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1 TESTATE AMOEBAE COMMUNITY ANALYSIS AS A TOOL TO ASSESS BIOLOGICAL
2 IMPACTS OF PEATLAND USE

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ABSTRACT

As most ecosystems, peatlands have been heavily exploited for different human purposes. For example, in Finland the majority is under forestry, agriculture or peat mining use. Peatlands play an important role in carbon storage, water cycle, and are a unique habitat for rare organisms. Such properties highlight their environmental importance and the need for their restoration. To monitor the success of peatland restoration sensitive indicators are needed. Here we test whether testate amoebae can be used as a reliable bioindicator for assessing peatland condition. To qualify as reliable indicators, responses in testate amoebae community structure to ecological changes must be stronger than random spatial and temporal variation.

In this study, we simultaneously assessed differences between the effects of seasonality, intermediate scale spatial variation and land uses on living testate amoebae assemblages in natural, forested and restored peatlands. We expected the effects of seasonality on testate amoebae communities to be less pronounced than those of land use and within site variation.

On average, natural sites harboured the highest richness and density, while the lowest numbers were found at forestry sites. Despite small changes observed in taxa dominance and differences in TA community structure between seasons and years at some sites, spatial heterogeneity, temperature, pH, nor water table depth seemed to significantly affect testate amoebae communities. Instead, observed differences were related to type of land use, which explained 75% of the community variation. Our results showed that testate amoebae community monitoring is a useful tool to evaluate impacts of human land use on boreal peatlands.

Keywords: Bioindicators, boreal peatlands, forestry, land uses, peatland restoration.

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INTRODUCTION

While peatlands of the boreal and subarctic regions cover only 3% of the global area, they store ca. 500Gt of C corresponding to one third of the terrestrial carbon storage (Yu 2011, 2012). Roughly 50

% of the total Finnish peatland area has been used for forestry, followed by 2.6% for agriculture, and 0.6% for peat mining (Lappalainen 1996; Vasander et al. 2003). Peatland use in Finland is mainly concentrated to the central and southern parts of the country where less than 25% of the peatland area is pristine (Aapala et al. 1996).

Besides their importance as carbon storage, peatlands play an important role in balancing the water cycle. They are also a unique habitat for many organisms including many rare and endangered species and form repositories of paleontological information through the accumulation and storage of remains of flora, fauna, and atmospheric particles (Gorham 1991; Barber 1993). Thanks to these properties, there is an increased attention on the environmental importance of peatlands and the need for restoration of impacted areas to regain lost ecosystem services (e. g. specific biodiversity, carbon sink, etc.; Lunn and Burlton 2013). To accurately determine whether ecosystem structure and functioning are indeed moving towards near-pristine state following restoration attempts, sensitive, yet robust indicators are needed.

To date, there is no evidence that any specific indicator (be it biological, chemical, or physical) outperforms others in indicating changes in peatlands or their restoration success (Chapman et al. 2003). Indeed, in the case of biological indicators, it is well known that different taxa respond differently to ecosystem dynamics and environmental gradients (Francez et al. 2000). However, assessments of peatland plant community composition to study restoration success have shown some promising results (e. g. Haapalehto et al. 2011; Laine et al. 2011; Hedberg et al. 2012; Poulin et al. 2013) but little is known about responses of other important components of peatland ecosystems. Recently, the search for efficient indicators has directed the focus on testate amoebae because they possess several beneficial qualities (e. g. Mitchell et al. 1999; Charman 2001; Koenig et al. 2015; Daza Secco et al. 2016). First, testate amoebae (TA) are shell-building protists (Charman 1999). found in a wide range of habitats e.g. soils, lakes, rivers, they are very strongly associated with peatland plants and especially abundant in *Sphagnum* mosses (Tolonen 1986). Second, TA density can be as high as 16×10^6 individuals per m^2 and even in boreal environments TA can produce several generations per year (Sleigh 1989). Third, TA are a vital component of the microorganism community in *Sphagnum*-dominated peatlands where they account for almost half of the community in terms of biomass (Gilbert et al. 1998). Lastly seasonality is generally not expected to significantly affect the TA communities since they can survive throughout the year through their encysting capacity (Gilbert and Mitchell 2006). These factors could make TA a valuable

tool in the assessment of short -and long- term responses of peatlands to land use changes (e. g. Koenig et al. 2015; Daza Secco et al. 2016). However peatland microorganisms can exhibit microtopographic transitions at scales of few centimetres (Mitchell et al. 2000b) and previous studies on temporal (Warner et al. 2007) and spatial TA community variation (Mitchell et al. 2000b) highlight the need to account for spatial variation when trying to assess seasonal community changes. Thus, to which degree spatial or temporal variation in community structure may confound the use of TA in routine monitoring of peatlands is yet unknown and warrants study.

In this study, we simultaneously assess differences between the effects of seasonality, intermediate scale spatial variation and land uses on living TA assemblages in i) natural peatlands, ii) forested peatlands and iii) restored peatlands. We expected the effects of seasonality on TA communities to be less pronounced than those of land use and within site variation.

MATERIALS AND METHODS

Study sites

Study sites were chosen to represent three stages of peatland use: pristine, forested, and restored. We assumed that if restoration was successful, sites restored many decades ago were likely to resemble natural sites more than sites actively forested ones.

All the studied peatlands are raised bogs, which represent the prevalent peatland type within a mosaic of *Sphagnum*-dominated peatlands, coniferous forests and lakes in the boreal zone of central Finland. Sites were chosen based on their similar characteristics such as elevation, mean annual temperature, and mean annual precipitation (Table 1). Each land use was represented by two peatlands: Riihineva and Aittosuo (natural), Lahnanen and Ruuskanlampi (forestry), and Aitoneva60 and Aitoneva80 (restored).

