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**Title:** Testate amoebae community analysis as a tool to assess biological impacts of peatland use

**Year:** 2018

**Version:** Accepted version (Final draft)

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**Please cite the original version:**

Daza Secco, E., Haimi, J., Högmander, H., Taskinen, S., Niku, J., & Meissner, K. (2018). Testate amoebae community analysis as a tool to assess biological impacts of peatland use. *Wetlands Ecology and Management*, 26(4), 597-611. <https://doi.org/10.1007/s11273-018-9594-y>

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

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## 31 ABSTRACT

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

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

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

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

52

## 53 ACKNOWLEDGEMENTS

54 We thank Yuri Mazei for his guidance on testate amoebae taxonomy and Merle Pöntynen for her  
55 valuable work in the field. Emmanuela Daza Secco thanks the Maj and Tor Nessling foundation for the  
56 financial support.

57

## 58 INTRODUCTION

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

61 % of the total Finnish peatland area has been used for forestry, followed by 2.6% for agriculture, and  
62 0.6% for peat mining (Lappalainen 1996; Vasander et al. 2003). Peatland use in Finland is mainly  
63 concentrated to the central and southern parts of the country where less than 25% of the peatland area is  
64 pristine (Aapala et al. 1996).

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

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

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

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

102

## 103 MATERIALS AND METHODS

### 104 Study sites

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

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

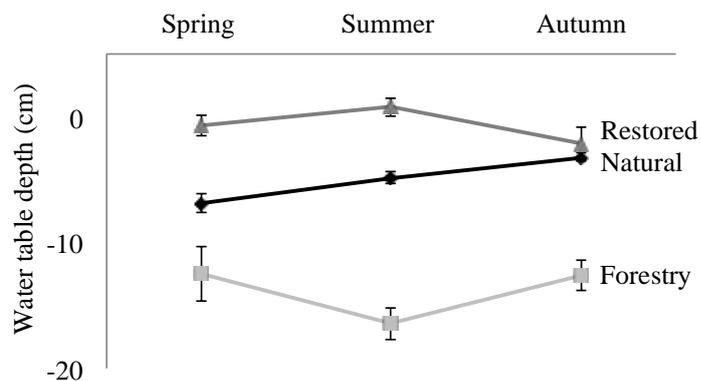
114

115 Table 1. Sampling sites coordinates and elevation by land use. Elevation is given in meters above sea  
 116 level. Land use refers to: Natural: peatlands not under direct human influence, Forestry: peatlands used  
 117 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

118

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



130  
 131 **Fig. 1** Average water table depth by season for the three land uses. Values correspond to measurements  
 132 during three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling  
 133 plots/site at each sampling time. Restored: peatlands previously under human use but restored either 60  
 134 or 80 years ago, Natural: peatlands not under human use, Forestry: peatlands used for forestry. Y-axis:  
 135 water table depth given in centimetres below ground level. Points represent mean values; bars represent  
 136 SE ( $\pm 1$ ).

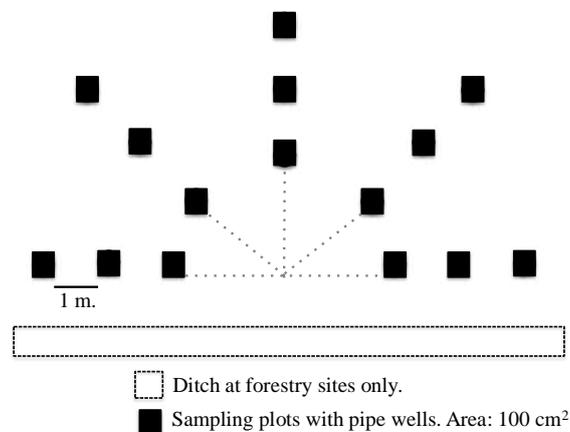
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138 Field sampling and sample processing

