

Taxonomic and functional traits responses of *Sphagnum* peatland testate amoebae to experimentally manipulated water table

Isabelle Koenig^{a,*,1}, Matthieu Mulot^{a,c,1}, Edward A.D. Mitchell^{a,b}

^a Laboratory of Soil Biodiversity, University of Neuchâtel, Rue Emile-Argand 11, CH-2000, Neuchâtel, Switzerland

^b Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000, Neuchâtel, Switzerland

^c Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR 7144, Adaptation et Diversité en Milieu Marin, Équipe EPEP, Station Biologique de Roscoff, F-29680, Roscoff, France

A B S T R A C T

Keywords:

Testate amoebae
Mesocosm experiment
Functional diversity
Functional traits
Sphagnum peatland
Biomonitoring
Water table depth

Biomonitoring tools are useful to assess the impact of environmental changes on the functioning of ecosystems. Existing tools mostly require species identification, thus allowing to estimating changes in biodiversity, and possibly inferring ecosystem functioning, using functional diversity and traits based approaches.

Testate amoebae are good indicators of surface moisture conditions in *Sphagnum* peatlands and are routinely used in palaeoecology. Their shells (tests), on which identification is based, can also be used to define functional traits and thus to infer changes in ecosystem functioning.

We investigated the response of testate amoeba communities to manipulated water table depth (wet: -4 cm, intermediate: -15 cm, and dry: -25 cm) over time (seven time points, 19 months) using mesocosms by comparing two approaches: community structure and functional traits responses, using a combination of morphological (biovolume, length, aperture size and position) and physiological (mixotrophy/heterotrophy, shell material) traits.

This is the first study investigating the effect of water table depth on testate amoeba assemblages over time using a mesocosm approach. Taxonomical and functional approaches showed similar response patterns, confirming that water level acted as a strong environmental filter. After one year *Hyalosphenia papilio* decreased in the dry treatment, and the community structure shifted towards a dominance of dry indicators (*Nebela tinctoria* complex, *Corythion dubium*, *Euglypha compressa*) and the selected functional traits (smaller, heterotrophic, compressed species, with a ventral aperture) corresponded to drought adaptations.

In line with recent observational and transfer function studies exploring the use of testate amoebae functional traits, our experimental results illustrate how well-selected traits could be used to monitor the impact of present and past climatic changes on *Sphagnum* peatlands.

1. Introduction

Biomonitoring tools are useful to assess the impact of environmental changes on the functioning of ecosystems. Finding and testing relevant proxies to monitor the evolution of ecosystems are therefore priorities in applied ecological research (Bartkowski et al., 2015; Laurila-Pant et al., 2015). In addition to describing biodiversity itself, good bioindicators should also inform about ecosystem services. Biomonitoring approaches are now accepted as standard tools to assess environmental quality (European Commission, 1999; Finlayson, 1994).

Functional diversity (FD) is a useful tool to model ecosystem functioning. Based on the relationship between environmental constraints

and community structure, through functional traits, FD allows assessing the impact of environmental changes on both community structure and ecosystem functioning (Bockstael et al., 2000; Cadotte et al., 2011; Keddy, 1992; Woodward and Diament, 1991). Species traits may explain both the effects of environmental changes on community structure, (response traits) and the feedback from species presence on ecosystem functioning (effect traits) (Lavorel and Garnier, 2002). Traits used for FD analysis should ideally relate to niche characteristics and/or directly or indirectly to ecosystem processes (Mlambo, 2014; Violle et al., 2007). The FD approach was initially developed for macroscopic organisms such as plants, fishes and soil invertebrates, for which different sets of traits have been validated and more recently for

* Corresponding author.

E-mail addresses: Isabelle.koenig@unine.ch (I. Koenig), Matthieu.mulot@unine.ch (M. Mulot).

¹ Joint first authors (equal overall contribution).

microorganisms (Arrieira et al., 2015; Barnett et al., 2007; Fournier et al., 2016, 2015, 2012; Jassey et al., 2016; Lamentowicz et al., 2015; Marcisz et al., 2016; van Bellen et al., 2017; Violle et al., 2011). Our focus here is on the impact of climate change on testate amoebae living in *Sphagnum* peatlands.

