

Impact of two hot and dry summers on the community structure and functional diversity of testate amoebae in an artificial bog, illustrating their use as bioindicators of peatland health

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SUMMARY

Ongoing climate warming threatens the survival of bogs at the warm/dry limit of their distribution (*e.g.* in central Europe), and jeopardises the restoration of damaged bogs even more. Because vegetation changes can be slow, early indicators of hydrological change such as testate amoebae are useful. We used testate amoeba community structure and community weighted mean of functional traits to monitor the impact of two very hot and dry summers on a small (around 100 m²) artificial peatland constructed in the botanic garden of Neuchâtel, Switzerland. We collected analogous samples in a naturally regenerating cutover peatland at 1000 m a.s.l. in the Jura Mountains as a reference. The comparison of living and dead assemblages in the botanic garden showed an increased representation of smaller testate amoeba taxa (*Corythion dubium*, small *Euglypha* sp.) with a small pseudostome (indicative of dry conditions) and a loss of mixotrophy in 2015, followed by a weaker further shift in 2016. Nevertheless, the testate amoeba community structure in 2016 still indicated a dry *Sphagnum* bog. Testate amoeba analysis allows rapid assessment of peatland health and/or restoration success. The comparison of living and dead assemblages makes it possible to observe changes within a season in a single sampling campaign.

KEY WORDS: biomonitoring, community weighted mean, functional traits, *Sphagnum* peatlands

INTRODUCTION

Sphagnum-dominated peatlands are ecosystems with permanently waterlogged soils, low nutrient availability and high acidity (Mitsch & Gosselink 2000). Anoxic conditions hamper organic matter mineralisation leading to carbon accumulation. Northern peatlands constitute an important carbon stock of 500 ± 100 Gt, representing one-third of all soil C trapped on an average of 3 % of the total land area (Gorham 1991, Yu 2012). Peatlands are threatened by direct impacts such as peat extraction (Ramsar 2013) and ongoing climate change, resulting in the release of C and thus feeding back positively to warming (Davidson & Janssens 2006, Fleischer *et al.* 2016). Peatlands contain a relatively small number of highly specialised taxa which are well adapted to the characteristically harsh ecological conditions (*e.g.* *Sphagnum* spp., *Eriophorum vaginatum*, *Andromeda polifolia*). However, these species are poor competitors (MacArthur & Wilson 1967) and when conditions change (*e.g.* due to nutrient inputs, drought or drainage) less specialised and more competitive species (*e.g.* *Molinia caerulea*, *Betula pendula/pubescens*) colonise, leading to losses of biodiversity and C-sequestration function (Chapman

et al. 2003). Peatlands also represent invaluable archives of past environmental changes and human history (Buckland 1993). Understanding global change effects on peatlands is, therefore, relevant at the global (C-cycling) and local/regional (biodiversity conservation, preservation of peat archives) scales. To achieve this, a combination of observational, experimental and modelling approaches are used. Our focus here is on an experimental and observational study making use of an artificial peatland built in a botanic garden for outreach, conservation and research purposes.

In 2014, a small (*ca.* 100 m²) experimental raised bog was constructed in the botanic garden of Neuchâtel, Switzerland (Figure A1 in Appendix) and planted with characteristic species collected from regional natural and regenerating cutover bogs. High moisture and low nutrient contents are needed to ensure the viability of specific raised bog plants and associated microbial communities (Andersen *et al.* 2013). Recreating a lowland peatbog is challenging, because the local climate at 530 m a.s.l. is warmer and drier than in the mountains at 1000 m a.s.l., where most Swiss peatlands are located. Peatlands including raised bogs once existed at lower altitudes in Switzerland but were almost totally destroyed

during the last two centuries (Grünig 1994). During 2015, extended periods of drought occurred in Switzerland (MétéoSuisse 2016). This was followed by a humid winter and spring, with more than 750 mm (75 % of the mean annual amount) of rainfall during the first half of 2016 in Neuchâtel, and then by a short but intensive heatwave in the summer of 2016 (MétéoSuisse 2017). In the autumn of 2015 the experimental bog was very dry with deep cracks in the peat and extremely low water table (the lowest average for five measurement points was recorded on 08 August 2015 at -59.7 cm; Figure A2). The rainy spring of 2016 provided suitable conditions for the recovery of most of the bog vegetation. A vegetation survey indeed showed that most of the vascular plants and bryophytes survived (Mitchell *et al.* unpublished data), although the most fragile species including *Drosera rotundifolia* were lost. However, the water table has not reached the high levels of spring 2015 for any extended period since (Figure A2), suggesting that an appropriate water level for long-term survival of the bog vegetation cannot be maintained in this artificial ecosystem. Therefore, the meteorological conditions of 2015 and 2016 represented a first real challenge for this experimental bog, as well as a valuable ‘unintentional experiment’ opportunity to test the resistance of characteristic bog communities to unfavourable conditions.

To assess the impact of these two climatically challenging years on the bog, in 2015 and 2016 we analysed the patterns of testate amoeba communities in *Sphagnum* patches of different sizes spanning a broad range of shading by vascular plants. Testate amoebae are unicellular protists that build shells known as ‘tests’. They are the dominant micro-eukaryotes in *Sphagnum* peatlands (Gilbert & Mitchell 2006). Owing to their diversity (more than 100 taxa can be found in peatlands), relatively short generation times (weeks to months - allowing communities to adapt to changing conditions), their community turnover along environmental gradients, and the decay-resistant tests they produce, testate amoebae are useful indicators for monitoring changes in environmental conditions (Mitchell *et al.* 2008). Testate amoebae are widely used as palaeohydrological indicators (Booth 2002, Payne *et al.* 2008, Swindles *et al.* 2009, Qin *et al.* 2013, Lamentowicz *et al.* 2015, Gałka *et al.* 2017, Kołaczek *et al.* 2017) and increasingly also as biomonitors in peatland monitoring and restoration (Davis & Wilkinson 2004, Booth 2008, Laggoun-Defarge *et al.* 2008, Sullivan & Booth 2011, Daza Secco *et al.* 2016). Indeed, they have been shown to be more accurate than vegetation as reflectors of water table depth and hydrochemistry in *Sphagnum* peatlands

(Koenig *et al.* 2015) and to respond rapidly to changes in hydrology and peatland restoration (Buttler *et al.* 1996, Lamentowicz *et al.* 2013, Marcisz *et al.* 2014a, Daza Secco *et al.* 2016).

Testate amoebae can be used as bioindicators using the classical taxonomical approach as well as the functional traits approach; here we explore both. Functional diversity and community weighted mean of traits are meaningful tools for measuring and estimating ecosystem functioning (Lavorel & Garnier 2002, Ricotta & Moretti 2011, Laliberté *et al.* 2014). The functional diversity approach assumes that species morphology and life history traits reflect and affect niche dimensions (Kearney *et al.* 2010, Villéger *et al.* 2011, Mouillot *et al.* 2013). Well-selected traits can be used to assess the impact of stress or disturbance on communities by revealing changes in niche dimensions (Moretti & Legg 2009, Mouillot *et al.* 2013, Fournier *et al.* 2015) and, through these effects on communities, inform on ongoing ecological processes (*e.g.* changes in C cycling due to alterations of the food web structure). This approach was first developed for plants and animals and is now being explored for microorganisms such as ciliates (Barnett *et al.* 2013) and testate amoebae (Fournier *et al.* 2015, 2016; van Bellen *et al.* 2016). The functional traits approach partly overcomes identification difficulties and provides data that are more directly informative ecologically than change in taxonomic composition (Booth 2008, Lamentowicz *et al.* 2013, 2015; Fournier *et al.* 2015, Koenig *et al.* 2015, Daza Secco *et al.* 2016). For example, Fournier *et al.* (2012) demonstrated that testate amoeba functional traits were related to soil moisture in a floodplain, Marcisz *et al.* (2016) showed that the proportion of mixotrophic taxa decreased and the proportion of small taxa with a hidden (plagiostomic) pseudostome (shell aperture) increased in response to fire and peat extraction in two palaeoecological studies in Poland, and Koenig *et al.* (2018) observed a decrease of mixotrophic taxa and an increase in small taxa with a ventral or ventral-central pseudostome following lowering of the water level in a mesocosm study.

In this study we used testate amoeba community composition and community weighted mean (CWM) of traits to assess the impact of extreme climate events on the artificial experimental peatland in the botanic garden of Neuchâtel, Switzerland. We hypothesised that: 1) the community structure and CWM of functional traits of testate amoebae from *Sphagnum* patches would change in response to the severe droughts of 2015 and 2016; and 2) that the magnitude of this effect would be higher in small *Sphagnum* patches with low shading by vascular

plants than in larger, more shaded patches because larger, more shady patches would provide more stable and favourable conditions for testate amoebae. We expected shifts in CWM to reflect changes in moisture conditions and to inform on changes in ecosystem functioning. We compared these temporal patterns with the testate amoeba communities of similar *Sphagnum* patches sampled in a naturally recovering peatland located at 1000 m a.s.l. in the Jura Mountains, used as a reference. We expected to see clearer differences in the lowland experimental site due to more stressful climatic conditions.

METHODS

Experimental bog and study design

The experimental bog was built in autumn 2014 in the botanic garden (BG) of Neuchâtel (530 m. a.s.l.). A 60 cm layer of clay-rich sediments (marls) was overlaid with 0.5–1.5 m of peat collected from a degraded peatland in the Jura Mountains near

Neuchâtel. The primary purpose of the experimental bog is to show to the public an example of these threatened ecosystems - 90 % of the original mire area of Switzerland has been converted to agricultural land or lost to peat extraction, and 90 % of the remaining 10 % is degraded (Grünig 1994) - and use it as an educational tool. A second aim is to maintain some regionally rare and endangered plant species such as *Betula nana* L. *ex-situ*. The third aim is to conduct experimental studies on aspects of peatland restoration under challenging climatic conditions. Water table level (relative to bog surface) was measured manually, in five dipwells inserted in different parts of the bog, at weekly intervals.

To compare our experimental results with a more natural reference, we selected the Bois-des-Lattes (BdL) peatland, which is Switzerland's largest remaining peatland (Vallée des Ponts-de-Martel, 1000 m a.s.l.; Figure 1, Table 1). This site was exploited intensively for peat extraction until the mid-twentieth century and is now protected by law. In 2015, drainage ditches were filled in to raise the

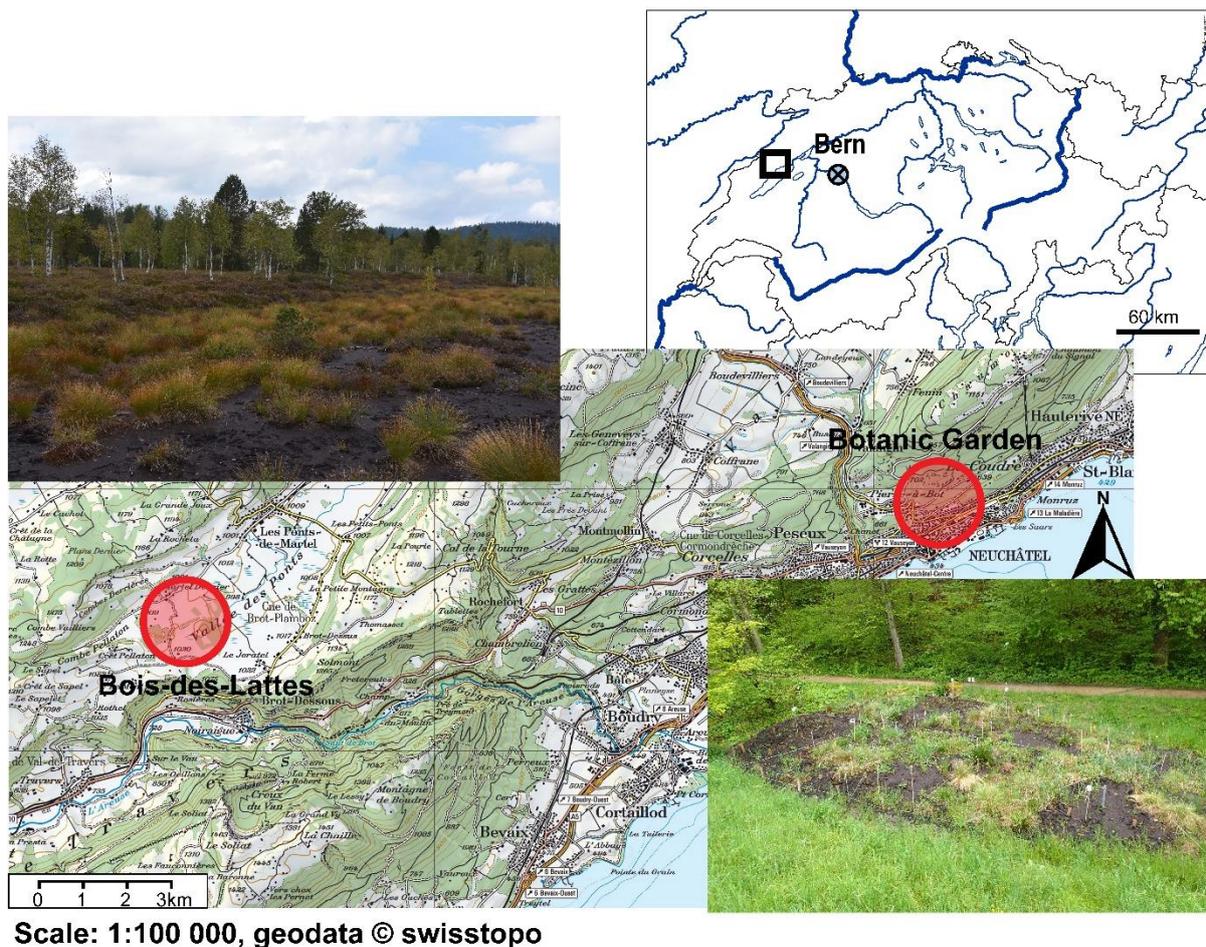


Figure 1. Locations and pictures of the experimental bog at the botanic garden in Neuchâtel and the Bois-des-Lattes peatland, Vallée des Ponts-de-Martel (Neuchâtel), Switzerland.

water table in a formerly extracted zone. This allowed spontaneous recolonisation by bog vegetation in patches of different sizes.