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

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

151



153

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

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

164

165 Data analysis

166 TA number of taxa and relative abundances were calculated for each site (using plot averages),  
167 and averaged by land use. Concentrations of TA in 10 cm<sup>3</sup> of fresh *Sphagnum* were estimated using the  
168 *Lycopodium* counts as an external marker (Stockmarr 1971). The Shannon Wiener diversity index  
169 (Shannon and Weaver 1963) was used to estimate TA diversity and a non-parametric Kruskal-Wallis H  
170 analysis (Van Hecke 2013) was used to check for temporal differences in TA richness and diversity. TA  
171 taxa not frequently found were excluded from ordination analysis to avoid noise. We applied a model-  
172 based ordination method as suggested in Hui et al. (2015) and Warton et al. (2015) to visualize the main  
173 patterns between different sampling sites in terms of the taxa composition. A model-based ordination  
174 approach offers several advantages over traditional distance-based ordination methods such as non-  
175 metric multidimensional scaling (NMDS). The main advantage is that models can be used to account for  
176 important features such as the mean-variance relationship. For a thorough comparison of model-based  
177 and traditional ordination methods, see Hui et al. (2015). A latent variable model with two latent  
178 variables was fitted to the data assuming a negative binomial distribution for the TA density. An  
179 ordination plot was then produced based on the bivariate latent variables. As the samples were collected  
180 in three seasons, we first fitted latent variable models to data sets corresponding to different seasons  
181 separately. Lastly, a latent variable model with covariates was fitted in order to identify drivers of amoeba  
182 community composition.

183

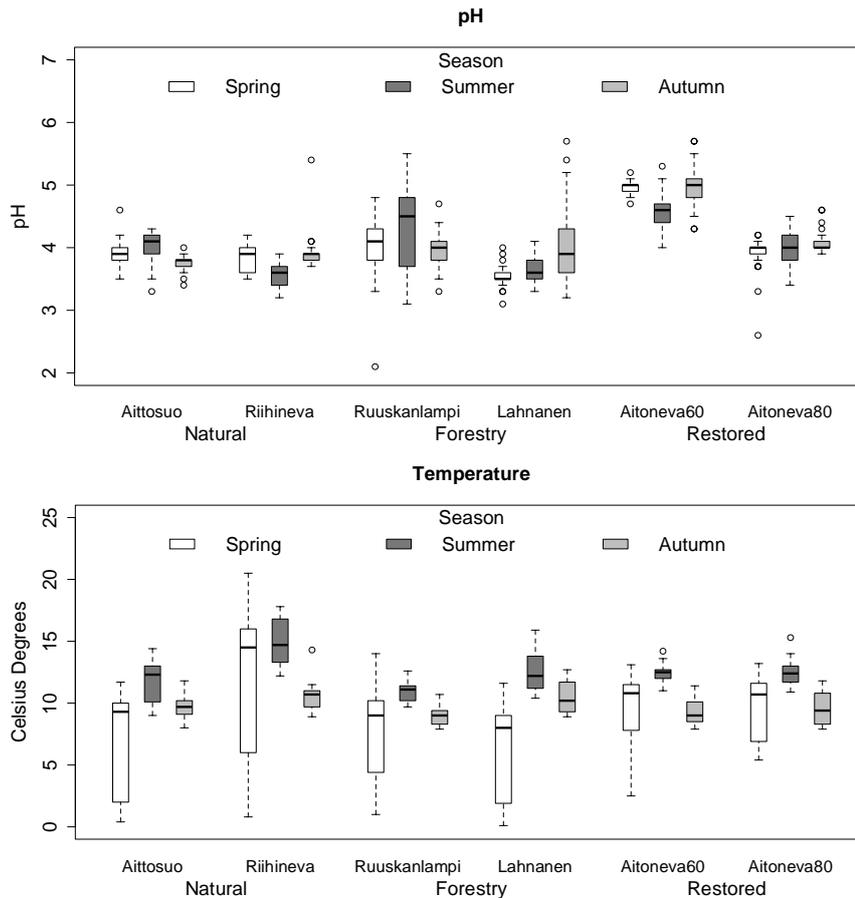
## 184 RESULTS

### 185 Environmental variables

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

189

190



191

192 **Fig. 3** Environmental variables (surface water pH and temperature) in relation to land use and season of

193 sampling. Values correspond to measurements during three years (2013-2015) at each site, during spring,

194 summer and autumn for a total of 15 sampling plots/site at each sampling time. Natural: peatlands not

195 under human use, Forestry: peatlands used for forestry, Restored: peatlands previously under human use

196 but restored either 60 or 80 years ago. Circles: outliers, upper whisker: maximum value excluding

197 outliers, upper box line: upper quartile, middle line inside box: median, lower box line: lower quartile,

198 lower whisker: minimum value excluding outliers.