Sphagnum-dominated peatlands are key ecosystems in the global carbon cycle as C stores and sinks (Froking and Roulet, 2007; Turunen et al., 2002), but are threatened by direct human impact (peat harvesting, drainage) and climatic change (warming, drought) (Belyea and Malmer, 2004; Bonn et al., 2016; Briones et al., 2014; MEA, 2005). Northern peatlands represent an important carbon stock of 500 ± 100 gigatons, representing ca. $\frac{1}{3}$ of all soil carbon trapped in ca. 3% of total land area (Gorham, 1991; Yu, 2012). The key to peat accumulation is the low decomposition rate, which is driven by the anoxic conditions in the usually water-logged soils (Belyea and Malmer, 2004; Rydin and Jeglum, 2006). *Sphagnum*-dominated peatlands, are primarily situated in high latitudes where documented and predicted climate warming are greatest (Hassol, 2004; IPCC, 2013). Understanding their response to climatic change is therefore a research priority (Belyea and Malmer, 2004; Pachauri and Mayer, 2015; Rydin and Jeglum, 2006; Yu, 2006), for their potential impact on carbon cycle at the global scale as well as for biodiversity conservation at the regional scale, especially in marginal climatic setting for their development at mid-latitudes.

As the vegetation of peatlands is dominated by perennial plants, shifts in vegetation in response to environmental changes are slow. By contrast, soil microorganisms, including protists such as testate amoebae, have short generation times and are thus well adapted to being used as early warning indicators of environmental changes (Buttler et al., 1996; Gilbert et al., 1998; Laggoun-Defarge et al., 2008). Even within stable conditions such as found in raised bogs, testate amoebae were shown to be better indicators of micro-environmental gradient (hummock-pool) than plants (Koenig et al., 2015). Testate amoebae are a polyphyletic group of protists building a shell (i.e. “test”). They are known to be well correlated with environmental gradients like soil moisture, water table depth, pH, or nutrients content (Booth and Zygmunt, 2005; Jassey et al., 2011; Swindles et al., 2009). Their decay-resistant shell, well preserved in peat and sediments after the organisms’ death, allows past climates to be reconstructed by inferring ancient local conditions through testate amoeba community structures (Chambers et al., 2012; Talbot et al., 2010). In addition, testate amoebae functional traits can also be defined in accordance with functional diversity assumptions and be used for biomonitoring or paleoreconstruction (Fournier et al., 2015; Jassey et al., 2016; Kajukała et al., 2016; van Bellen et al., 2017).

The structure of testate amoeba communities living in peatlands has long been shown to be well correlated to factors such as water table depth and pH (Amesbury et al., 2013; Jassey et al., 2011; Marcisz et al., 2014; Mazei et al., 2007; Mieczan, 2009; Swindles et al., 2009; Wilken et al., 2013). The informative power of testate amoebae relies strongly on the accuracy of identification. However, due to taxonomic uncertainties (Booth, 2008; Mitchell et al., 2013, 2008; Payne et al., 2012), a significant yet unknown fraction of the data used in ecological studies contain errors. Although numerical experiments showed that such taxonomic inconsistencies generally have little effect on ecological interpretation, (Payne et al., 2011), it is nevertheless desirable to develop bioindication tools that are robust to potential identification biases.

The approach we are developing is to analyse the patterns of testate amoeba functional traits along environmental gradients and how they respond to experimental manipulations of key environmental factors, which were identified as major potential drivers of community structure based on observational studies. In a bioindication perspective, it is essential to understand the dynamic processes involved in environmental filtering in order to test the causality of relationships and determine at which temporal scale organisms can be used as bioindicators

– and this can only be achieved using experimental approaches (Marcisz et al., 2014).

The key driver – and environmental filter – we selected is water table depth, which generally emerges as the strongest explanatory variable in ecological studies of testate amoeba communities (Mitchell et al., 2008) and is also one of the main factors controlling the vegetation composition and functioning of peatland ecosystems (Rydin and Jeglum, 2006).