In order to assess how vegetation patch size and shading by vascular plants influenced the survival of testate amoebae during a drought period, we selected *Sphagnum* carpets of various sizes with different degrees of vascular plant cover. We measured the diameter of each selected patch (average of several measurements if the patch was of irregular shape) and estimated vascular plant cover using the Londo semi-quantitative scale (Londo 1976). At the Bois-des-Lattes reference site, *Sphagnum* patches on a large area (~8 ha) of formerly cutover peat surface were selected to encompass the whole range of potential vascular plant cover (10–100 % at both sites) and patch diameter (6–35 cm, *i.e.* available range, at BG and 13–45.5 cm at BdL where patches smaller than 13 cm could not be found).

In the BG peatland, about ten randomly picked *Sphagnum* moss shoots *per* patch were collected for testate amoeba extraction on 16 September 2015 (BG 2015) and 27 October 2016 (BG 2016). Similarly, on 16 September 2016, we collected 12 samples at the Bois-des-Lattes. For all samples, the top 3 cm of the moss (including the capitulum) was collected and fixed (on the same day) with a 4 % solution of glutaraldehyde (C₅H₈O₂) to allow differentiation of living and recently dead amoeba assemblages (Mitchell & Gilbert 2004).

Testate amoebae

Testate amoebae were extracted by standard sieving (150 µm) and back sieving (15 µm) (Charman 2001, Booth *et al.* 2010). Rose Bengal was added to stain the cytoplasm and thus differentiate living from dead individuals. One tablet of *Lycopodium clavatum* (standard preparation by Department of Quaternary Geology, Lund University, Sweden, batch N° 938934, 10,679 ± 400 spores *per* tab) was added for density calculation (Stockmarr 1971, Booth *et al.* 2010). Testate amoebae were identified at morpho-species level under light microscopy at 200× and 400× magnification. Because this work was partly done by undergraduate project students, the identification of some taxa was challenging. To ensure consistency and avoid possible bias due to taxonomic confusion (Heger *et al.* 2009, Payne *et al.* 2011) the following grouping of taxa was applied: *Centropyxis aerophila* includes *C. aerophila*, *C. cassis*, *C. platystoma* and *Cyclopyxis kahli*; *Corythion dubium* includes *C. delamarei*; *Cryptodiffugia* sp. includes several other small taxa (*C. oviformis*, *C. minuta*); *Diffugia lucida* type includes all *Diffugia* larger than 50 µm and *Diffugia*

pulex type includes *Diffugia* and other taxa smaller than 50 µm with a shell made of xenosomes (*e.g.* *Cryptodiffugia sacculus*, *Schoenbornia humicola* and *Pseudodiffugia* sp.); *Euglypha* taxa are split into two groups, namely large taxa (> 60 µm) and small taxa (< 60 µm); taxa from the *Nebela tinctoria-collaris* group (Kosakyan *et al.* 2012) are split between *N. tinctoria* (< 95 µm, *N. tinctoria* s.str., *N. gimlii*, *N. guttata*, *N. rotunda*) and *N. collaris* (> 95 µm, *N. collaris*, *N. bohémica*); *Phryganella acropodia* includes *Cyclopyxis eurystoma*. Morphotaxa were grouped into three classes defined by water table depth optima (< 15 cm, 15–30 cm, > 30 cm) based on several datasets (Jura, Poland and Pechora, Russia) (Mitchell *et al.* 1999, Lamentowicz & Mitchell 2005, Lemonis 2012). We counted a total of 100 individuals (dead and alive) *per* sample from BdL. At BG, limited amounts of *Sphagnum* were sampled to avoid significantly impacting the already stressed vegetation and we aimed for minimum total counts of 50 individuals, which have been shown to provide valuable ecological information (Payne & Mitchell 2008). In all cases counting was stopped after a maximum of two hours. Therefore, although the taxonomic resolution of this study is not ‘state of the art’ it does allow us to demonstrate the information that can be derived by non-specialists.

Functional diversity

The community weighted mean (CWM) of functional traits was calculated separately on living and dead assemblages. Morphometric traits were measured randomly during counting under light microscopy. As test length and biovolume were correlated (adjusted R² = 69 %, calculated on data from a previous study, Koenig unpublished), we used test length as a proxy for biovolume because length is easier to measure. Community weighted mean of traits represents the mean value of each trait weighted by the species’ relative abundance (Lavorel & Garnier 2002, Dray & Legendre 2008, Suding *et al.* 2008). Functional traits are assumed to reflect the local ecological conditions and can be used to estimate the niche dimension through the functional niche (multi-dimensional space in which traits are axes) (Villéger *et al.* 2011). We selected traits that are expected to respond to changes in humidity, as listed (1–6) below.

1) Mixotrophy: binary (1 = mixotrophy, 0 = heterotrophy). The presence of photosymbionts is interpreted as an adaptation to wet oligotrophic conditions such as those existing in bog pools. Mixotrophy is both a response trait (mixotrophs being indicative of wet conditions; Mitchell *et al.* (1999)) and an effect trait (mixotrophs have been shown to

enhance carbon fixation (Stoecker *et al.* 2009, Jassey *et al.* 2015)).

- 2) Test compression: binary (1=clearly compressed, 0=approximately round in cross-section). Compressed tests are expected to favour survival in a thin water film but at the cost of increasing the quantity of test material required relative to amoeba biovolume (Bonnet 1964).
- 3) Origin of test material: factor with four levels (protein, idiosomes, xenosomes prey, xenosomes particles). Testate amoeba shells are composed of organic material (protein test), secreted silica plates (idiosomes), or made of particles found in the environment (xenosomes, either prey skeleton re-used in test construction or inorganic particles). The presence of taxa is related to the availability of test material (Gilbert & Mitchell 2006, Schwind *et al.* 2016).
- 4) Pseudostome position: factor with three levels (terminal, ventral, ventral-central). The position of the pseudostome could reflect an adaptation to moisture as an exposed (terminal) pseudostome is better adapted to wet environments and a hidden one to drier conditions (Bonnet 1964).
- 5) Pseudostome size: quantitative (range 4.5–55.8 μm). The pseudostome size is an effect trait as it determines the range of prey - taxa with a large pseudostome occupy higher trophic positions in microbial food webs (*i.e.* they are predators of other protists and micro-eukaryotes) while taxa with a small pseudostome are mainly bacterivorous (Jassey *et al.* 2013b).
- 6) Test length: quantitative (range 18.2–153.7 μm). Used as a proxy for biovolume. This is a response trait - larger taxa have longer generation times than small taxa and are thus expected both to be more sensitive to perturbation and to recover more slowly from perturbations. As larger taxa generally also have a large pseudostome this is also, to some extent, an effect trait.

Numerical analyses

All analyses were based on relative abundance after removal of rare taxa (*i.e.* mean relative abundance < 1 %, Table A1) or on density. Differences in mean values between sites or between living and dead assemblages were tested using the Wilcoxon rank sum test because the data followed neither normality nor homoscedasticity criteria. Statistical tests between living and dead assemblages were considered as repeated measurements. The same applied to the BG 2015 and BG 2016 datasets, as the same patches were sampled. Pairwise comparison for CWM of each trait between sites employed the Wilcoxon rank test with Holm correction and exact

p-value. Student's t-test was used to test the differences between environmental variables (patch size, vascular cover) as the data conformed to the assumptions for parametric analyses.

We performed β -diversity analyses between all pairs of testate amoeba assemblages (relative abundance data) using the Bray-Curtis index, which is equivalent to the Sørensen-Dice diversity index for abundance data, with bias adjustment (Baselga & Orme 2012, Oksanen 2015). Finally, to test our hypothesis that shade (provided by vascular plant cover) and patch size should protect the testate amoeba community from drought, we plotted the β -diversity between living and dead assemblages for each site *versus* the gradient of 1) vascular plant cover expressed as a percentage of the maximum for the site, 2) patch size as a percentage of the maximum for the site, and 3) the average of both.

All analyses were carried out with R statistical software (R Core Team 2016). β -diversity indices were calculated with the package betapart (Baselga & Orme 2012) and community weighted mean of functional traits was calculated using the package FD (Laliberté *et al.* 2014).

RESULTS

Climate and sampled moss patches at the two sites

The average climate is sunnier, colder and wetter at the Bois-des-Lattes (BdL) reference site than at the botanic garden (BG) (Table 1), on the basis of annual mean values for 1981–2010 which differ (BdL - BG) by +700 Kwh m^{-2} of solar radiation, $-4.5\text{ }^{\circ}\text{C}$, +520 mm of precipitation, +250 cm of accumulated snow and +32 days with snow cover (MétéoSuisse 2017). The years 2015 and 2016 were hotter and drier than the 1980–2010 average at both BdL and BG. In Neuchâtel, 2015 was the hottest year since 1864 (1.29 $^{\circ}\text{C}$ above the 1981–2010 mean) and included the second hottest summer after 2003. In north-west Switzerland, total hours of sunlight was 125 % and precipitation 73 % of the mean for 1981–2010 (MétéoSuisse 2016). The situation in 2016 was less extreme: average temperature was 0.6 $^{\circ}\text{C}$ above the mean for 1981–2010, but total hours of sunlight and precipitation were within the 1981–2010 norms. However, this lack of overall difference was due to the cold and very wet spring. The second half of the year was warmer with a short but record summer heatwave and an extremely warm September (2.7 $^{\circ}\text{C}$ above the 1981–2010 mean in Neuchâtel) (MétéoSuisse 2017). Overall, BdL was only slightly less impacted than BG during the climate anomaly in 2015 and 2016.

Vascular plant cover did not differ significantly between BdL and BG (Student's *t*-value = -0.072, *p*-value = 0.943) but *Sphagnum* patches were on average significantly larger in BdL (*t*-value = 3.903, *p*-value < 0.001). In BG, neither mean patch size nor mean vascular plant cover differed significantly between 2015 and 2016 (Student's paired *t*-value = 1.36, *p*-value = 0.21 for patch size and *t*-value = -1.035, *p*-value = 0.33 for vascular cover). BdL samples were mostly *Sphagnum magellanicum* (66.6 %), and to a lesser extent *S. fallax* and *S. rubellum* (both 16.6 %). The BG samples were mainly *S. fallax* (44.4 %) and *S. magellanicum* (33.3 %). Overall, most of the *Sphagnum* mosses sampled were alive (BG 2015: 77.8 %, BG 2016: 91.7 %, BdL: 83.3 %), the remaining being half dry or possibly already dead.

Testate amoeba density and community structure

The density of testate amoebae (number of individuals *per* gram of dry *Sphagnum*) differed significantly between sites and sampling dates. It was higher in BdL (10.8, with se (standard error) 1.2×10^4 g^{-1}), lower in BG 2016 ($3.59 \pm 0.86 \times 10^4$ g^{-1}), and lowest in BG 2015 ($2.68 \pm 0.25 \times 10^4$ g^{-1} , Figure 2). The proportion of living individuals was highest in BG 2016 (62 ± 6 %), lower in BdL, (43 ± 2.7 %), and lowest in BG 2015 (25 ± 0.7 %).

We identified a total of 24 morphotaxa, and after removing rare taxa (mean relative abundance < 1%), 17 morphotaxa remained for further analyses (Figure 3 and Table A1). Overall, the most abundant taxa were *Corythion dubium* (10.9 %), small *Euglypha* sp. (10.2 %), *Hyalosphenia papilio* (9.6 %), *Diffugia pulex* group (9.5 %), *Phryganella acropodia*

Table 1. Location and long-term (1981–2010) climatic characteristics (MétéoSuisse 2016, 2017) of the two study sites.

Site	GPS		Altitude ^a m a.s.l.	Global radiation ^a Kwh m ⁻² year ⁻¹	Mean annual temperature ^b °C	Average precipitation ^b mm year ⁻¹	Total amount of snow ^b cm year ⁻¹	Days with snow cover ^b days year ⁻¹
	Latitude	Longitude						
BG	46° 59' 06" N	6° 56' 08" E	529	375	10.2	978	45.6	13.4
BdL	46° 58' 03" N	6° 42' 03" E	1000	1073	5.7 ^c	1500 ^c	300 ^c	45.5 ^c

^a Geoportal of Service d'Information du Territoire Neuchâtelois (SITN); ^b Mean value 1981–2010, from Federal Office of Meteorology and Climatology MétéoSuisse; ^c rounded mean of measurements at La Brévine and la Chaux-de-Fonds, located 11 km to the west and 12 km to the north-east of Bois-des-Lattes, respectively.