199

200 TA community data

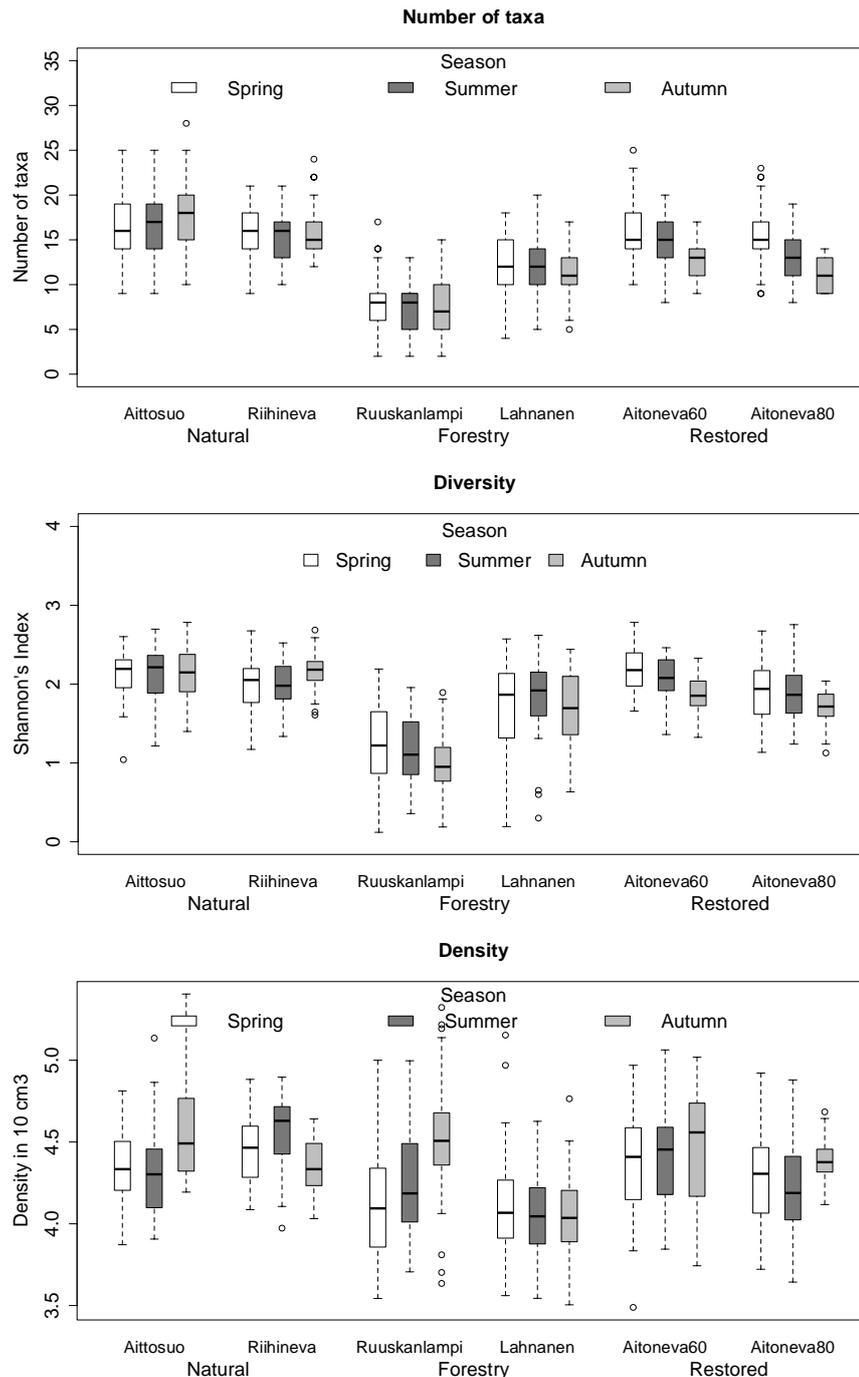
201 We found altogether 62 TA taxa in our study. The highest number of taxa was found at natural

202 sites except in spring 2013 when number of taxa was highest at restored sites (Fig. 4). On average,

203 forestry sites harboured the lowest number of taxa (Fig. 4). TA densities showed a less distinct pattern.