Table 1. Sampling sites coordinates and elevation by land use. Elevation is given in meters above sea level. Land use refers to: Natural: peatlands not under direct human influence, Forestry: peatlands used for forestry, Restored: peatlands previously under human use but restored either 60 or 80 years ago.

Land use	Coordinates		Elevation (m.a.s.l.)	Temperature (mean annual °C)	Precipitation (mean annual mm)
	Longitude	Latitude			
Natural	25° 28' 9-24° 37' 53" E	61° 50' 43"-62° 45' 15" N	ca. 150	3	600
Forestry	25° 28' 9-24° 37' 53" E	61° 50' 43"-62° 45' 15" N	ca. 150	3	600
Restored	23° 18' 19.3248" E	62° 10' 52.1544" N	ca. 100	4	650

While *Sphagnum* mosses mainly dominated at natural sites, Aittosuo had a higher coverage of shrubs and trees whereas mosses almost exclusively covered Riihineva. At the forestry sites Lahnanen and Ruuskanlampi, vegetation was mainly composed of brown and *Sphagnum* mosses with high presence of trees and bushes. Lahnanen was mainly a dry site surrounded by ditches while Ruuskanlampi displayed a patchwork of flooded and very dry spots and highly diversified microhabitats. Restored sites Aitoneva were previously used for peat extraction and were restored either 60 (Aitoneva60) or 80 (Aitoneva80) years ago by blocking ditches and rewetting the sites to restore previous hydrological conditions in order to allow natural peatland species to recolonise. Aitoneva60 particularly, displayed the highest water table of all sites, and it was flooded most of the year with small streams crossing the site. On average, the highest water table depths were found at restored sites while lowest at forestry sites (Fig. 1).

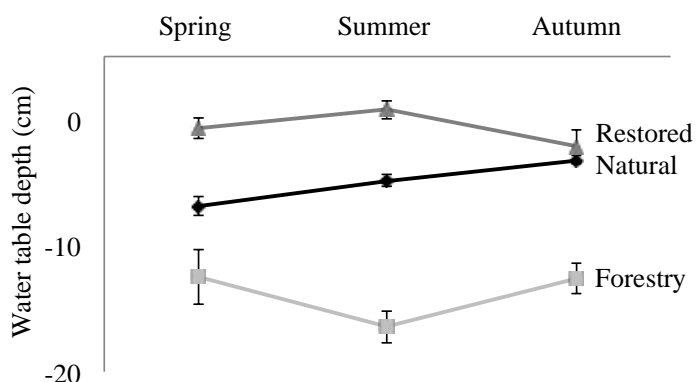


Fig. 1 Average water table depth by season for the three land uses. Values correspond to measurements during three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling plots/site at each sampling time. Restored: peatlands previously under human use but restored either 60 or 80 years ago, Natural: peatlands not under human use, Forestry: peatlands used for forestry. Y-axis: water table depth given in centimetres below ground level. Points represent mean values; bars represent SE (± 1).

Field sampling and sample processing

Starting from a randomly selected point, fifteen 100 cm²-sampling plots were placed at each site and distributed in three concentric semicircles of five plots each with ca. 1m spacing (Fig. 2). For water table depth measurements (WTD), 2cm diameter polypropylene pipe wells with 2mm slits at every 3cm were placed at the top-left corner of each sampling plot. Water table depth was recorded as negative

values when the water level was below ground (top of moss layer), and as positive values when it was above ground (flooded plots). Water temperature and water pH were measured in the middle of each sampling plot using a VWR pH meter 1000H. For TA samples, bryophyte mass was collected using a knife, including only the stem and capitulum of the mosses (ca. 10 cm) in order to obtain mainly living TA (see Booth et al. 2010). Samples were stored in Ziploc bags at 5°C before further analysis. TA samples were taken during spring (May), summer (July), and autumn (September) in three consecutive years (2013, 2014 and 2015). WTD, pH and water temperature from the peat layer were measured during each TA sampling.

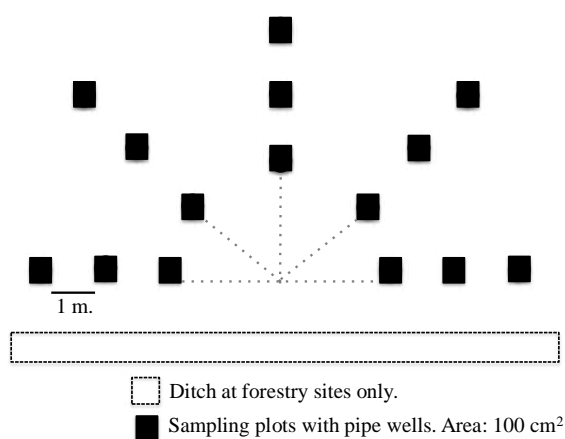


Fig. 2 Schema of the sampling design for testate amoebae, pH, temperature and water table depth.

TA samples were analysed during the following days immediately after sampling in order to account only for the living TA. Sample processing was carried using the protocol proposed by Booth et al. (2010). Each sample was boiled for ca. 10 minutes in distilled water with one tablet of *Lycopodium clavatum* spores (batch 1031) standard preparation from Lund University (Sweden). To remove coarse materials, samples were sieved through 300µm mesh and further filtered onto a 7µm mesh to retain TA and transferred into a centrifuge tube. TA were centrifuged at 3000rpm for five minutes and stored in distilled water. Counting and identification of living TA (empty shells were not taken into account) was done using a 40X magnification (Olympus BX41 microscope). TA were identified mainly to species or species groups based on characteristics of the shell following a number of different taxonomic keys (e.g. Charman et al. 2000; Meisterfeld 2002; Clark 2003; Mazei and Tsyanov 2006).