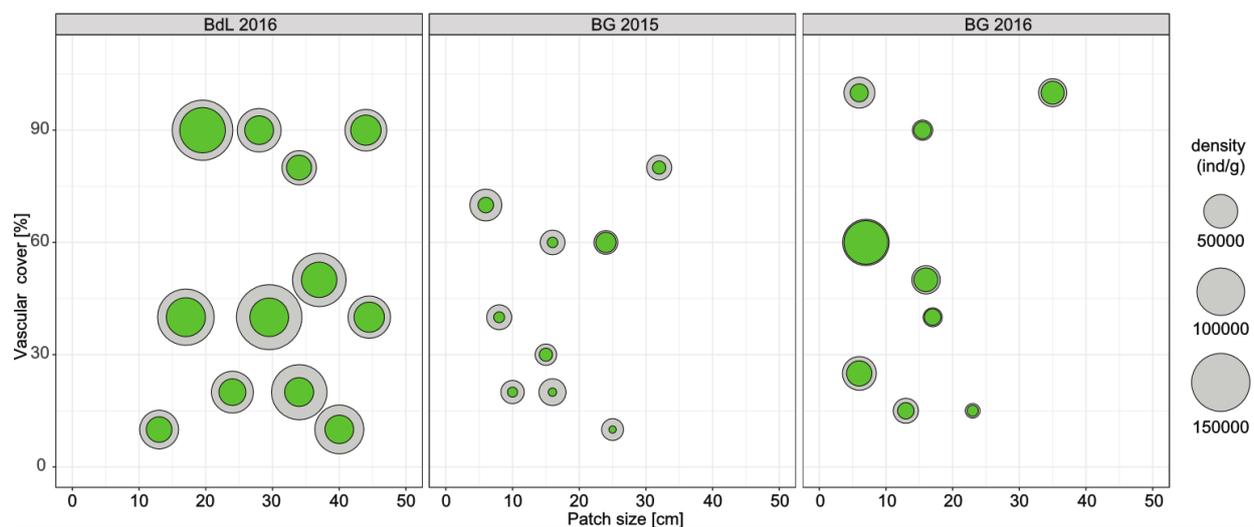


Figure 2. Biplots of overall density of testate amoebae assemblages in the Neuchâtel Botanic Garden experimental peatland (BG 2015, BG 2016) and the Bois-des-Lattes reference peatland (BdL) in relation to *Sphagnum* patch diameter (x axis) and percentage vascular plant cover (y axis). The area of each circle is proportional to the testate amoeba density: grey circle = total density of dead + living assemblages; superposed (green) circle = density of living assemblages. The differences in mean patch size and mean vascular plant cover between the two BG sampling dates were not significant (based on Student's *t*-test).

(9.3 %) and *Nebela tincta* (9.1 %); Table A1). Large *Euglypha* sp. was the most frequent taxon (found in 93.3 % of samples) but was not abundant (4.5 %). Other frequent taxa were *C. dubium*, small *Euglypha* sp., *Nebela tincta*, *N. collaris*, *P. acropodia*, *Assulina muscorum* and *H. papilio* (60–83.3 %). The smallest

taxa were the most abundant (*D. pulex*, *P. acropodia*, small *Euglypha* and *N. tincta*, $5.6\text{--}10.3 \times 10^3 \text{ g}^{-1}$). *Archerella flavum* was never found alive and could be a contaminant from the adjacent bare peat. In BdL 2016, the most abundant morphotaxa were *Diffflugia pulex* (22 %, of which 48.9 % was alive),

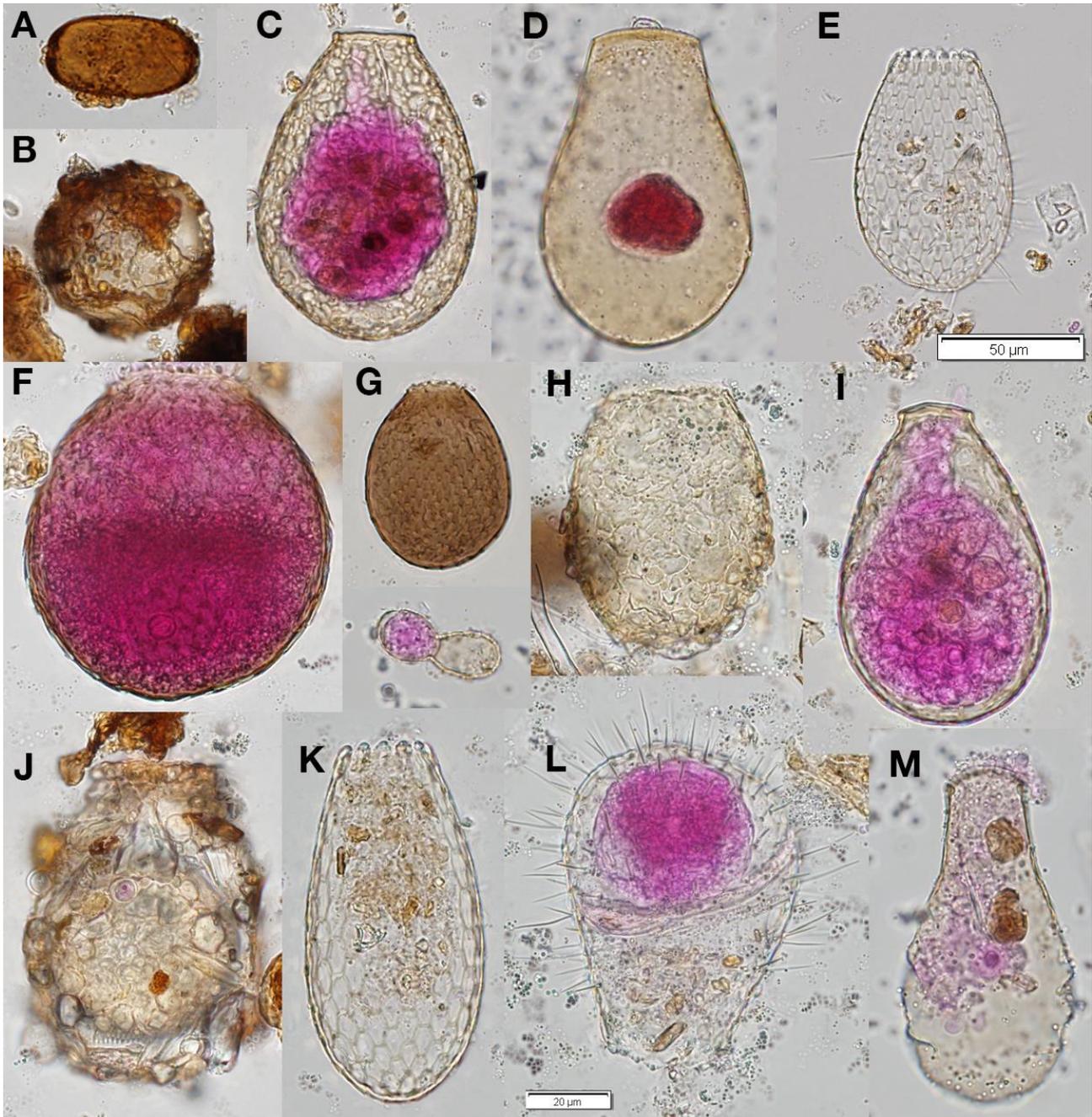


Figure 3. Light microscopy pictures (bright field and differential interference contrast) of selected testate amoeba species from the botanic garden (BG) and Bois-des-Lattes (BdL) peatlands. A: *Archerella flavum*, B: *Diffflugia globulosa* (included in *Phryganella acropodia* type), C: *Nebela collaris*, D: *Hyalosphenia papilio*, E: *Euglypha compressa*, F: *Assulina scandinavica* (included in *A. seminum* type), G: *Assulina muscorum*, H: *Heleopera sylvatica*, I: *Nebela tincta* type, J: *Diffflugia* cf. *lucida*, K: *Euglypha* cf. *tuberculata*, L: *Euglypha* cf. *ciliata*, M: *Hyalosphenia elegans*. Note the two scales used: images A–E were taken at 200× magnification, scale bar = 50 µm; images F–M were taken at 400× magnification, scale bar = 20 µm.

P. acropodia (14.2 %, 46.1 % alive), *N. tincta* (9.9 %, 44.7 % alive) and large *Euglypha* (9.1 %, 42.7 % alive). All other taxa each accounted for less than 7 % of the total community (Table 2). In BG

2015 (Table 2) only two species were found, mostly alive: *C. dubium* (relative abundance 15.3 %, 80.5 % of individuals living) and *A. muscorum* (2 % relative abundance, 50 % alive). *Hyalosphenia papilio*

Table 2: Descriptive statistics of testate amoeba communities (dead and living pooled together) from the experimental bog in Neuchâtel Botanic Garden (BG) and the Bois-des-Lattes peatland (BdL), with density (*per gram of dry Sphagnum*) (upper table) and relative abundance (lower table) presented separately for each site. Morphotaxa are ordered according to water table depth class (WTD, estimated on the basis of previous studies in Europe (Mitchell *et al.* 1999, Lamentowicz & Mitchell 2005, Lemonis 2012); se = standard error.

Morphotaxa	WTD	BdL					BG 2015					BG 2016				
		mean	se	median	min	max	mean	se	median	min	max	mean	se	median	min	max
<i>Archerella flavum</i>	<15	4120	1108	3638	0	13074	341	112	481	0	794	219	148	0	0	1177
<i>Euglypha</i> "big taxa"	<15	5873	1456	3398	998	14517	715	355	0	0	3116	2208	929	1239	0	7525
<i>Hyalosphenia elegans</i>	<15	5324	2465	2192	0	29714	2510	707	2353	0	6037	133	104	0	0	929
<i>Hyalosphenia papilio</i>	<15	5462	3485	499	0	42864	6157	2136	2886	0	18733	754	530	0	0	4767
<i>Assulina seminulum</i>	15–30	2375	639	1413	0	6049	603	341	0	0	3064	403	209	0	0	1559
<i>Centropyxis aerophila</i>	15–30	1151	500	0	0	4840	2486	755	1677	0	6232	3187	1755	0	0	12713
<i>Euglypha</i> "small taxa"	15–30	9859	2308	6877	1497	27361	1709	568	1022	335	5608	5993	2563	2477	0	22362
<i>Heleopera rosea</i>	15–30	1715	1328	0	0	16168	787	554	0	0	4986	192	127	0	0	929
<i>Heleopera sylvatica</i>	15–30	1013	411	645	0	4975	706	546	0	0	4986	105	105	0	0	941
<i>Hyalosphenia minuta</i>	15–30	2659	1163	0	0	11007	0	0	0	0	0	0	0	0	0	0
<i>Nebela collaris</i>	15–30	6079	1742	4133	0	20283	374	374	0	0	3367	6145	3793	1881	0	35868
<i>Assulina muscorum</i>	>30	3172	1285	1448	0	15084	734	545	0	0	4985	3741	1925	808	0	17872
<i>Corythion dubium</i>	>30	1853	551	1001	0	5788	4035	1758	1004	0	15282	6032	2267	3179	0	21832
<i>Cryptodiffugia sp</i>	>30	4626	1063	4619	0	13074	1237	863	0	0	7215	0	0	0	0	0
<i>Diffugia pulex</i>	>30	25183	5926	19070	0	78445	0	0	0	0	0	658	413	0	0	3531
<i>Nebela tincta</i>	>30	9723	2388	9077	499	25982	3976	1013	3689	0	10198	1784	1727	0	0	15595
<i>Phryganella acropodia</i>	>30	15762	3732	11082	2935	52157	0	0	0	0	0	3720	1280	1881	0	9535

Morphotaxa	WTD	BdL					BG 2015					BG 2016				
		mean	se	median	min	max	mean	se	median	min	max	mean	se	median	min	max
<i>Archerella flavum</i>	<15	3.9%	1.0%	3.2%	0.0%	10.9%	1.3%	0.4%	1.4%	0.0%	3.4%	0.6%	0.4%	0.0%	0.0%	3.4%
<i>Euglypha</i> "big taxa"	<15	5.9%	1.5%	4.1%	0.6%	18.0%	2.3%	1.0%	0.0%	0.0%	7.0%	4.8%	1.6%	3.8%	0.0%	15.1%
<i>Hyalosphenia elegans</i>	<15	4.5%	1.6%	2.3%	0.0%	15.7%	10.4%	3.2%	9.5%	0.0%	31.0%	0.6%	0.4%	0.0%	0.0%	3.4%
<i>Hyalosphenia papilio</i>	<15	4.7%	2.8%	0.8%	0.0%	33.8%	24.1%	8.3%	14.3%	0.0%	71.8%	1.7%	1.3%	0.0%	0.0%	11.5%
<i>Assulina seminulum</i>	15–30	2.2%	0.6%	1.4%	0.0%	6.7%	2.1%	1.3%	0.0%	0.0%	11.5%	1.7%	1.1%	0.0%	0.0%	10.0%
<i>Centropyxis aerophila</i>	15–30	1.2%	0.5%	0.0%	0.0%	6.1%	8.4%	2.1%	8.6%	0.0%	19.1%	9.1%	4.6%	0.0%	0.0%	33.7%
<i>Euglypha</i> "small taxa"	15–30	9.1%	1.6%	7.4%	2.3%	20.2%	5.7%	1.4%	3.8%	1.3%	12.7%	16.0%	6.7%	12.8%	0.0%	65.5%
<i>Heleopera rosea</i>	15–30	1.5%	1.0%	0.0%	0.0%	11.9%	2.2%	1.4%	0.0%	0.0%	11.3%	0.6%	0.4%	0.0%	0.0%	3.4%
<i>Heleopera sylvatica</i>	15–30	0.9%	0.3%	0.8%	0.0%	3.7%	2.0%	1.3%	0.0%	0.0%	11.3%	0.2%	0.2%	0.0%	0.0%	1.9%
<i>Hyalosphenia minuta</i>	15–30	2.9%	1.4%	0.0%	0.0%	14.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Nebela collaris</i>	15–30	5.9%	1.5%	4.9%	0.0%	18.3%	1.9%	1.9%	0.0%	0.0%	16.7%	12.2%	4.0%	8.0%	0.0%	37.7%
<i>Assulina muscorum</i>	>30	3.2%	1.2%	2.2%	0.0%	14.3%	2.0%	1.3%	0.0%	0.0%	11.3%	15.4%	6.2%	3.6%	0.0%	46.0%
<i>Corythion dubium</i>	>30	2.1%	0.9%	0.9%	0.0%	11.2%	15.3%	7.0%	3.8%	0.0%	63.3%	18.1%	5.9%	12.0%	0.0%	58.9%
<i>Cryptodiffugia sp</i>	>30	4.1%	0.8%	4.4%	0.0%	7.6%	5.6%	4.1%	0.0%	0.0%	35.7%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Diffugia pulex</i>	>30	22.0%	3.7%	19.9%	0.0%	41.7%	0.0%	0.0%	0.0%	0.0%	0.0%	2.3%	1.3%	0.0%	0.0%	10.3%
<i>Nebela tincta</i>	>30	9.9%	2.8%	10.1%	0.8%	35.0%	15.2%	3.7%	15.4%	0.0%	38.2%	2.0%	1.8%	0.0%	0.0%	16.4%
<i>Phryganella acropodia</i>	>30	14.2%	2.4%	12.6%	3.8%	32.1%	0.0%	0.0%	0.0%	0.0%	0.0%	11.9%	3.5%	12.5%	0.0%	29.3%