204 Some particularly high densities ( $>150,000/10\text{cm}^3$ ) of TA were recorded in autumn at Aittosuo and

205 Ruuskanlampi (natural and forestry, respectively; Fig. 4).



206

207

**Fig. 4** Number of taxa, Shannon Wiener diversity, and density, whisker boxplots for testate amoebae in

208

relation to land use and season of sampling. Values take into account correspond to measurements during

209

three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling

210

plots/site at each sampling time. Natural: peatlands not under human use, Forestry: peatlands used for

211

forestry extraction, Restored: Peatlands previously under human use but restored either 60 or 80 years

212

ago. Circles: outliers, upper whisker: maximum value excluding outliers, upper box line: upper quartile,

213

middle line inside box: median, lower box line: lower quartile, lower whisker: minimum value excluding

214 outliers. Density values were transformed to logarithmic scale, original values ranged between 3081,87  
215 and 253383,38 testate amoebae/10 cm<sup>3</sup> of fresh *Sphagnum*.

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

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

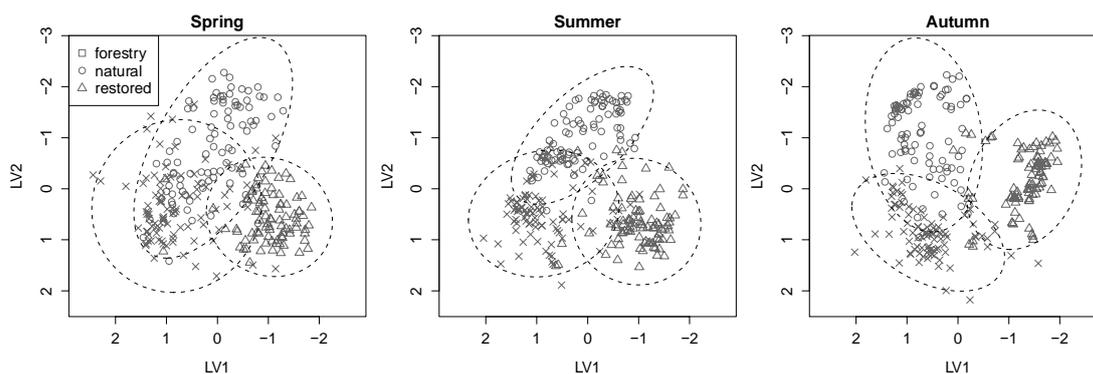
Land use	site	Variation between years						Variation between seasons					
		richness			Shannon Wiener diversity			richness			Shannon Wiener diversity		
		range	H	p	range	H	p	range	H	p	range	H	p
Natural	Riihineva	15.3-15.9	1.3	0.540	1.9-2.1	1.2	0.540	15.5-15.8	0.2	0.910	1.9-2.2	11	<b>0.005</b>
	Aittosuo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

228  
229 At natural sites, *Arcella catinus*, *Hyalosphenia papilio* and *Centropyxis aculeata* were the most  
230 abundant taxa representing 16.3%, 15.7% and 9.9% of the total average TA numbers, respectively. *A.*  
231 *catinus* represented up to 40% of communities at forestry sites. Other common taxa at forestry sites were  
232 *C. aculeata* (14%) and *Trigonopyxis arcula* (9.5%). At restored sites dominant taxa differed from those  
233 at the other types of peatlands, and showed the highest variability between seasons. Overall *Euglypha*  
234 *compressa*, *C. aculeata* and *Diffflugia globulosa* were the most abundant taxa representing on average  
235 41% of the communities at restored sites (appendix 1).