Data analysis

TA number of taxa and relative abundances were calculated for each site (using plot averages), and averaged by land use. Concentrations of TA in 10 cm³ of fresh *Sphagnum* were estimated using the *Lycopodium* counts as an external marker (Stockmarr 1971). The Shannon Wiener diversity index (Shannon and Weaver 1963) was used to estimate TA diversity and a non-parametric Kruskal-Wallis H analysis (Van Hecke 2013) was used to check for temporal differences in TA richness and diversity. TA taxa not frequently found were excluded from ordination analysis to avoid noise. We applied a model-based ordination method as suggested in Hui et al. (2015) and Warton et al. (2015) to visualize the main patterns between different sampling sites in terms of the taxa composition. A model-based ordination approach offers several advantages over traditional distance-based ordination methods such as non-metric multidimensional scaling (NMDS). The main advantage is that models can be used to account for important features such as the mean-variance relationship. For a thorough comparison of model-based and traditional ordination methods, see Hui et al. (2015). A latent variable model with two latent variables was fitted to the data assuming a negative binomial distribution for the TA density. An ordination plot was then produced based on the bivariate latent variables. As the samples were collected in three seasons, we first fitted latent variable models to data sets corresponding to different seasons separately. Lastly, a latent variable model with covariates was fitted in order to identify drivers of amoeba community composition.

RESULTS

Environmental variables

Environmental variables generally displayed clear patterns during all study years. On average, pH was highest at restored sites, especially at Aitoneva60. Surface water temperature of the peatlands was less variable between sites, but values were slightly higher at the natural site Riihineva (Fig. 3).

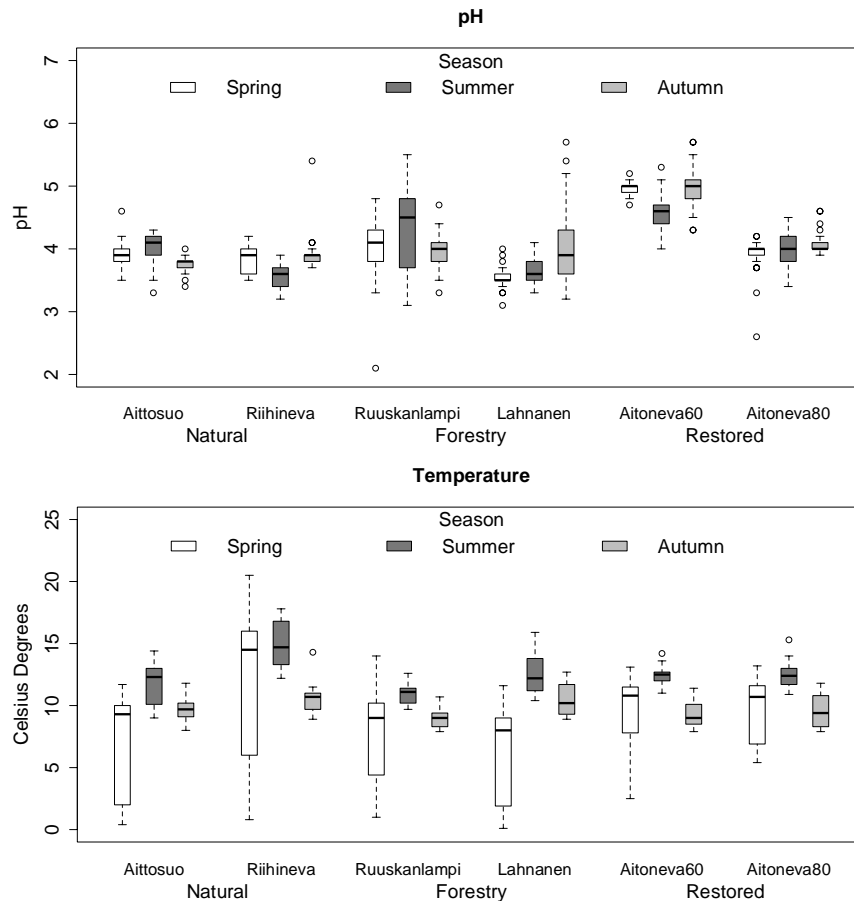


Fig. 3 Environmental variables (surface water pH and temperature) in relation to land use and season of sampling. Values correspond to measurements during three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling plots/site at each sampling time. Natural: peatlands not under human use, Forestry: peatlands used for forestry, Restored: peatlands previously under human use but restored either 60 or 80 years ago. Circles: outliers, upper whisker: maximum value excluding outliers, upper box line: upper quartile, middle line inside box: median, lower box line: lower quartile, lower whisker: minimum value excluding outliers.

TA community data

We found altogether 62 TA taxa in our study. The highest number of taxa was found at natural sites except in spring 2013 when number of taxa was highest at restored sites (Fig. 4). On average, forestry sites harboured the lowest number of taxa (Fig. 4). TA densities showed a less distinct pattern. Some particularly high densities ($>150,000/10\text{cm}^3$) of TA were recorded in autumn at Aittosuo and Ruuskanlampi (natural and forestry, respectively; Fig. 4).