was the most abundant taxon (24.1 %, Table 2), but only 8.6 % of individuals were alive. The next most abundant taxa were *N. tincta* (15.2 %, 20.9 % alive) and *Hyalosphenia elegans* (10.4 %, 15.6 % alive). In BG 2016 (Table 2) the overall community composition was different from that of BG 2015. The most abundant taxon was still *C. dubium* (18.1 %, 72.6 % alive) but followed by small taxa of *Euglypha* sp (16 %, 70.3 % alive), *A. muscorum* (15.4 %, 60 % alive), *Nebela collaris* (12.2 %, 65.6 % alive), *P. acropodia* (11.9 %, 43.8 % alive), and *Centropyxis aerophila* (9.1 %, 67.3 % alive). Other taxa each accounted for less than 5 % of the community. Except for *P. acropodia*, all of the most

abundant taxa (*i.e.* relative abundance > 10 %) had a higher proportion of living than dead individuals, but not significantly.

In BdL 2016, only three taxa related to dry conditions (excluding *A. flavum*, see above) reached significantly higher densities in the dead than in the living assemblage (Figure 4). Apart from these taxa, the dead and living communities were quite similar overall. In BG 2015, only *C. dubium* was more abundant in the living than in the dead assemblage, but this difference was not significant (Figure 4). All other taxa reached higher densities in the dead assemblage (significantly so for four morphotaxa: *H. papilio*, *C. aerophila*, small *Euglypha* sp. and

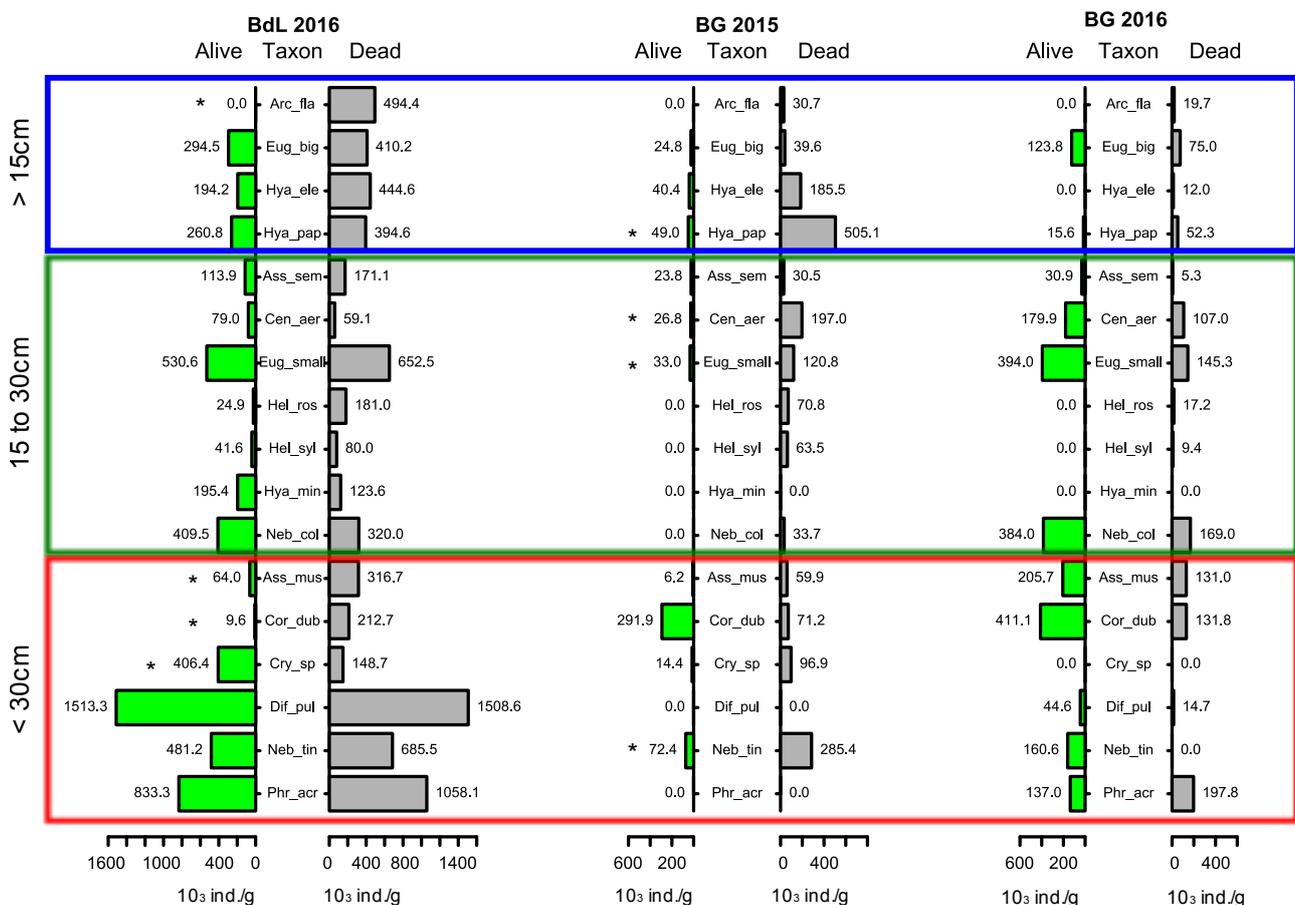


Figure 4. Density of testate amoeba taxa in the Neuchâtel Botanic Garden experimental peatland (BG 2015, BG 2016) and the Bois-des-Lattes reference peatland (BdL). For each dataset, the density of each morphotaxon is split between living (left, green) and dead (right, grey). The morphotaxa are arranged in three groups according to their water table depth optima: < 15 cm (top frame), 15–30 cm (middle frame), > 30 cm (bottom frame). Stars indicate significant differences in density between living and dead populations within the communities (Wilcoxon rank sum test p -value < 0.05). Morphotaxa abbreviations: Arc_fla: *Archerella flavum*; Ass_mus: *Assulina muscorum*; Ass_sem: *Assulina seminulum*; Cen_aer: *Centropyxis aerophila* type; Cor_dub: *Corythion dubium*; Cry_sp: *Cryptodiffugia* sp; Dif_pul: *Diffugia pulex* type; Eug_big: *Euglypha* ‘big taxa’; Eug_small: *Euglypha* ‘small taxa’; Hel_ros: *Heleopera rosea*; Hel_syl: *Heleopera sylvatica*; Hya_min: *Hyalosphenia minuta*; Hya_ele: *Hyalosphenia elegans*; Hya_pap: *Hyalosphenia papilio*; Neb_col: *Nebela collaris*; Neb_tin: *Nebela tincta*; Phr_acr: *Phryganella acropodia* type.

N. tincta). By comparison with the profile of the BdL community, the structure of the dead assemblage in BG 2015 seemed to be related to wetter conditions (especially due to the dominance of *H. papilio*). In BG 2016, a clear recovery occurred, as shown by the increased density of living individuals. However, the community shifted towards a dominance of taxa related to drier conditions than in BG 2015, but still within the range of the BdL community (Figure 4).

Community weighted mean of traits

The community weighted mean (CWM, Figure 5, Table A2) of traits in BG 2015 shifted significantly between the dead and living assemblages towards smaller taxa (Wilcoxon p-value = 0.044), more compressed (p-value = 0.021), with a smaller pseudostome (p-value = 0.013), an increased proportion of tests made of agglutinated particles (xenosomes, p-value = 0.013), and a loss of mixotrophy (p-value = 0.013). By contrast, there was no significant difference in CWM between living and dead assemblages in BG 2016 (Figure 5, Table A2). In BdL, the CWM did not differ significantly between living and dead assemblages, except for mixotrophy which was significantly lower in the living assemblage (Wilcoxon rank test p-value = 0.006; Figure 5, Table A2) and could be linked to *A. flavum*. The CWM of BG 2016 showed no significant differences from either BdL 2016 (living and dead assemblages) or the living assemblage of BG 2015 (Figure 5, Table A3).

How well do patch size and shading explain the shifts in communities?

To test if patch size or the vascular cover (\approx shading) offered protection against desiccation for the testate amoeba community, we calculated the β -diversity between the living and the dead assemblages, based on the assumption that the dead assemblage represented the community living in the sampling spot before the heatwave. For each site we plotted the β -diversity *versus* 1) the relative vascular plant cover, 2) the relative patch size, and 3) a combination of both (Figure 6). In BdL and BG 2016, no correlation appeared between protection against desiccation and β -diversity. More surprisingly, and contrary to our hypothesis, in BG 2015 the β -diversity between living and dead assemblages increased with shading, and to a lesser extent with increasing patch size (Figure 6).

DISCUSSION

Testate amoeba density and community composition in BdL and BG

Testate amoeba density was around three times higher in the Bois-des-Lattes peatland (BdL), a naturally regenerating bog in the Jura Mountains used here as a reference, than in the artificial peat bog in the botanic garden (BG) of Neuchâtel (Figure 2). Nevertheless, the densities at both sites were within the range reported from testate amoeba communities

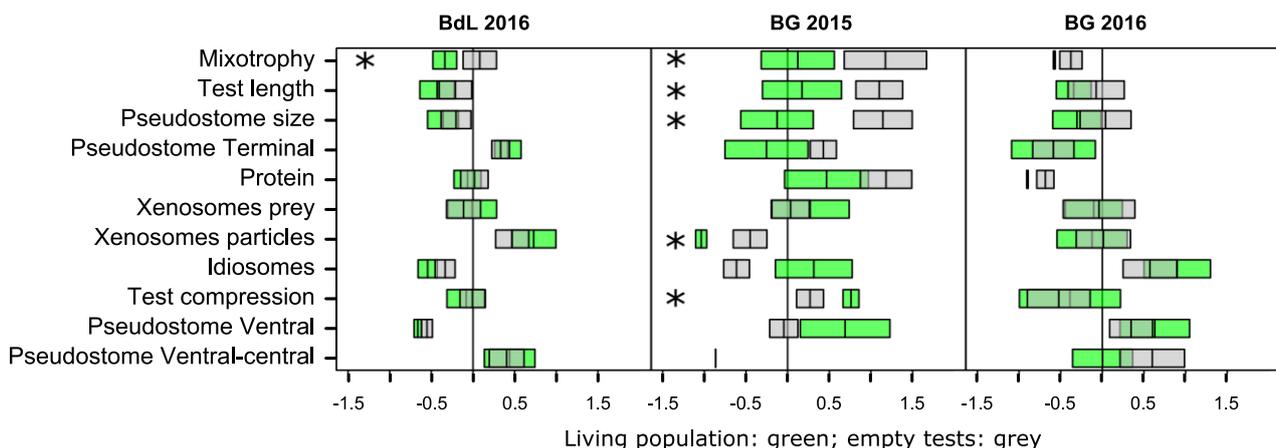


Figure 5. Boxplots of community weighted mean of traits in living and dead testate amoeba assemblages in the Neuchâtel Botanic Garden experimental peatland (BG 2015, BG 2016) and the Bois-des-Lattes reference peatland (BdL). Boxes represent standard error and vertical black lines the mean values of CWM for the sites (z-scored). Traits are arranged in order of decreasing humidity, with traits at the top expected to decrease with water stress and traits at the bottom to increase with water stress. Stars indicate significant trait differences between living and dead assemblages, based on a Wilcoxon rank test for paired value and exact p-value when available.