236

237 Community-environment relationships and community ordinations

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



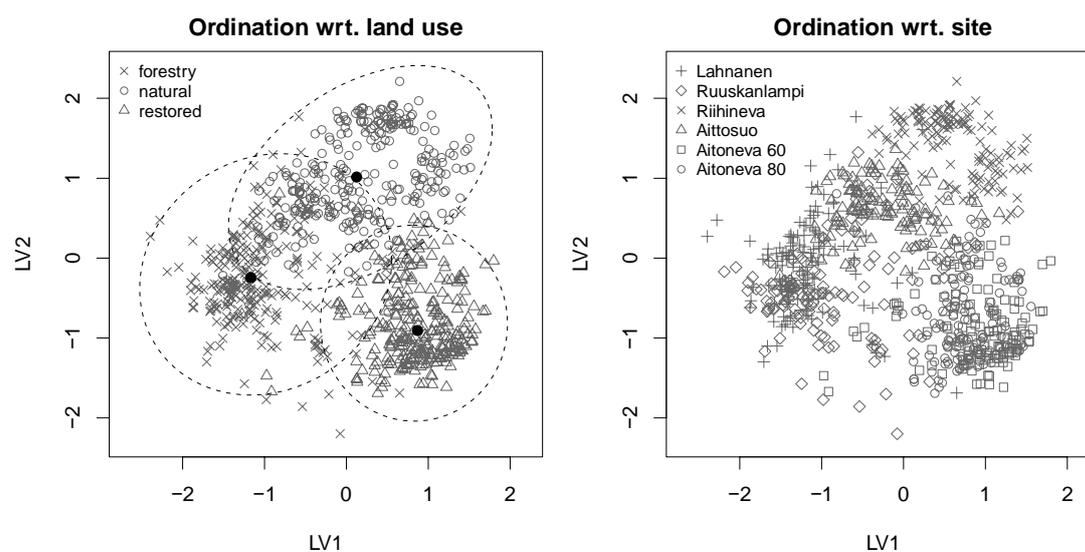
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241 **Fig. 5** The ordination of  $n=270$  sites based on generalized linear latent variable model without any  
 242 covariates assuming negative binomial distributed concentrations. The measurements were taken during  
 243 the years 2013-2015 and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

247

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

252



253

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

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

261

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

274

275

## 276 DISCUSSION

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

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

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

291 Forested sites are inherently different from *Sphagnum*-dominated peatlands, thus such differences  
292 together with the disturbances caused by forestry practices may have restricted the colonisation and  
293 survival of drought intolerant taxa, reducing both the TA taxa richness and their densities. The lowest  
294 water tables were in general recorded at forestry sites, where peat was also mainly drier. In *Sphagnum*  
295 dominated peatlands, peat moisture is considered the main factor affecting TA communities (Tolonen  
296 1986; Booth 2001) and wetter habitats have been found to harbour higher densities of TA (Fournier et  
297 al. 2012). Our results generally supported these findings as natural and restored sites showed both higher  
298 water table levels and TA densities. However, we found particularly high TA densities in autumn samples  
299 at natural and forestry sites. These high TA densities at individual plots were obviously not related to  
300 any of the measured environmental variables as these plots were particularly dry (WTD as low as -22cm  
301 in some cases) at the time of sampling. While we cannot entirely rule out human errors in sample  
302 processing which might have led to such high observed numbers, we feel that observed densities are  
303 more likely the result of other favourable environmental factors, such as nutrient concentrations that can  
304 either affect TA directly (Lamentowicz et al. 2011) or indirectly by controlling their food sources  
305 (Mitchell et al. 2004).

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

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

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

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

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

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

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

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

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

378 Low temporal variation has been attributed to the trophic diversity behaviour of TA and their  
379 capacity to resist changes in temperature and water content through encysting (e.g. Gilbert et al. 1998).  
380 On the other hand, studies of soil TA communities (e.g. Finlay and Fenchel 2004; Tysganov et al. 2013)  
381 have shown that climate-independent drivers of change i.e. local conditions such as soil moisture, either  
382 related to topography or geology (Tysganov et al. 2013) greatly affect the taxa number and abundance  
383 of soil TA. The differences in the responses of TA communities to large vs. local-scale environmental  
384 variation may also be related to the role of vegetation in regulating microclimatic conditions through  
385 shading, precipitation interception, etc. (Wookey et al. 2009; Graae et al. 2012). Other studies on the  
386 influence of seasonal fluctuations on TA community structure and composition (e.g. Lamentowicz et al.  
387 2013; Marcisz et al. 2014) have found differences mainly in TA density between seasons with highest  
388 values during spring. However, such a pattern was not observed in our results.

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

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Appendix 2. The ordination of n=810 sites based on generalized linear latent variable model without any

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covariates assuming negative binomial distributed concentrations. The measurements were taken during

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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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