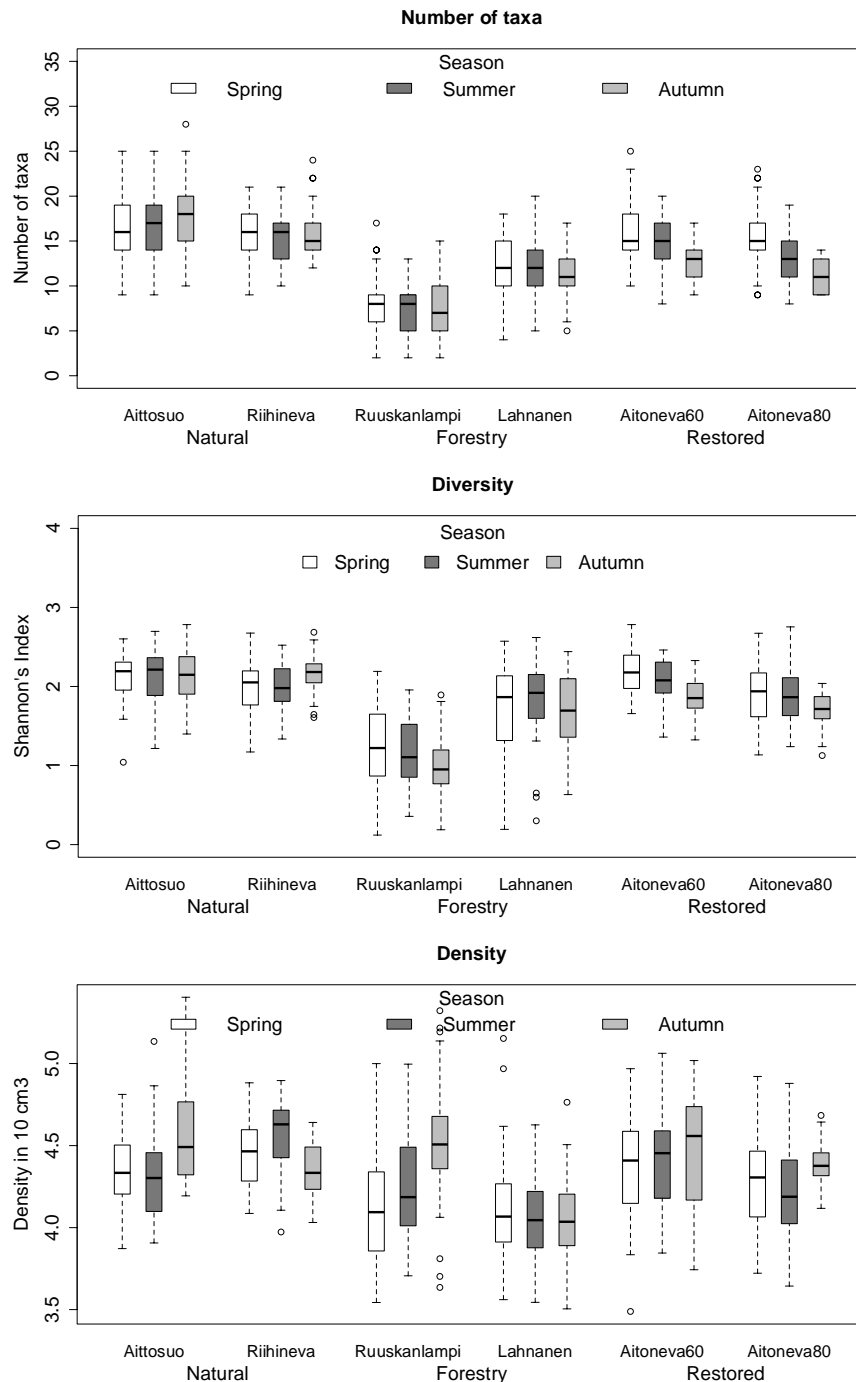


Fig. 4 Number of taxa, Shannon Wiener diversity, and density, whisker boxplots for testate amoebae in relation to land use and season of sampling. Values take into account correspond to measurements during three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling plots/site at each sampling time. Natural: peatlands not under human use, Forestry: peatlands used for forestry extraction, Restored: Peatlands previously under human use but restored either 60 or 80 years ago. Circles: outliers, upper whisker: maximum value excluding outliers, upper box line: upper quartile, middle line inside box: median, lower box line: lower quartile, lower whisker: minimum value excluding

outliers. Density values were transformed to logarithmic scale, original values ranged between 3081,87 and 253383,38 testate amoebae/10 cm³ of fresh *Sphagnum*.

Number of TA taxa and diversity were significantly affected by variations in environmental variables between years at Aittosuo (natural) and Lahnanen (forestry) sites, while at both restored sites the differences were related to seasonality. At Riihineva (natural) significant differences were observed just in taxa diversity between seasons, while at Ruuskanlampi (forestry) richness varied between years and diversity between seasons (Table 2).

Table 2 Non-parametric Kruskal-Wallis H tests results of testate amoebae taxa richness and Shannon's diversity index differences between years and seasons ($df=2$ for all comparisons). Significant differences in richness and diversity are marked in bold. Values take into account correspond to measurements during three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling plots/site at each sampling time. The ranges shown in the table refer to the site variation of the annual and seasonal means. Natural: peatlands not under human use, Forestry: peatlands used for forestry extraction, Restored: Peatlands previously under human use but restored either 60 or 80 years ago.

Land use	site	Variation between years						Variation between seasons					
		richness			Shannon Wiener diversity			richness			Shannon Wiener diversity		
		range	H	p	range	H	p	range	H	p	range	H	p
Natural	Riihineva	15.3-15.9	1.3	0.540	1.9-2.1	1.2	0.540	15.5-15.8	0.2	0.910	1.9-2.2	11	0.005
	Aittosuo	14.2-18.9	41	<0.001	1.9-2.2	31	<0.001	16.5-17.6	2.3	0.310	2.1-2.2	0.2	0.900
	Lahnanen	10.6-13.2	18	<0.001	1.5-1.9	9.6	0.018	11.1-11.8	2.2	0.330	1.7-1.8	2.6	0.280
Forestry	Ruuskanlampi	6.7-8.6	7.0	0.030	1.1-1.2	1.5	0.480	7.3-8.1	1.1	0.560	1-1.3	6.2	0.045
	Aitoneva60	14.5-14.8	0.1	0.960	2-2.1	1.7	0.440	12.8-16.1	26	<0.001	1.9-2.2	27	<0.001
Restored	Aitoneva80	12.6-13.7	2.9	0.230	1.8-1.9	4.2	0.120	11.2-15.6	44	<0.001	1.7-1.9	11	0.003

At natural sites, *Arcella catinus*, *Hyalosphenia papilio* and *Centropyxis aculeata* were the most abundant taxa representing 16.3%, 15.7% and 9.9% of the total average TA numbers, respectively. *A. catinus* represented up to 40% of communities at forestry sites. Other common taxa at forestry sites were *C. aculeata* (14%) and *Trigonopyxis arcuata* (9.5%). At restored sites dominant taxa differed from those at the other types of peatlands, and showed the highest variability between seasons. Overall *Euglypha compressa*, *C. aculeata* and *Diffugia globulosa* were the most abundant taxa representing on average 41% of the communities at restored sites (appendix 1).