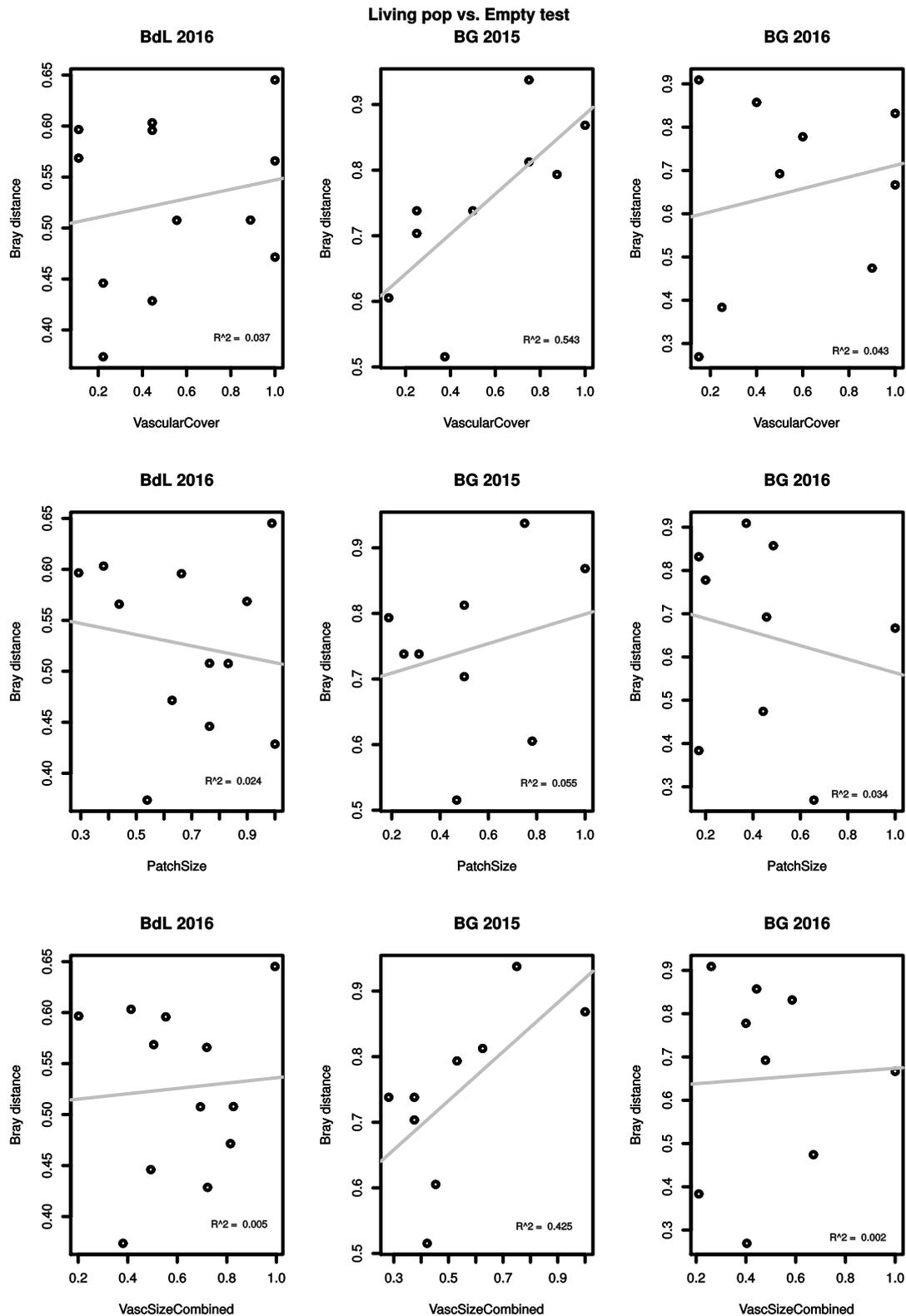


Figure 6: Biplots of β -diversity (Bray-Curtis distance index) calculated between living and dead testate amoeba assemblages from the Neuchâtel Botanic Garden experimental peatland (BG_2015, BG_2016) and the Bois-des-Lattes reference peatland (BdL) in relation to patch size, shading (% vascular plant cover) and a combination of the two, each given as a percentage of the maximum for the site. R² represents the linear regression coefficient for each dataset.

of *Sphagnum* peatlands (Mitchell *et al.* 1999, Mitchell & Gilbert 2004). The structure of testate amoeba communities differed between the two sites and between 2015 and 2016 in the botanic garden, but generally corresponded to communities reported from relatively dry *Sphagnum* peatlands (Mitchell *et al.* 1999, Opravilova & Hajek 2006, Smith *et al.* 2008, Lamentowicz *et al.* 2013).

In BdL 2016, the dominant morphotaxa (*Diffflugia pulex* group, *Phryganella acropodia* group, small *Euglypha* and *Nebela tincta*) mostly indicated low water table (*i.e.* below -25cm) (Mitchell *et al.* 1999, Bobrov *et al.* 2002, Lamentowicz *et al.* 2011). The compositions of living and dead communities were similar, only *C. dubium* and *A. muscorum* had significantly higher relative abundance in the dead than in the living assemblages while the reverse was true for *Cryptodiffflugia* sp. These taxa were not dominant, however (*i.e.* each <5 % of the overall community).

In BG 2015, most (75 %) of the testate amoeba community was dead. Some taxa were indicative for wet or relatively wet oligotrophic conditions (*H. papilio*, *H. elegans*; Mitchell *et al.* 1999, Smith *et al.* 2008), but the overall (living + dead) community was dominated by *C. dubium*, a taxon frequently reported from dry bog habitats (Mitchell *et al.* 1999) as well as from perturbed or stressed environments (*e.g.* dry mosses on walls and trees, tundra, *etc.*; Beyens *et al.* 1986, Bonnet 1991). A clear shift occurred with dying-out of testate amoebae related to wet conditions (*H. papilio*, *H. elegans*) and dominance of *C. dubium* in the living community, illustrating the impact of the 2015 summer drought. In BG 2016, although the taxonomic composition of living and dead assemblages showed no significant difference, taxa related to dry conditions were more abundant in the living than in the dead assemblage (for example, *A. muscorum*, *C. dubium*, *N. collaris*, *N. tincta*), and the structure of the living community was similar to that of dry *Sphagnum* peatlands such as the BdL reference site.

Magnitude of the two summer droughts and effects on testate amoeba communities

By sampling the top 3 cm of *Sphagnum* mosses and counting the dead and living assemblages separately we were able to assess the recent changes in community structure (*i.e.* one growing season), and specifically how the communities and associated functional traits responded to two years of drought.

Although not identical, both sites experienced extreme climatic conditions in 2016, the summer of 2015 being even more extreme. We hypothesised that these conditions would impact on the testate amoeba

communities. The expected impact should logically be highest in BG 2015 for two reasons:

- 1) The moss patches planted in the experimental bog originated from both secondary and pristine bogs, and some were kept under very wet conditions in the botanic garden for a few years prior to building the bog. This is reflected by the high abundance of taxa such as *Hyalosphenia papilio* in the dead assemblage of BG 2015.
- 2) The drought and heatwave were most extreme in 2015. Thus, the contrast between the 'original' conditions experienced by the communities and the conditions to which they were exposed was strongest in 2015. In 2016, the climate was less extreme and communities had already partly adapted to the drier conditions. Thus, a lesser shift in community structure could be expected.

In agreement with this, our results showed that the testate amoeba communities from the botanic garden clearly shifted in 2015, as shown by the contrast between dead and living assemblages and the high mortality (more than 75 %). The community structure shifted towards taxa related to drier conditions such as *C. dubium* (alive: 80.5 %) and lost taxa related to humid conditions (*H. papilio* alive: 8.6 %, *H. elegans* alive: 15.6 %, *H. rosea* alive: 0 %, *N. collaris* alive: 0 %; Figure 4) (Mitchell *et al.* 1999, Bobrov *et al.* 2002, Booth 2002, Lamentowicz *et al.* 2011).

In contrast, communities from the bog in the botanic garden did not change much more in 2016, probably because their structure already corresponded to the dry conditions. Indeed, the most abundant taxa in the living community in 2016 were *C. dubium* (20.1 %), small *Euglypha* (18.1 %) and *A. muscorum* (16.6 %) (Figure 4), all of which tolerate relatively dry conditions and nutrient inputs (Mitchell *et al.* 1999, Booth 2001, Mitchell & Gilbert 2004, Opravilova & Hajek 2006). Nevertheless, the percentage of living individuals increased markedly (+232 %). The adaptation to new conditions was, therefore, mostly achieved during the year 2015.

The community weighted means (CWM) of traits of the BG 2016 whole community and the BG 2015 living assemblages were more similar to the whole community weighted mean of the BdL reference site. This result suggests that, although the taxonomic composition of communities differed among the three sample sets, the abiotic conditions (*e.g.* moisture content) - which are assumed to control communities through ecological filtering - were comparable to those of a cutover bog in an early stage of spontaneous regeneration. By contrast, the CWM of traits from BG 2015 indicated that the original testate amoeba community was adapted to more

humid conditions than those found in *Sphagnum* patches in Bois-des-Lattes. The strong contrast between these initially much wetter conditions and the drought experienced during the summer 2015 explains the observed dramatic effect on testate amoeba communities. These results add to the small but growing body of experimental evidence showing rapid (*i.e.* within one season or year) shifts in testate amoeba communities in response to experimental manipulation of water table depth (Marcisz *et al.* 2014b, Mulot *et al.* 2017, Koenig *et al.* 2018).

Testate amoeba community shifts in the two study sites versus patch size and shading

We hypothesised that the testate amoeba community would survive better in large moss patches with high vascular plant cover because this combination would a) maximise the potential storage of water (due to higher overall volume of the mosses) and b) minimise water loss. Reduced water loss is due to the lower surface-to-volume ratio of larger moss carpets and the reduced water vapour gradient between the surface and the air directly above mosses under the shade of vascular plants. To assess how patch size and shading influenced the magnitude of the shift in testate amoeba communities, we calculated the β -diversity between living and dead assemblages as a measure of changes in community structure. We did not find any significant correlation in BdL and BG 2016 between β -diversity and patch size and/or shading. In BG 2015, contrary to our hypothesis, β -diversity was lower in open (unshaded) patches than in shaded ones (Figure 6). Our explanation for this apparent contradiction is that, under very dry conditions, the amoebae could barely survive in the less shaded patches but they may have been able to remain active in the shaded patches where, nevertheless, the dry-adapted taxa (e.g. *Corythion*, *Euglypha*) would thrive better and increase their relative abundance in the community. As a result, the community shift measured as β -diversity was higher in the more shaded moss patches. This is further supported by the fact that very few living individuals remained in open patches (Figure 3). Thus, the living assemblage corresponded to the remains of the original community without any (or with only very little) replacement of taxa. Therefore, it is quite likely that a similar study conducted in a natural peatland would yield different results, perhaps more in line with our hypothesis.

Evolution of the testate amoeba functional niche

Functional traits were selected in relation to water stress. When conditions become drier, the thickness of the water film becomes thinner and the nutrient

content and prey availability change. A compressed and small test with a ventral or ventral-central pseudostome theoretically allows the amoeba to stay active even in a thin water film (Fournier *et al.* 2012, Marcisz *et al.* 2016, Payne *et al.* 2016). Under drier conditions, the populations of bacteria and fungi increase, and this is reflected in smaller pseudostome size (Jassey *et al.* 2013a, 2013b; Bragazza *et al.* 2016, Gavazov *et al.* 2017). Finally, mixotrophy is expected to decrease as nutrient availability increases due to higher mineralisation, thus reducing the competitive advantage of mixotrophs over heterotrophs (Stoecker 1998, Jassey *et al.* 2015). In BG 2015 the functional niche clearly differed between dead and living assemblages, indicating a shift towards drier conditions with a decrease in mixotrophy, test length and pseudostome size and an increase in shell compression (Jassey *et al.* 2013a, 2013b; Fournier *et al.* 2015, Marcisz *et al.* 2016).

The relationship between pseudostome position and water level has so far been explored using mesocosm experiments, field experiments and observational studies along humidity gradients in natural peatlands (Fournier *et al.* 2016, Marcisz *et al.* 2016, Payne *et al.* 2016). Our results suggest that it could be interesting to monitor specifically functional traits in regenerated bogs to assess adaptations to local constraints and in natural peatlands to assess the impact of the increasingly frequent occurrence of drought and/or heatwaves. As adaptations are trade-offs between niche filters, competition, access to nutrients, *etc.* (Vandermeer 1972, Tilman 1982, Holyoak *et al.* 2005, Kearney *et al.* 2010), long term studies could highlight temporal patterns from the stressed stage to the stable community weighted mean of traits.

The shift in test material is more explainable as protein test was mainly related, in the botanic garden, to the presence of *Hyalosphenia* ssp. which disappeared completely between 2015 and 2016. In BdL the dominance in CWM of xenosome tests should be related to a broader presence of mineral particles and to a more complex community structure, including the presence of testate amoebae occupying different trophic levels such as bacterivorous, fungivorous but also micro-eukaryote predators (Gilbert & Mitchell 2006, Jassey *et al.* 2012, Geisen *et al.* 2015).

The CWM of mixotrophy calculated on living assemblages was highest in BG 2015, almost nil in BG 2016 and intermediate in BdL. Mixotrophy was shown to vary seasonally (Marcisz *et al.* 2016) and in relation to sunlight (Schönborn 1965). In our case, as we sampled during the same season (autumn) and after a long sunny period, mixotrophy should be high

if it was locally suitable (Schönborn 1965, Marcisz *et al.* 2016). Here we observed the opposite, which is consistent with drier and less oligotrophic local conditions where mixotrophy is not advantageous (Jassey *et al.* 2013b).

The CWMs of test length and pseudostome size were small in both BG and BdL, indicating that water stress was high in both sites and that the community was mainly dominated by bacterivorous testate amoeba (Fournier *et al.* 2015, Marcisz *et al.* 2016, van Bellen *et al.* 2016).

The dead assemblages were expected to reflect the shape of the original community (Mitchell *et al.* 2008). So far, we do not know if this ‘old’ community was viable in the climatic context of the botanic garden, regardless of the unexpected drought. The comparison between all three datasets, particularly when only living assemblages were considered, indicated few differences in CWM, illustrating the rapidity of community shifts and the pertinence of separating living and dead assemblages.

