were at least occasionally present in brown moss or vascular plant-dominated samples. There appeared to be taxa that clearly preferred *Sphagnum* habitats characteristic of well-developed mire sites. For instance *Heleopera rosea, Hyalosphenia elegans, Nebela tincta* and *Planocarina carinata* only occurred in samples that contained at least small 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.
Testate amoeba taxa relationship with pH

The pH of the sample plots varied between 3.92 and 6.72. Acidity increased along the succession sequence from the seashore towards the bog stage (declined pH in Table 1 and Fig. A.1). Considering the pH scale is logarithmic, most of the taxa had quite large pH ranges, wider than 1.5 pH units, and many taxa, such as *Euglypha* spp., had a range larger than 2 pH units (Fig. 4 and Fig. A.1c). However, some taxa clearly seemed to thrive in a narrow pH range, such as *Difflugia pristis* type (~ 4.9-5.1), *N. militaris* (~ 4.5) and *Planocarina carinata* (~ 4.6-4.75).

Some taxa, for example, *A. seminulum*, *Valkanovia elegans* and *H. papilio* were more commonly found in lower pH environments, while other taxa like *C. cassis* type were found more in high pH conditions (Fig. A.1c).

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

Taxon diversity

In SJ1-6 Shannon diversity index values fell between 1.5 and 2.5 while at SJ0 they were between 0.1 and 1.5 (Fig. 5). There was an increasing trend in diversity with successional stage up to the fen stage (SJ4), after which there was a decline towards the ombrotrophic end stage (SJ6). The 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
the Siikajoki samples. It had relatively narrow pH range < 1 pH units but in contrast a relatively large WTD range, ca. 30 cm (Fig. 4). It was mainly detected in fen sites SJ3 and SJ4. In the ordinations it was positioned at the minerotrophic high pH end of the succession gradient. This roughly corresponds with few earlier observations where the taxon has been found in poor acid fen habitats (Opravilová and Hájek 2006) and minerotrophic pools (Mitchell et al. 2000).

The Siikajoki data also revealed divergent habitat constraint patterns for several testate amoeba species whose ecology has been reported to be well known (cf. Charman et al. 2000 and references therein). For instance, *Hyalosphenia papilio* which has often been classified as a wet species in ombrotrophic mires (Swindles et al. 2009), was often found in hummock plots with WTD 30 cm or deeper. This agrees with observations of Opravilová and Hájek (2006) whose data show that *Hyalosphenia papilio* can have a relatively large ecological range. In addition, *Nebela militaris* and especially *Corythion-Trinema* spp. that have been classified as dry taxa (Tolonen et al. 1992; Warner and Charman 1994; Opravilová and Hájek 2006; Swindles et al. 2009; Lamentowicz et al. 2011) were abundantly present also in some relatively wet habitats in Siikajoki (Fig. 4 and A.1b). In the case of *Nebela militaris*, pH seemed to be more important than WTD; the highest abundances were detected in habitats with pH ~4.5, whereas the WTD range was > 40 cm, and it was plotted within the medium wet bog species cluster (Fig. 2b). *Corythion-Trinema* spp. occurred in drier habitats in more acidic conditions (Fig. 4 and A.1c), but in higher pH conditions, it was present throughout wetter locations (Fig. 4 and A.1c). In the ordination *Corythion-Trinema* spp. was associated with various types of sites from young meadows, including plots dominated by vascular plants, to fen stage mires. In the bog sites SJ6 and SJ5 these species occurred in both dry and wet habitats. This agrees with the observation by Paulson (1952-1953) whose data showed presence of *Corythion-Trinema* spp. in various different bog
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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Conflict of Interest: All of the authors declare no conflict of interest.
References


Mitchell, E.A.D., Gilbert, D., Butler, A., Grosvernier, P., Albinsson, C., Rydin, H., Heijmans,
Can testate amoebae (Protozoa) and other micro-organisms help to overcome biogeographic bias in large scale global change research? In: Visconti, G., Beniston, M., Iannorelli, E. D., Barba, D. (Eds.), Global Change and Protected Areas. Advances in Global Change Research 9, 301-310.


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.

<table>
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<th>Site type</th>
<th>SJ0</th>
<th>SJ1</th>
<th>SJ2</th>
<th>SJ3</th>
<th>SJ4</th>
<th>SJ5</th>
<th>SJ6</th>
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<tr>
<td>Peat depth (cm)</td>
<td>0</td>
<td>0-10</td>
<td>10</td>
<td>30-50</td>
<td>70-100</td>
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<td>180-240</td>
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<td>Site age (yr)</td>
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<td>170-200</td>
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<td>670-700</td>
<td>1070-1300</td>
<td>2410-2520</td>
<td>~3000</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>9.4-13.5</td>
<td>11.7-19.5</td>
<td>14.5-23.4</td>
<td>17.5-38.7</td>
<td>15.9-47.0</td>
<td>25.0-52.5</td>
<td>42.3-65.8</td>
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<tr>
<td>WTD (cm)</td>
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<td>-2 – 3.5</td>
<td>0.5 – 4.5</td>
<td>-13.5 – 30.0</td>
<td>-7.0 – 22.0</td>
<td>-0.5 – 27.8</td>
<td>8.0 – 54.0</td>
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<tr>
<td>No. of samples</td>
<td>6 (1)</td>
<td>13 (7)</td>
<td>6</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>9</td>
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</table>
Figure captions:

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

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

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

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

**Fig. 5.** Shannon diversity index box plot and Whittaker diversity index of testate amoebae taxon diversity variations along the succession gradient. Median, minimum and maximum values are indicated for the box plots.
Fig. 3
Fig. 4
Fig. 5
Electronic supplement materials

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

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

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

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<tr>
<th>Code</th>
<th>Full name</th>
<th>Code</th>
<th>Full name</th>
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<td><em>maximum WTD</em></td>
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**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.