Community-environment relationships and community ordinations

In the ordination, the different land uses clustered separately, suggesting that they differed in their TA taxa composition but seasonality did not seem to affect TA communities (Fig. 5).

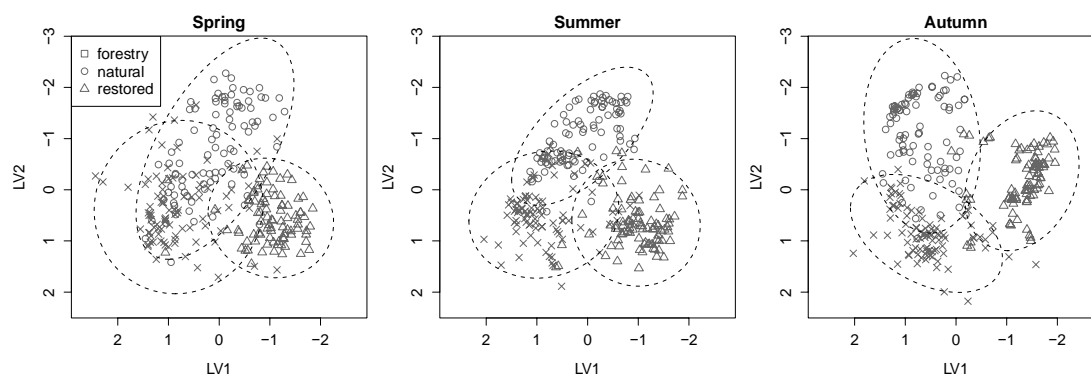


Fig. 5 The ordination of $n=270$ sites based on generalized linear latent variable model without any covariates assuming negative binomial distributed concentrations. The measurements were taken during the years 2013-2015 and three different seasons. Here the ordinations are shown separately for spring, summer and autumn. The sites in ordination are labelled according to the land use (Natural: peatlands not under human use, Forestry: peatlands used for forestry, Restored: Peatlands previously under human use but restored either 60 or 80 years ago).

As the seasons did not affect the ordination, we fitted a latent variable model to the whole dataset (Fig. 6) resulting in a similar pattern as in Fig. 5. When plotting by sites instead of land use, sites still mainly grouped by land use. The natural site Riihineva displayed a different pattern, where all the plots grouped separately from the other sites (Fig. 6).

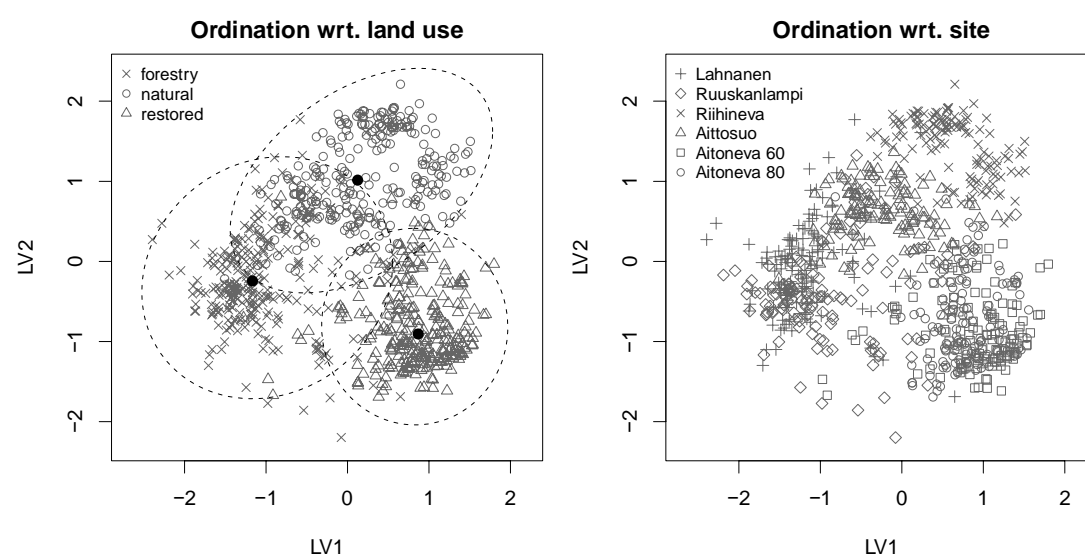


Fig. 6 The ordination of $n=810$ sites based on generalized linear latent variable model without any covariates assuming negative binomial distributed concentrations. The measurements were taken during

the years 2013-2015 and three different seasons. The sites in left ordination plot are labeled according to the land use (Natural: peatlands not under human use, Forestry: peatlands used for forestry, Restored: Peatlands previously under human use but restored either 60 or 80 years ago), and in right ordination plot according to the sampling site. In left ordination plot, the spatial medians for ordination points corresponding to each land use are shown as black dots.