Taken together, these results indicate a high mineralisation rate, a low capacity for C fixation and, indeed, that these peatlands are likely to be C sources (Jassey *et al.* 2013a, 2015; Bragazza *et al.* 2016). The comparison of BG with the BdL reference site revealed that the structure of the community, based on functional traits, indicated comparable drivers (*i.e.* hydric stress) in both BdL and BG.

Development of the botanic garden bog and future challenges

When we sampled at the botanic garden in 2015, the bog showed clear signs of impact from the long and dry summer, with deep cracks in the peat. The very low density of living amoebae in BG 2015 and the shift in testate amoeba community structure suggested that the peatland was in a very critical condition. Even with the heavy rainfall of the following winter and spring, the water level in the bog barely returned to the surface and the ‘pools’ remained dry. Despite this, the specific bog vegetation generally remained alive until 2016 and most of the *Sphagnum* patches survived, although they tended to become smaller and drier than in 2015 (data not shown). The evolution of testate amoeba communities reflected the macroscopic state of the *Sphagnum* patches and confirmed that the humidity had been reduced. The functional composition of the testate amoeba assemblage is a more direct reflector of bog wetness than is the assemblage itself, and thus offers potential for comparing sites at larger scales, as was done for peatland vegetation across Europe by Robroek *et al.* (2017).

Our results show that it will be very challenging

to maintain the artificial bog in the botanic garden. A key condition for active bog is the long-term maintenance of waterlogged conditions (Mitsch & Gosselink 2000). After the 2015 drought, the water table in the experimental bog hardly ever returned to the soil surface. These low-water-table conditions stimulate peat mineralisation, releasing nutrients for plant growth and favouring non-bog vegetation including plant species from the surrounding meadows and forest. Without intensive weeding, several specific peatland plant species may not remain (*e.g.* *Drosera rotundifolia* disappeared), although some characteristic bog species such as *Vaccinium oxycoccos* can grow well on relatively dry bogs (Ecker *et al.* 2008, Graf *et al.* 2010).

This study had some limitations. The sampling in BG was made with parsimony to preserve as much as possible of the bog. The density of testate amoebae was very low and, in some samples, we could find only a few (less than ten) living individuals. Secondly, we compared the community structure with that at BdL. This choice was sensible because some of the BG *Sphagnum* patches came from this peatland. However, some *Sphagnum* patches also came from other sites and from more natural settings. Nevertheless, our results show that such comparative studies are valuable, although ideally it would be better to compare *Sphagnum* patches from the same site (BdL), some of which had been transplanted into the experimental site.

Despite these reservations, the testate amoeba community structure and the community weighted mean of traits gave coherent results and allowed their interpretation in terms of ongoing processes. Moreover, the selected traits seem pertinent to the assessment of drought impact on *Sphagnum*-inhabiting microorganisms and are consistent with the macroscopic state of the experimental bog. By developing a set of traits that are easy for non-specialists to measure, like the presence of photosymbionts (mixotrophy), test length and/or the position of the pseudostome, monitoring tools could be developed for use alongside classical vegetation relevés in permanent plots - which are expected to react more slowly to environmental changes (Mitchell *et al.* 2000, Artz *et al.* 2008, Haapalehto *et al.* 2011, Daza Secco *et al.* 2016) - to follow the evolution of peatland ecosystems.

CONCLUDING REMARKS

Botanic gardens are privileged places for plant conservation and public information but are less often used for field experiments such as the one

presented here. Incorporating experimental ecosystems such as peatlands, even if they are not as spectacular as tropical greenhouses, could increase public awareness regarding the challenges of biodiversity and ecosystem conservation and restoration, as well as emphasise the usefulness of such systems for experimental research. Moreover, providing easy access to environments like peatlands brings to the attention of a wide public some rare (possibly locally, as is the case for peatlands in Switzerland) ecosystems which deserve protection. Using soil microorganisms as indicators for the management of these ecosystems also nicely illustrates how different spatial and temporal scales should be integrated in ecology.

ACKNOWLEDGMENTS

We would like to thank the Service Faune Forêt Nature (SFFN) of Neuchâtel and in particular Sébastien Tchantz for sampling in the protected site of Bois-des-Lattes, students of the Biogeosciences Master's programme of the Universities of Neuchâtel and Lausanne who helped with collecting the BdL samples, Blaise Mulhauser, Sylvian Guenat and Laurent Oppliger for their contribution to creating the experimental bog and Jérémy Tritz for recording the water table depth with the help of other collaborators from the botanic garden. This project was funded internally by Neuchâtel Botanic Garden and the University of Neuchâtel.

REFERENCES

- Andersen, R., Chapman, S.J. & Artz, R.R.E. (2013) Microbial communities in natural and disturbed peatlands: A review. *Soil Biology and Biochemistry*, 57, 979–994.
- Artz, R.R.E., Chapman, S.J., Siegenthaler, A., Mitchell, E.A.D., Buttler, A., Bortoluzzi, E., Gilbert, D., Yli-Petays, M., Vasander, H. & Francez, A.-J. (2008) Functional microbial diversity in regenerating cutover peatlands responds to vegetation succession. *Journal of Applied Ecology*, 45, 1799–1809.
- Barnett, A.J., Finlay, K. & Beisner, B.E. (2013) Functional diversity of crustacean zooplankton communities: towards a trait-based classification. *Freshwater Biology*, 58, 1755–1765.
- Baselga, A. & Orme, C.D.L. (2012) betapart: an R package for the study of beta diversity. *Methods in Ecology and Evolution*, 3, 808–812.
- Beysens, L., Chardez, D., De Landtsheer, R., De Bock, P. & Jacques, E. (1986) Testate amoebae populations from moss and lichen habitats in the Arctic. *Polar Biology*, 5, 165–173.
- Bobrov, A., Charman, D. & Warner, B. (2002) Ecology of testate amoebae from oligotrophic peatlands: specific features of polytypic and polymorphic species. *Biology Bulletin*, 29, 605–617.
- Bonnet, L. (1964) Le peuplement thécamobien des sols (The testate amoeba population of soils). *Revue d'Écologie et de Biologie du Sol*, 1, 123–408 (in French).
- Bonnet, L. (1991) Écologie de quelques Euglyphidae (Thécamoebiens, Filosea) des milieux édaphiques et paraédaphiques. I: Genres *Corythion* et *Trinema* (Ecology of some Euglyphidae (Thecamoebians, Filosea) from edaphic and paraedaphic environments. I: *Corythion* and *Trinema*). *Bulletin de la Société d'Histoire Naturelle de Toulouse*, 127, 7–13 (in French).
- Booth, R.K. (2001) Ecology of testate amoebae (Protozoa) in two Lake Superior coastal wetlands: Implications for paleoecology and environmental monitoring. *Wetlands*, 21, 564–576.
- Booth, R.K. (2002) Testate amoebae as paleoindicators of surface-moisture changes on Michigan peatlands: modern ecology and hydrological calibration. *Journal of Paleolimnology*, 28, 329–348.
- Booth, R.K. (2008) Testate amoebae as proxies for mean annual water-table depth in *Sphagnum*-dominated peatlands of North America. *Journal of Quaternary Science*, 23, 43–57.
- Booth, R.K., Lamentowicz, M. & Charman, D. (2010) Preparation and analysis of testate amoebae in peatland palaeoenvironmental studies. *Mires and Peat*, 7(02), 1–7.
- Bragazza, L., Buttler, A., Robroek, B.J.M., Albrecht, R., Zaccone, C., Jassey, V.E.J. & Signarbieux, C. (2016) Persistent high temperature and low precipitation reduce peat carbon accumulation. *Global Change Biology*, 22, 4114–4123.
- Buckland, P.C. (1993) Peatland archaeology: a conservation resource on the edge of extinction. *Biodiversity & Conservation*, 2, 513–527.
- Buttler, A., Warner, B.G., Grosvernier, P. & Matthey, Y. (1996) Vertical patterns of testate amoebae (Protozoa: Rhizopoda) and peat-forming vegetation on cutover bogs in the Jura, Switzerland. *New Phytologist*, 134, 371–382.
- Chapman, S., Buttler, A., Francez, A., Laggoun-Defarge, F., Vasander, H., Schloter, M., Combe, J., Grosvernier, P., Harms, H., Epron, D., Gilbert, D. & Mitchell, E.A.D. (2003) Exploitation of northern peatlands and biodiversity maintenance:

- a conflict between economy and ecology. *Frontiers in Ecology and the Environment*, 1, 525–532.
- Charman, D.J. (2001) Biostratigraphic and palaeo-environmental applications of testate amoebae. *Quaternary Science Reviews*, 20, 1753–1764.
- Davidson, E.A. & Janssens, I.A. (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440, 165–173.
- Davis, S.R. & Wilkinson, D.M. (2004) The conservation management value of testate amoebae as ‘restoration’ indicators: speculations based on two damaged raised mires in northwest England. *The Holocene*, 14, 135–143.
- Daza Secco, E., Haapalehto, T., Haimi, J., Meissner, K. & Tahvanainen, T. (2016) Do testate amoebae communities recover in concordance with vegetation after restoration of drained peatlands? *Mires and Peat*, 18(12), 1–14.
- Dray, S. & Legendre, P. (2008) Testing the species traits-environment relationships: the fourth-corner problem revisited. *Ecology*, 89, 3400–3412.
- Ecker, K., Kuchler, M., Feldmeyer-Christe, E., Graf, U. & Waser, L.T. (2008) Predictive mapping of floristic site conditions across mire habitats: Evaluating data requirements. *Community Ecology*, 9, 133–146.
- Fleischer, E., Khashimov, I., Hölzel, N. & Klemm, O. (2016) Carbon exchange fluxes over peatlands in Western Siberia: Possible feedback between land-use change and climate change. *The Science of the Total Environment*, 545–546, 424–433.
- Fournier, B., Malysheva, E., Mazei, Y., Moretti, M. & Mitchell, E.A.D. (2012) Toward the use of testate amoeba functional traits as indicator of floodplain restoration success. *European Journal of Soil Biology*, 49, 85–91.
- Fournier, B., Lara, E., Jassey, V.E. & Mitchell, E.A.D. (2015) Functional traits as a new approach for interpreting testate amoeba palaeo-records in peatlands and assessing the causes and consequences of past changes in species composition. *The Holocene*, 25, 1375–1383.
- Fournier, B., Coffey, E.E.D., van der Knaap, W.O., Fernandez, L.D., Bobrov, A. & Mitchell, E.A.D. (2016) A legacy of human-induced ecosystem changes: spatial processes drive the taxonomic and functional diversities of testate amoebae in *Sphagnum* peatlands of the Galapagos. *Journal of Biogeography*, 43, 533–543.
- Galka, M., Tobolski, K., Górski, A. & Lamentowicz, M. (2017) Resilience of plant and testate amoeba communities after climatic and anthropogenic disturbances in a Baltic bog in Northern Poland: Implications for ecological restoration. *The Holocene*, 27 (1), 130–141.
- Gavazov, K., Ingrisch, J., Hasibeder, R., Mills, R.T.E., Buttler, A., Gleixner, G., Pumpanen, J. & Bahn, M. (2017) Winter ecology of a subalpine grassland: Effects of snow removal on soil respiration, microbial structure and function. *Science of the Total Environment*, 590, 316–324.
- Geisen, S., Rosengarten, J., Koller, R., Mulder, C., Urich, T. & Bonkowski, M. (2015) Pack hunting by a common soil amoeba on nematodes: Pack-hunting protists attacking nematodes. *Environmental Microbiology*, 17, 4538–4546.
- Gilbert, D. & Mitchell, E.A.D. (2006) Microbial diversity in *Sphagnum* peatlands. *Developments in Earth Surface Processes*, 9, 287–318.
- Gorham, E. (1991) Northern peatlands: Role in the carbon cycle and probable responses to climatic warming. *Ecological Applications*, 1, 182–195.
- Graf, U., Wildi, O., Kuchler, M. & Ecker, K. (2010) Five-year changes in Swiss mire vegetation. *Botanica Helvetica*, 120, 15–27.
- Grünig, A. (1994) *Mires and Man: Mire Conservation in a Densely Populated Country - the Swiss Experience*. Excursion Guide and Symposium Proceedings of the 5th Field Symposium of the International Mire Conservation Group (IMCG) to Switzerland 1992. Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, 415 pp.
- Haapalehto, T.O., Vasander, H., Jauhiainen, S., Tahvanainen, T. & Kotiaho, J.S. (2011) The effects of peatland restoration on water-table depth, elemental concentrations, and vegetation: 10 years of changes. *Restoration Ecology*, 19, 587–598.
- Heger, T.J., Mitchell, E.A.D., Ledeganck, P., Vincke, S., Van de Vijver, B. & Beyens, L. (2009) The curse of taxonomic uncertainty in biogeographical studies of free-living terrestrial protists: a case study of testate amoebae from Amsterdam Island. *Journal of Biogeography*, 36, 1551–1560.
- Holyoak, M., Leibold, M.A. & Holt, R.D. (2005) *Metacommunities: Spatial Dynamics and Ecological Communities*. The University of Chicago Press, Chicago, USA, 526 pp.
- Jassey, V.E.J., Shimano, S., Dupuy, C., Toussaint, M.-L. & Gilbert, D. (2012) Characterizing the feeding habits of the testate amoebae *Hyalosphenia papilio* and *Nebela tinctoria* along a narrow “fen-bog” gradient using digestive vacuole content and ¹³C and ¹⁵N isotopic analyses. *Protist*, 163, 451–464.
- Jassey, V.E.J., Chiapuisio, G., Binet, P., Buttler, A.,

- Laggoun-Defarge, F., Delarue, F., Bernard, N., Mitchell, E.A.D., Toussaint, M.L., Francez, A.J. & Gilbert, D. (2013a) Above- and belowground linkages in *Sphagnum* peatland: climate warming affects plant-microbial interactions. *Global Change Biology*, 19, 811–823.
- Jassey, V.E.J., Meyer, C., Dupuy, C., Bernard, N., Mitchell, E.A.D., Toussaint, M.-L., Metian, M., Chatelain, A.P. & Gilbert, D. (2013b) To what extent do food preferences explain the trophic position of heterotrophic and mixotrophic microbial consumers in a *Sphagnum* peatland? *Microbial Ecology*, 66, 571–580.
- Jassey, V.E.J., Signarbieux, C., Hättenschwiler, S., Bragazza, L., Buttler, A., Delarue, F., Fournier, B., Gilbert, D., Laggoun-Défarge, F., Lara, E., Mills, R.T.E., Mitchell, E.A.D., Payne, R.J. & Robroek, B.J.M. (2015) An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. *Scientific Reports*, 5(16931), 1–10.
- Kearney, M., Simpson, S.J., Raubenheimer, D. & Helmuth, B. (2010) Modelling the ecological niche from functional traits. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 3469–3483.
- Koenig, I., Feldmeyer-Christe, E. & Mitchell, E.A.D. (2015) Comparative ecology of vascular plant, bryophyte and testate amoeba communities in four *Sphagnum* peatlands along an altitudinal gradient in Switzerland. *Ecological Indicators*, 54, 48–59.
- Koenig, I., Mulot, M. & Mitchell, E.A.D. (2018) Taxonomic and functional traits responses of *Sphagnum* peatland testate amoebae to experimentally manipulated water table. *Ecological Indicators*, 85, 342–351.
- Kołodziej, P., Karpińska-Kołodziej, M., Marcisz, K., Gałka, M. & Lamentowicz, M. (2017) Palaeohydrology and the human impact on one of the largest raised bogs complex in the Western Carpathians (Central Europe) during the last two millennia. *The Holocene*, 28, 595–608.
- Kosakyan, A., Heger, T.J., Leander, B.S., Todorov, M., Mitchell, E.A.D. & Lara, E. (2012) COI barcoding of Nebelid testate amoebae (Amoebozoa: Arcellinida): extensive cryptic diversity and redefinition of the Hyalospheniidae Schultze. *Protist*, 163, 415–434.
- Laggoun-Défarge, F., Mitchell, E.A.D., Gilbert, D., Disnar, J.-R., Comont, L., Warner, B.G. & Buttler, A. (2008) Cut-over peatland regeneration assessment using organic matter and microbial indicators (bacteria and testate amoebae). *Journal of Applied Ecology*, 45, 716–727.
- Laliberté, E., Legendre, P. & Shipley, B. (2014) *FD: Measuring Functional Diversity from Multiple Traits, and Other Tools for Functional Ecology*. <https://cran.r-project.org/web/packages/>
- Lamentowicz, M. & Mitchell, E.A.D. (2005) The ecology of testate amoebae (protists) in *Sphagnum* in north-western Poland in relation to peatland ecology. *Microbial Ecology*, 50, 48–63.
- Lamentowicz, Ł., Gabka, M., Rusinska, A., Sobczynski, T., Owsiany, P.M. & Lamentowicz, M. (2011) Testate amoeba (Arcellinida, Euglyphida) ecology along a poor-rich gradient in fens of western Poland. *International Review of Hydrobiology*, 96, 356–380.
- Lamentowicz, M., Bragazza, L., Buttler, A., Jassey, V.E.J. & Mitchell, E.A.D. (2013) Seasonal patterns of testate amoeba diversity, community structure and species–environment relationships in four *Sphagnum*-dominated peatlands along a 1300 m altitudinal gradient in Switzerland. *Soil Biology and Biochemistry*, 67, 1–11.
- Lamentowicz, M., Gałka, M., Lamentowicz, Ł., Obremska, M., Kühl, N., Lücke, A. & Jassey, V.E.J. (2015) Reconstructing climate change and ombrotrophic bog development during the last 4000 years in northern Poland using biotic proxies, stable isotopes and trait-based approach. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 418, 261–277.
- Lavorel, S. & Garnier, E. (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 16, 545–556.
- Lemonis, N. (2012) *Paléocécologie d'une Tourbière de Russie du Nord par une Approche Multi-indicateurs (Thécambies, Macrorestes de Plantes, Pollens et Étude de la Tourbe) (Peatland Palaeoecology of a Northern Russian Peatland through a Multi-indicators Approach (Thecamoebians, Botanical Macroremains, Pollens and Peat Study))*. Master thesis, University of Neuchâtel, Switzerland, 90 pp. (in French).
- Londo, G. (1976) Decimal scale for relevés of permanent quadrats. *Vegetatio*, 33, 61–64.
- MacArthur, R.H. & Wilson, E.O. (1967) *The Theory of Island Biogeography*. Princeton University Press, Princeton, NJ, USA, 203 pp.
- Marcisz, K., Lamentowicz, Ł., Słowińska, S., Słowiński, M., Muszak, W. & Lamentowicz, M. (2014a) Seasonal changes in *Sphagnum* peatland testate amoeba communities along a hydrological gradient. *European Journal of Protistology*, 50, 445–455.
- Marcisz, K., Fournier, B., Gilbert, D., Lamentowicz, M. & Mitchell, E.A.D. (2014b) Response of

- Sphagnum* peatland testate amoebae to a 1-year transplantation experiment along an artificial hydrological gradient. *Microbial Ecology*, 67, 810–818.
- Marcisz, K., Colombaroli, D., Jassey, V.E.J., Tinner, W., Kołaczek, P., Gałka, M., Karpińska-Kołaczek, M., Słowiński, M. & Lamentowicz, M. (2016) A novel testate amoebae trait-based approach to infer environmental disturbance in *Sphagnum* peatlands. *Scientific Reports*, 6(33907), 1–11.
- MétéoSuisse (2016) *Bulletin Climatologique Année 2015 (2015 Climatological Bulletin)*. Federal Office of Meteorology and Climatology MétéoSuisse, Geneva, 10 pp. (in French).
- MétéoSuisse (2017) *Bulletin Climatologique Année 2016 (2016 Climatological Bulletin)*. Federal Office of Meteorology and Climatology MétéoSuisse, Geneva, 10 pp. (in French).
- Mitchell, E.A.D. & Gilbert, D. (2004) Vertical micro-distribution and response to nitrogen deposition of testate amoebae in *Sphagnum*. *The Journal of Eukaryotic Microbiology*, 51, 480–490.
- Mitchell, E.A.D., Buttler, A.J., Warner, B.G. & Gobat, J.M. (1999) Ecology of testate amoebae (Protozoa: Rhizopoda) in *Sphagnum* peatlands in the Jura mountains, Switzerland and France. *Ecoscience*, 6, 565–576.
- Mitchell, E.A.D., Buttler, A., Grosvernier, P., Rydin, H., Albinsson, C., Greenup, A.L., Heijmans, M., Hoosbeek, M.R. & Saarinen, T. (2000) Relationships among testate amoebae (Protozoa), vegetation and water chemistry in five *Sphagnum*-dominated peatlands in Europe. *New Phytologist*, 145, 95–106.
- Mitchell, E.A.D., Charman, D.J.G. & Warner, B.G. (2008) Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. *Biodiversity and Conservation*, 17, 2115–2137.
- Mitsch, W.J. & Gosselink, J.G. (2000) *Wetlands*. Third edition, John Wiley & Sons, New York, 920 pp.
- Moretti, M. & Legg, C. (2009) Combining plant and animal traits to assess community functional responses to disturbance. *Ecography*, 32, 299–309.
- Mouillot, D., Graham, N.A., Villéger, S., Mason, N.W. & Bellwood, D.R. (2013) A functional approach reveals community responses to disturbances. *Trends in Ecology & Evolution*, 28, 167–177.
- Mulot, M., Marcisz, K., Grandgirard, L., Lara, E., Kosakyan, A., Robroek, B.J., Lamentowicz, M., Payne, R.J. & Mitchell, E.A.D. (2017) Genetic determinism vs. phenotypic plasticity in protist morphology. *The Journal of Eukaryotic Microbiology*, 64, 729–739.
- Oksanen, J. (2015) *Vegan: an introduction to ordination*, 12 pp. Online at: <https://cran.r-project.org/web/packages/vegan/vignettes/intro-vegan.pdf>
- Opravilova, V. & Hajek, M. (2006) The variation of testacean assemblages (Rhizopoda) along the complete base-richness gradient in fens: A case study from the Western Carpathians. *Acta Protozoologica*, 45, 191–204.
- Payne, R.J. & Mitchell, E.A.D. (2008) How many is enough? Determining optimal count totals for ecological and palaeoecological studies of testate amoebae. *Journal of Paleolimnology*, 42, 483–495.
- Payne, R.J., Charman, D.J., Matthews, S. & Eastwood, W.J. (2008) Testate amoebae as palaeohydrological proxies in Sürmene Agacbası Yaylası peatland (Northeast Turkey). *Wetlands*, 28, 311–323.
- Payne, R.J., Lamentowicz, M. & Mitchell, E.A.D. (2011) The perils of taxonomic inconsistency in quantitative palaeoecology: experiments with testate amoeba data. *Boreas*, 40, 15–27.
- Payne, R.J., Creevy, A., Malysheva, E., Ratcliffe, J., Andersen, R., Tsyganov, A.N., Rowson, J.G., Marcisz, K., Zielińska, M., Lamentowicz, M., Lapshina, E.D. & Mazei, Y. (2016) Tree encroachment may lead to functionally-significant changes in peatland testate amoeba communities. *Soil Biology and Biochemistry*, 98, 18–21.
- Qin, Y., Mitchell, E.A.D., Lamentowicz, M., Payne, R.J., Lara, E., Gu, Y., Huang, X. & Wang, H. (2013) Ecology of testate amoebae in peatlands of central China and development of a transfer function for paleohydrological reconstruction. *Journal of Paleolimnology*, 50, 319–330.
- R Core Team (2016) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Ramsar (2013) *The Ramsar Convention Manual: A Guide to the Convention on Wetlands (Ramsar, Iran, 1971)*. Sixth edition, Ramsar Convention Secretariat, Gland, Switzerland, 109 pp.
- Ricotta, C. & Moretti, M. (2011) CWM and Rao's quadratic diversity: a unified framework for functional ecology. *Oecologia*, 167, 181–188.
- Robroek, B.J.M., Jassey, V.E.J., Payne, R.J., Martí, M., Bragazza, L., Bleeker, A., Buttler, A., Caporn, S.J.M., Dise, N.B., Kattge, J., Zając, K., Svensson, B.H., van Ruijven, J. & Verhoeven,

- J.T.A. (2017) Taxonomic and functional turnover are decoupled in European peat bogs. *Nature Communications*, 8(1161), 1–9.
- Schönborn, W. (1965) Untersuchungen über die Zoochlorellen-Symbiose der Hochmoor-Testacean (Analysis of Zoochlorella symbiosis in peatland testate amoebae). *Limnologica*, 173–176 (in German).
- Schwind, L.T.F., Arriera, R.L., Bonecker, C.C., Lansa-Tôha, F.A. & Amodêo, F. (2016) Chlorophyll-a and suspended inorganic material affecting the shell traits of testate amoebae community. *Acta Protozoologica*, 2016, 145–154.
- Smith, H.G., Bobrov, A. & Lara, E. (2008) Diversity and biogeography of testate amoebae. *Biodiversity and Conservation*, 17, 329–343.
- Stockmarr, J. (1971) Tablets with spores used in absolute pollen analysis. *Pollen et Spores*, 13, 615–621.
- Stoecker, D.K. (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *European Journal of Protistology*, 34, 281–290.
- Stoecker, D.K., Johnson, M.D., de Vargas, C. & Not, F. (2009) Acquired phototrophy in aquatic protists. *Aquatic Microbial Ecology*, 57, 279–310.
- Suding, K.N., Lavorel, S., Chapin, F.S., Cornelissen, J.H.C., Díaz, S., Garnier, E., Goldberg, D., Hooper, D.U., Jackson, S.T. & Navas, M.-L. (2008) Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology*, 14, 1125–1140.
- Sullivan, M.E. & Booth, R.K. (2011) The potential influence of short-term environmental variability on the composition of testate amoeba communities in *Sphagnum* peatlands. *Microbial Ecology*, 62, 80–93.
- Swindles, G.T., Charman, D.J., Roe, H.M. & Sansum, P.A. (2009) Environmental controls on peatland testate amoebae (Protozoa: Rhizopoda) in the North of Ireland: Implications for Holocene palaeoclimate studies. *Journal of Paleolimnology*, 42, 123–140.
- Tilman, D. (1982) *Resource Competition and Community Structure*. Princeton University Press, Princeton, NJ, USA, 296 pp.
- van Bellen, S., Mauquoy, D., Payne, R.J., Roland, T.P., Hughes, P.D.M., Daley, T.J., Loader, N.J., Street-Perrott, F.A., Rice, E.M. & Pancotto, V.A. (2016) An alternative approach to transfer functions? Testing the performance of a functional trait-based model for testate amoebae. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 468, 173–183.
- Vandermeer, J.H. (1972) Niche theory. *Annual Review of Ecology and Systematics*, 3, 107–132.
- Villéger, S., Novack-Gottshall, P.M. & Mouillot, D. (2011) The multidimensionality of the niche reveals functional diversity changes in benthic marine biotas across geological time: Long-term functional diversity changes. *Ecology Letters*, 14, 561–568.
- Yu, Z.C. (2012) Northern peatland carbon stocks and dynamics: a review. *Biogeosciences*, 9, 4071–4085.