To visualize whether the sites align along any of the measured gradients we plotted the sites with shadings corresponding to their respective pH, temperature values, and WTD (Appendix 2). However, none of the variables seemed to affect the ordination i.e. any of the covariates did not affect testate amoebae community structure and composition. To evaluate the amount of variation in testate amoebae taxa caused by different predictors, we used ratios of traces of residual covariance matrices from generalized linear latent variable models as a measure for total variation (Warton et al. 2015). Adding the land use as a covariate to the null model reduced the trace from 497 to 142. Thus, the land use alone explained approximately 71.5% of the covariation across species. Further, the pH alone explained 14.8%, the temperature 2.6% and water table depth 2.3% of the total covariation. To compare the locations of the scatterplots related to different land uses we determined the spatial medians for the sets (see Fig. 6) and tested for differences in spatial medians. The locations differed significantly (Spatial signs test, $p < 0.0001$; Oja and Randles 2004).

DISCUSSION

Overall, our results did not show large variations in environmental variables between the land uses except that the highest values of water table depth were recorded at restored and the lowest at forestry sites. Testate amoebae community structure and composition differed between seasons only at the restored sites. However, when all data were analysed together, differences seemed unaffected by seasons or years. Additionally, testate amoebae community structure and composition did not respond to the measured environmental variable gradients (pH, temperature and WTD). Instead, the land use seemed to explain most of the variation between TA communities.

On average, natural peatlands Riihineva and Aittosuo harboured the highest number of taxa, and displayed highest diversity and TA density, while the lowest values were found at forestry sites. Wanner

and Xylander (2005) found in mineral soils that higher TA taxa richness might be result of a longer time for cumulative colonisation without substantial species replacement, which can also be the explanation for the higher taxa richness found at our natural sites. Additionally, TA commonly occur in highest numbers in wet mosses coverage (Charman 2001), habitat more frequently found at natural sites compared to forestry and restored sites.

Forested sites are inherently different from *Sphagnum*-dominated peatlands, thus such differences together with the disturbances caused by forestry practices may have restricted the colonisation and survival of drought intolerant taxa, reducing both the TA taxa richness and their densities. The lowest water tables were in general recorded at forestry sites, where peat was also mainly drier. In *Sphagnum* dominated peatlands, peat moisture is considered the main factor affecting TA communities (Tolonen 1986; Booth 2001) and wetter habitats have been found to harbour higher densities of TA (Fournier et al. 2012). Our results generally supported these findings as natural and restored sites showed both higher water table levels and TA densities. However, we found particularly high TA densities in autumn samples at natural and forestry sites. These high TA densities at individual plots were obviously not related to any of the measured environmental variables as these plots were particularly dry (WTD as low as -22cm in some cases) at the time of sampling. While we cannot entirely rule out human errors in sample processing which might have led to such high observed numbers, we feel that observed densities are more likely the result of other favourable environmental factors, such as nutrient concentrations that can either affect TA directly (Lamentowicz et al. 2011) or indirectly by controlling their food sources (Mitchell et al. 2004).

Taxa dominance was relatively constant among study years and seasons at natural and forestry sites. The high abundance of *A. catinus* at forestry sites and *H. papilio* at natural sites for example, agrees with common findings suggesting that these taxa are potential indicators of dry and wet conditions, respectively (e.g. Charman and Warner 1992; Mitchell et al. 1999; Bobrov et al. 2002; Galka et al. 2012).

The highest abundances of *A. catinus* at natural sites were recorded at the driest plots at Aittosuo where they represented more than 50% of the TA communities. Plots in Aittosuo varied in their hydrological characteristics, some plots being very dry (water level as low as 30cm below ground) compared to the average water level found in this study. However, *A. catinus* was commonly found at all sites, even at the flooded restored sites, but in much lower abundances. This suggests that *A. catinus* could display broad moisture tolerance in Finnish peatlands, as also found by Daza Secco et al. (2016).

It should be also noted that the taxonomic keys used in this study group some morphologically similar species into one taxon, which may increase its range of environmental tolerance (for more details see Booth 2001). The highest abundances of *H. papilio* were recorded at Riihineva site where they represented more than 40% of the TA community. Riihineva is a site particularly different in its plant composition. The most remarkable characteristic of this site is the absence of vascular plants while mosses are dominating and creating a homogeneous mat. In fact, in a study by Booth and Zygmunt (2005) *H. papilio* was restricted to floating peat mats, suggesting that hydrological stability of such peat mats is of importance for this taxon. These habitat characteristics were unique to Riihineva and might also explain why TA communities from Riihineva sites grouped particularly separated from the other sites.

Compared to forestry and natural sites, taxa dominance was more variable between seasons at restored sites where the two dominant taxa were *E. compressa* and *D. globulosa*. These findings agree with other studies (e.g. Bobrov et al. 1999; Booth 2002) that have found the spiny shells of the spined forms of *Euglypha* help them to restrict their sinking and movements during interstitial water flows to in the wet habitats they are commonly associated with (Bobrov et al. 2002). Both *E. compressa* and *D. globulosa* were common and generally abundant also at natural sites, but very scarce or totally absent from forestry sites, suggesting a low tolerance of dry conditions. In contrast, *C. aculeata* was abundant at all sampling sites regardless of their hydrological conditions. Taxa such as *C. aculeata*, with an intermediate moisture optimum, are common along a wide range of the moisture gradient (Booth 2001). It should be pointed that also *C. aculeata* may be similar as *A. catinus*, grouping different species together, and thus influencing its observed tolerance range.

Although pH, temperature and WTD are often the most important variables driving TA communities, their effect was not able to mask the effect of land use in our study. Hence, it is possible that TA community structure is driven by other variables such as nutrient concentrations in peat water or biotic interactions more directly linked to the land use. Some studies have found an important relationship between TA and nutrients (e. g. Mitchell et al. 2000a; Jauhiainen 2002; Mitchell et al. 2004). Calcium for example, directly affects some TA species due to its importance in the shell building process (Lamentowicz et al. 2011). While the direct role of other nutrients in the regulation of TA communities is still poorly understood, Mitchell et al. (2004) suggested that nutrients indirectly affect TA via affecting the presence and abundance of their prey organisms.

After restoration measurements, it is often inherently expected that both environmental variables and biological communities move towards the pristine conditions of a site, recreating ecosystems that preceded human activities (Choi 2004). However, in our ordinations, sites clustered separately by land use, and natural and restored sites did not show higher similarities between them than they did to forestry sites. A combination of random forces such as percolating rainwater, convective transport into atmosphere, burrowing and other moving efforts, may greatly determine the spatial distributions of dormant protists such as TA (Finlay et al. 2001). Hence, the high similarities in taxa composition between natural and forestry sites might be explained by their geographical proximity, as they were more closely located to each other than to restored sites.

A commonly expected main outcome of most ecological restoration programs is that the restored ecosystem reverts back to its pristine conditions; however, the validity of this expectation has recently been openly debated (Wortley et al. 2013). In some wetlands and peatland studies that evaluated restoration success, no conclusive evidence for changes towards a target community after restoration was found (e.g. Zedler and Callaway 1999; Moreno-Mateos et al. 2012). Given such results, evaluations of restoration success should account for: i) the unpredictability of ecological succession, ii) the difficulties to determine the pre-disturbance state of the ecosystem, and the fact that iii) ecological change might be irreversible (Choi 2004). Irreversibility of ecological damage has been previously observed in both diversity and ecosystem services that usually remain lower in the restored than in the reference ecosystems (Wortley et al. 2013; Gałka et al 2017). These findings also highlight the importance of studies including peat core sampling in order to recognise the TA community structure and composition before disturbance, and evaluate whether the observed changes following restoration truly move towards the original states of sites (Gałka 2017).

The importance of discriminating between the large-scale climatic effects i.e. seasonal variation, and the local-scale changes caused by environmental disturbance (e.g. drainage) on TA communities has been pointed out earlier (e. g. Mitchell et al. 2000b; Warner et al. 2007; Talbot et al. 2010). Here, we found that despite small changes in taxa dominance (especially at restored sites), changes in TA community structure and composition related to land use strongly overrode effects of seasonality and random spatial variation, explaining 75% of the differences between the TA communities. Land use has been previously reported to greatly affect the microorganisms' communities in wetland environments. For example, Hartman et al. 2008 found that wetland restoration significantly influenced the bacterial

community composition when comparing restored vs. reference wetlands even when taking into account soil chemistry and wetland type. The lack of influence of seasonal variation has been previously reported for TA in peatlands (e. g. Gilbert et al. 1998) and TA in other habitat types (e.g. Schönborn 1986).

Low temporal variation has been attributed to the trophic diversity behaviour of TA and their capacity to resist changes in temperature and water content through encysting (e.g. Gilbert et al. 1998). On the other hand, studies of soil TA communities (e.g. Finlay and Fenchel 2004; Tysganov et al. 2013) have shown that climate-independent drivers of change i.e. local conditions such as soil moisture, either related to topography or geology (Tysganov et al. 2013) greatly affect the taxa number and abundance of soil TA. The differences in the responses of TA communities to large vs. local-scale environmental variation may also be related to the role of vegetation in regulating microclimatic conditions through shading, precipitation interception, etc. (Wookey et al. 2009; Graae et al. 2012). Other studies on the influence of seasonal fluctuations on TA community structure and composition (e.g. Lamentowicz et al. 2013; Marcisz et al. 2014) have found differences mainly in TA density between seasons with highest values during spring. However, such a pattern was not observed in our results.

In conclusion, our results showed that in boreal peatlands, the magnitude of the response of TA communities to human-induced environmental changes is higher than their response to local spatial and seasonal environmental variation. Additionally, other studies have also shown the advantages of using not only TA community structure and composition (e. g. Koenig et al. 2015; Daza Secco et al. 2016) but also their functional traits (Marcisz et al. 2016) as bioindicators of peatland disturbance. Here, we suggest the use of TA communities as an efficient tool for assessment and monitoring of ecological changes in boreal peatlands caused by human disturbances. Further, TA can and should be used over broad spatial and temporal scales commonly applied in routine monitoring. An especially attractive property of TA is their robustness against short-term climatic events. This greatly expands the timeframe over which samples from the same year can be combined in analyses, which is an especially helpful feature in the boreal region, where weather conditions from spring to autumn can change drastically.

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647 APPENDIXES

648 Appendix 1. Testate amoebae taxa mean relative abundances. Values correspond to percentage of

649 presence of each taxa relative to the total of testate amoebae found.