Submitted 09 Jan 2018, revision 14 Apr 2018
 Editor: Frank Chambers

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Appendix



Figure A1. The experimental raised bog at Neuchâtel Botanic Garden in spring 2015. Top: general view on 04 May, during the very wet spring period; right inset: *Vaccinium myrtillus*; left inset: *Vaccinium oxycoccos*. Left-column pictures show gradual drying of the ‘fen’ area from 04 May (top) through 13 May (middle) to 02 June (bottom); during that period, the water table dropped from 19 cm above ground level to 8 cm below ground level. The remaining pictures show (from left to right in each case), top row: *Eriophorum vaginatum*, *Betula nana*; middle row: *Sphagnum* patch with *Drosera rotundifolia* and the first cracks appearing on bare peat, *Polytrichum strictum*; bottom row: *Pinus mugo* subsp. *uncinata*, *Drosera rotundifolia*.

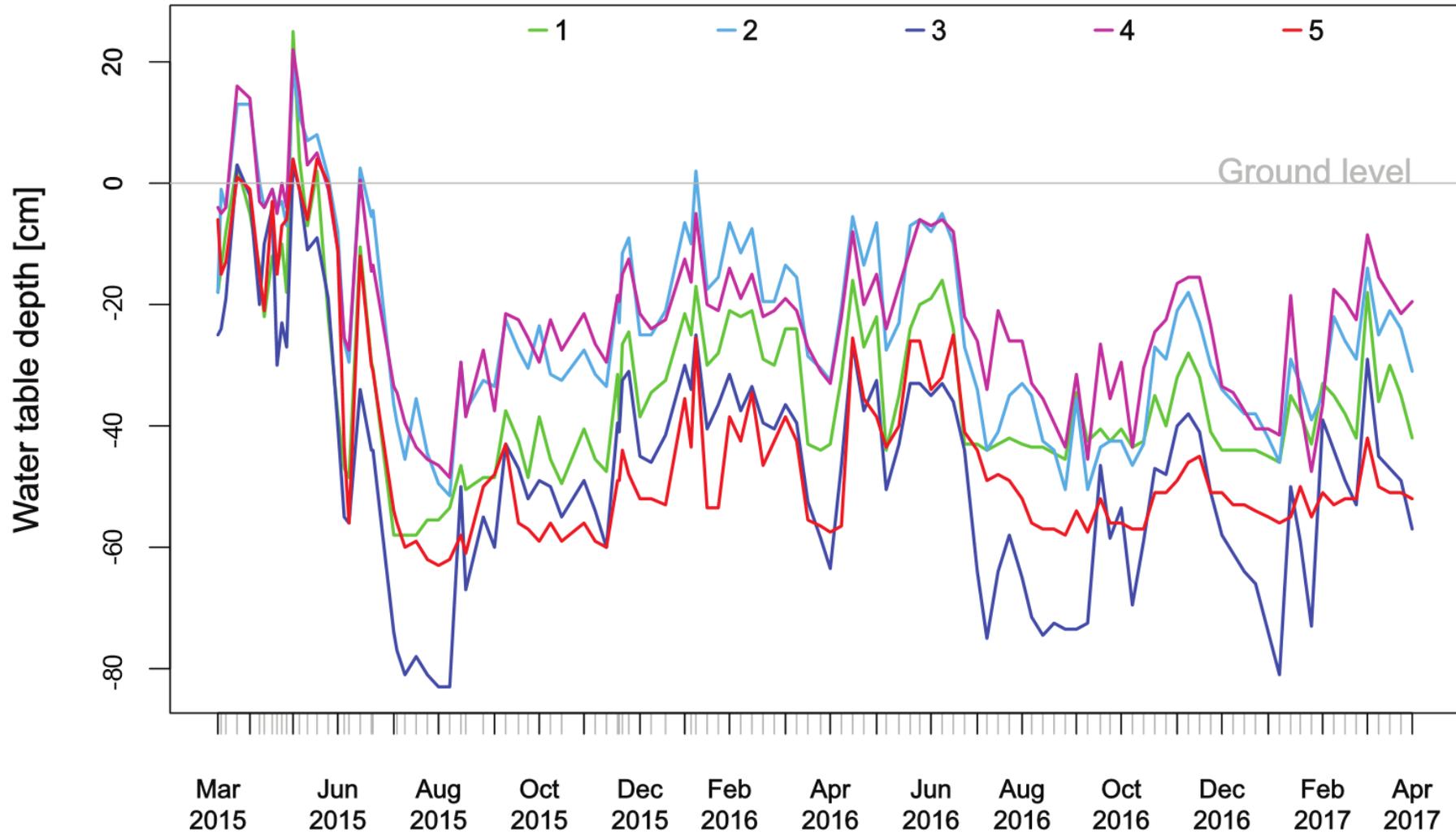


Figure A2. Fluctuation of water table depth measured in five dipwells in the experimental peatland at Neuchâtel Botanic Garden between March 2015 and April 2017. The horizontal line represents ground level (*i.e.* peat surface or top of moss carpet). The peatland was very wet during the spring of 2015, then the water table dropped sharply during the summer drought of 2015. The water level recovered during winter and the spring of 2016, but then dropped again in the summer of 2016 and did not recover afterwards.

Table A1. Descriptive statistics for the whole testate amoeba dataset (living individuals and empty tests sampled at the Neuchâtel Botanic Garden experimental peatland (BG 2015 and BG 2016) and the Bois-des-Lattes (BdL) reference site). Morphotaxa are listed in order of decreasing abundance, and the low-abundance (< 1 %) taxa in the box at the bottom of the Table were discarded before the analyses. Density is expressed as number of individuals *per* gram of dry *Sphagnum*, n = number of sampling plots where the taxa occurred, N = total number of sampling plots (30).

Morphotaxa	Frequency	Relative abundance (%)					Density (g ⁻¹)				
	n/N	mean	se	median	min	max	mean	se	median	min	max
<i>Corythion dubium</i>	83.3%	10.9	3.0	3.7	0	63.3	3761	912	1968	0	21832
<i>Euglypha</i> “small taxa”	73.3%	10.2	2.2	7.4	0	65.5	6254	1331	4597	0	27361
<i>Hyalosphenia papilio</i>	56.7%	9.6	3.2	1.7	0	71.8	4258	1558	731	0	42864
<i>Diffugia pulex</i>	46.7%	9.5	2.4	0.0	0	41.7	10271	3234	0	0	78445
<i>Phryganella acropodia</i>	63.3%	9.3	1.8	8.1	0	32.1	7421	1980	3416	0	52157
<i>Nebela tincta</i>	73.3%	9.1	1.9	6.1	0	38.2	5617	1269	2401	0	25982
<i>Nebela collaris</i>	66.7%	6.6	1.6	3.7	0	37.7	4387	1379	1663	0	35868
<i>Assulina muscorum</i>	60.0%	6.5	2.2	1.8	0	46.0	2611	797	885	0	17872
<i>Centropyxis aerophila</i>	56.7%	5.7	1.6	1.6	0	33.7	2163	605	725	0	12713
<i>Hyalosphenia elegans</i>	60.0%	5.1	1.3	1.5	0	31.0	2922	1061	707	0	29714
<i>Euglypha</i> “big taxa”	93.3%	4.5	0.8	3.6	0	18.0	3226	759	1561	0	14517
<i>Cryptodiffugia sp</i>	43.3%	3.3	1.3	0.0	0	35.7	2222	612	0	0	13074
<i>Archerella flavum</i>	56.7%	2.1	0.5	1.2	0	10.9	1816	558	567	0	13074
<i>Assulina seminulum</i>	56.7%	2.0	0.5	0.9	0	11.5	1252	323	608	0	6049
<i>Heleopera rosea</i>	30.0%	1.5	0.6	0.0	0	11.9	980	556	0	0	16168
<i>Hyalosphenia minuta</i>	16.7%	1.2	0.6	0.0	0	14.3	1064	513	0	0	11007
<i>Heleopera sylvatica</i>	40.0%	1.0	0.4	0.0	0	11.3	648	237	0	0	4986
<i>Trinema lineare</i>	20.0%	0.7	0.4	0.0	0	10.7	223	101	0	0	2155
<i>Diffugia lucida</i>	23.3%	0.4	0.1	0.0	0	2.9	257	103	0	0	2420
<i>Trigonopyxis arcula</i>	20.0%	0.3	0.1	0.0	0	1.9	217	101	0	0	2487
<i>Nebela militaris</i>	16.7%	0.2	0.1	0.0	0	2.0	228	100	0	0	2155
<i>Arcella catinus</i>	6.7%	0.2	0.2	0.0	0	4.3	65	48	0	0	1324
<i>Bullinularia indica</i>	6.7%	0.1	0.1	0.0	0	2.1	53	37	0	0	914
<i>Amphitrema wrightianum</i>	6.7%	0.1	0.0	0.0	0	0.9	82	57	0	0	1244

Table A2. Wilcoxon rank test (V) for each functional trait between living and dead testate amoeba assemblages in the Neuchâtel Botanic Garden experimental peatland (BG 2015 and BG 2016) and Bois-des-Lattes reference site (BdL). Significant p-values (<0.05) are shown in **bold** type.

CWM	BdL		BG 2015		BG 2016	
	V	p-value	V	p-value	V	p-value
Mixotrophy	55	0.006	44	0.008	9	0.201
Test length	63	0.064	40	0.039	30	0.426
Pseudostome size	55	0.233	44	0.008	33	0.25
Pseudostome terminal	27	0.38	27	0.234	22	1
Test protein	47	0.569	35	0.164	14	0.106
Xenosomes prey	32	0.622	19	0.944	22	0.624
Xenosomes particles	20	0.151	44	0.008	21	0.726
Idiosomes	61	0.092	6	0.055	13	0.301
Test compression	38	0.97	1	0.021	12	0.8
Pseudostome ventral-central	43	0.791	0	NA	23	0.151
Pseudostome ventral	59	0.129	9	0.234	15	0.426

Table A3. Pairwise comparison (Wilcoxon rank test with “Holm” correction for the p-value) of the community weighted mean (CWM) of selected traits between pairs of datasets (Bois-des-Lattes: BdL; Neuchâtel Botanic Garden: BG (2015 and 2016); living assemblages: A; dead assemblages: D). Significant p-values (<0.05) are shown in **bold** type.

Mixotrophy	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	0.2709	-	-	-	-
BG 2015_A	1	1	-	-	-
BG 2015_D	0.0315	0.7584	0.031	-	-
BG 2016_A	1	0.0195	0.604	0.023	-
BG 2016_D	1	0.4289	0.604	0.023	0.604
Test length	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	1	-	-	-	-
BG 2015_A	1	1	-	-	-
BG 2015_D	0.0068	0.0131	0.195	-	-
BG 2016_A	1	1	1	0.047	-
BG 2016_D	1	1	1	0.219	1
Pseudostome size	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	1	-	-	-	-
BG 2015_A	1	1	-	-	-
BG 2015_D	0.014	0.014	0.047	-	-
BG 2016_A	1	1	1	0.047	-
BG 2016_D	1	1	1	0.156	0.75
Pseudostome terminal	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	1	-	-	-	-
BG 2015_A	1	1	-	-	-
BG 2015_D	1	1	0.936	-	-
BG 2016_A	1	1	1	0.195	-
BG 2016_D	0.034	0.02	1	0.023	1

Test protein	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	1	-	-	-	-
BG 2015_A	1	1	-	-	-
BG 2015_D	0.0449	0.0663	0.211	-	-
BG 2016_A	0.0014	0.0014	0.09	0.045	-
BG 2016_D	0.0471	0.04	0.104	0.023	0.211
Xenosomes prey	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	1	-	-	-	-
BG 2015_A	1	1	-	-	-
BG 2015_D	1	1	1	-	-
BG 2016_A	1	1	1	1	-
BG 2016_D	1	1	1	1	1
Xenosomes particles	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	1	-	-	-	-
BG 2015_A	0.0029	0.0029	-	-	-
BG 2015_D	0.0559	0.0798	0.047	-	-
BG 2016_A	0.8749	0.9915	0.237	1	-
BG 2016_D	0.8749	1	0.112	1	1
Idiosomes	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	1	-	-	-	-
BG 2015_A	1	1	-	-	-
BG 2015_D	1	1	0.219	-	-
BG 2016_A	0.094	0.373	0.387	0.039	-
BG 2016_D	0.084	0.373	0.734	0.023	0.602
Test compression	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	1	-	-	-	-
BG 2015_A	0.023	0.011	-	-	-
BG 2015_D	1	1	0.13	-	-
BG 2016_A	1	1	0.32	1	-
BG 2016_D	1	1	0.13	0.3	1
Pseudostome ventral-central	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	0.254	-	-	-	-
BG 2015_A	0.211	1	-	-	-
BG 2015_D	0.031	0.148	0.98	-	-
BG 2016_A	0.015	0.052	1	0.98	-
BG 2016_D	0.015	0.052	1	0.98	1
Pseudostome ventral	BdL_A	BdL_D	BG 2015_A	BG 2015_D	BG 2016_A
BdL_D	1	-	-	-	-
BG 2015_A	0.0011	0.0011	-	-	-
BG 2015_D	0.0011	0.0011	-	-	-
BG 2016_A	0.9224	0.9358	0.18	0.18	-
BG 2016_D	1	1	0.11	0.11	0.18