Species	Natural			Forestry			Restored		
	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn
<i>Amphitrema flavum</i>	2.00	1.43	1.98	0.23	0.10	0.00	0.08	0.06	0.02
<i>A. wrightianum</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
<i>Arcella catinus</i>	13.52	16.94	18.48	40.65	38.06	40.92	4.92	7.29	5.40
<i>A. gibbosa</i>	0.03	0.00	0.00	0.05	0.00	0.00	0.77	0.82	0.02
<i>A. vulgaris</i>	0.07	0.09	0.10	0.19	0.31	0.09	4.29	6.76	11.85
<i>A. discoides</i>	0.32	0.15	0.07	2.91	2.21	3.07	6.57	4.32	2.49
<i>Argynnia vitraea</i>	0.03	0.09	0.02	0.03	0.00	0.00	0.50	0.65	0.55
<i>Asulina muscorum</i>	1.87	1.35	1.55	3.65	3.75	4.56	0.43	0.54	0.34
<i>A. seminulum</i>	0.32	0.31	0.41	0.11	0.23	0.34	0.00	0.00	0.00
<i>Bulinularia indica</i>	3.54	2.76	2.91	2.04	1.27	1.99	0.16	0.02	0.04
<i>Centropyxis aculeata</i>	9.49	8.79	11.31	12.27	14.85	15.96	11.12	13.45	14.32
<i>C. cassis</i>	1.49	0.72	0.83	4.11	4.22	6.12	1.17	0.25	0.40
<i>C. ecornis</i>	0.38	0.40	0.16	0.01	0.40	0.14	0.02	0.00	0.00
<i>C. platystoma</i>	0.01	0.07	0.00	0.06	0.04	0.00	0.01	0.09	0.01
<i>Cyclopyxis arcelloides</i>	4.55	2.03	3.74	2.51	2.33	1.57	5.28	4.44	2.99
<i>Cryptodiffugia oviformis</i>	0.03	0.08	0.06	0.17	0.61	0.20	0.08	0.09	0.00
<i>Cyphoderia ampulla</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
<i>Diffugia rubescens</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.08	0.05	0.00
<i>D. bacillifera</i>	0.24	0.29	0.68	0.80	0.06	0.20	8.01	8.34	13.88
<i>D. oblonga</i>	0.06	0.20	0.23	0.23	0.39	0.02	4.37	5.15	5.32
<i>D. lucida</i>	0.93	0.74	0.67	0.20	0.01	0.04	2.64	1.48	0.78
<i>D. pristis</i>	0.03	0.00	0.00	0.07	0.00	0.00	0.59	0.07	0.00
<i>D. globulosa</i>	1.69	3.25	2.56	1.92	1.73	1.62	9.88	11.26	14.32
<i>D. bacillarium</i>	0.07	0.00	0.01	0.03	0.00	0.00	0.79	0.25	0.04
<i>D. acuminata</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00
<i>D. lithophila</i>	0.16	0.02	0.00	0.01	0.06	0.00	1.41	0.68	0.30
<i>D. leidy</i>	1.53	1.49	2.19	0.03	0.06	0.00	0.08	0.19	0.23
<i>Euglypha strigosa</i>	7.30	5.98	6.49	1.27	2.53	0.99	11.35	3.75	1.03
<i>E. compressa</i>	2.83	7.95	6.94	0.54	0.16	0.64	8.81	14.23	16.79
<i>E. tuberculata</i>	0.32	0.21	0.19	0.39	1.24	0.47	0.62	0.43	0.00
<i>E. rotunda</i>	0.27	0.20	0.22	0.85	0.24	0.56	0.41	0.37	0.12
<i>E. cristata</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
<i>Heleopera rosea</i>	0.82	0.94	0.62	0.81	0.37	1.21	0.44	0.17	0.04
<i>H. sphagni</i>	10.33	12.34	6.87	0.55	0.79	0.17	0.15	0.15	0.00
<i>H. petricola</i>	1.85	3.84	2.30	0.19	0.28	0.07	0.11	0.03	0.07
<i>H. sylvatica</i>	0.30	0.25	0.23	0.72	0.42	0.05	0.02	0.01	0.00
<i>Hyalosphenia minuta</i>	0.05	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
<i>H. papilio</i>	18.97	14.92	13.33	1.19	1.08	0.78	2.86	2.48	0.43
<i>H. subflava</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
<i>H. elegans</i>	0.79	0.50	0.67	0.00	0.00	0.00	0.01	0.00	0.00
<i>Lesquereusia spiralis</i>	0.03	0.00	0.00	0.03	0.09	0.02	0.22	0.16	0.16
<i>Nebela marginata</i>	0.46	0.59	0.46	0.13	0.05	0.00	3.68	5.92	3.71
<i>N. carinata</i>	2.74	2.34	3.35	0.04	0.08	0.00	0.23	0.36	0.36
<i>N. tubulosa</i>	0.02	0.01	0.00	0.00	0.00	0.00	1.34	0.07	0.00
<i>N. parvula</i>	1.75	1.41	0.95	1.14	0.77	0.09	2.20	2.00	1.14
<i>N. tincta</i>	0.88	0.85	1.20	3.54	3.47	3.78	0.34	0.08	0.04
<i>N. griseola</i>	1.27	1.31	1.13	0.51	0.00	0.00	0.03	0.01	0.00
<i>N. flabellulum</i>	0.01	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
<i>N. militaris</i>	0.12	0.20	0.37	0.57	1.21	0.50	0.04	0.01	0.00
<i>N. bohémica</i>	0.05	0.00	0.21	0.00	0.01	0.00	0.16	0.64	0.01
<i>Phryganella acropodia</i>	0.57	0.08	0.17	0.10	0.00	0.00	0.15	0.00	0.00
<i>Placosista spinosa</i>	0.32	1.40	0.78	0.04	0.01	0.01	0.01	0.33	0.00
<i>Pseudodiffugia fascicularis</i>	0.30	0.00	0.06	0.00	0.00	0.00	0.01	0.00	0.01
<i>Sphenoderia lenta</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.01	0.00
<i>Tracheleuglypha dentata</i>	0.00	0.00	0.00	0.08	0.06	0.06	0.10	0.10	0.13
<i>Trigonopyxis arcula</i>	3.12	2.40	2.70	10.81	10.62	7.31	0.49	0.57	0.50
<i>T. minuta</i>	0.04	0.35	0.87	0.13	1.46	2.01	0.00	0.08	0.17
<i>Trinema lineare</i>	0.76	0.50	1.50	1.44	2.34	2.38	1.51	1.61	1.62
<i>Trinema sp.</i>	1.31	0.23	0.44	2.42	2.04	2.06	0.50	0.19	0.10
<i>Sp. 1</i>	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00
<i>Sp. 2</i>	0.01	0.00	0.00	0.01	0.00	0.00	0.82	0.00	0.00
<i>Sp. 3</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 2. The ordination of n=810 sites based on generalized linear latent variable model without any covariates assuming negative binomial distributed concentrations. The measurements were taken during the years 2013-2015 and three different seasons. The sites in ordination plot are labelled according to the

654 land use (Natural: peatlands not under human use, Forestry: peatlands used for forestry, Restored:
655 Peatlands previously under human use but restored either 60 or 80 years ago), and coloured according to
656 pH, temperature and water table depth values.
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