We monitored the temporal pattern of the response of *Sphagnum* peatland testate amoeba communities and corresponding variations in traits to artificially manipulated water table. Our hypothesis was that the testate amoeba community weighted mean of traits (CWM: the abundance-weighted mean of the species trait values within a community) and community structure would exhibit similar responses to water level manipulation, but that the CWM would be more directly ecologically informative than community structure in understanding ecosystem functioning. The traits include shell and aperture size, which were shown to determine the trophic position of testate amoebae (Jassey et al., 2013a), with feedback on C and nutrient cycling, and mixotrophy, which was shown to relate to ecosystem carbon balance (Jassey et al., 2015).

2. Material and methods

2.1. Experimental design

We assessed the response of testate amoeba communities to water table depth using a mesocosm experiment. The mesocosms were plastic tanks filled with water (online supplementary material). In the centre of each tank a PVC tube (45 cm high, 12 cm diameter with regularly-spaced lateral holes to allow water movement) contained a peat core collected from a cutover bog using a large-diameter peat corer, topped with a 15 cm thick carpet of *Sphagnum fallax* (including the living mosses and underlying litter), collected from another peatland (Creux de l’Epral, 47°12’18.3”N; 006°56’05.83”E; altitude: 990 m) with an extensive homogeneous cover of this moss (see Mulot et al., 2015 for a complete description of the experiment setup). The water level in each mesocosm was adjusted to maintain three average water table depths (–4 cm, –15 cm, and –25 cm, hereafter referred to as “wet”, “intermediate” and “dry” treatments). Each treatment was replicated five times, giving a total of 15 mesocosms. Each carpet was seeded with a water extract from pool, hummock, and lawn (“bog microbial cocktail”) to provide the full community potential at the beginning of the experiment (Mulot et al., 2015). Therefore, the communities should be undistinguishable at T0 in all mesocosms. For the extraction of testate amoebae, the top three centimetres of *Sphagnum* mosses were first collected in August 2nd 2012 (T0), then after two month (T1; October 4th), four months (T2; November 29th), seven months (T3; March 3rd, 2013), twelve months (T4; August 11th), sixteen months (T5; December 15th), and eighteen months (T6; March 27th, 2014). Water levels were recorded twice a day using automatic custom piezometers as described in Mulot et al. (2015).

2.2. Testate amoeba isolation and characterisation

Testate amoebae were extracted by sieving and back-sieving through mesh filters (Booth et al., 2010; Jassey et al., 2011). A minimum of 100 individuals (living + dead) per sample were counted. The rationale for including living as well as dead individuals was that this corresponds to the community that eventually will be preserved in the peat and recovered in palaeoecological studies. Our focus here is thus not specifically on the living community and thus a potential limitation inherent to this choice is that by including the dead amoebae we expect some lag in the response to the experimental treatment. In some late dry samples, testate amoeba density was very low and only 50 individuals could be found. As the diversity in these samples was low

Table 1
Description, ecological meaning and references for the six functional traits selected for testate amoeba taxa.

Unit	Description	Type of trait	Ecological meaning	References
Biovolume μm^3	Volume of shell (90% occupied by the living amoeba)	Response	Related to the metabolic rate and the capacity of the food web to process energy.	Fournier et al. (2012), Laggoun-Defarge et al. (2008) and Makarieva et al. (2008)
Test length μm	Maximal length of the shell	Response	Proxy for biovolume (assuming isometry).	Fournier et al. (2012) and Lamentowicz et al. (2015)
Aperture position Factor	From a terminal aperture to the completely central one.	Response	Represents the ability to survive in thin water film and thus the ability to remain active and contribute to the food web in dry conditions.	Gilbert and Mitchell (2006)
Test material Factor	Protein, silica or agglutinated made (organic debris, diatom frustules, mineral particles)	Response	Availability of material and/or prey to construct the test. Source of material appear to be a major regulator of abundance and repartition of testate amoeba along <i>Sphagnum</i> parts.	Fournier et al. (2012)
Test compression Binary	0: not compressed, 1: compressed	Response	Survival potential in drier situations and thus potential contribution to the food web in dry conditions.	Fournier et al. (2012)
Mixotrophy Binary	Presence (1) or not (0) of photosynthetic endosymbionts	Effect	Mixotrophy is a key factor in oligotrophic conditions and plays a role in peatland C cycling.	Fournier et al. (2015) and Jassey et al. (2015, 2011)
Aperture size μm	Width of the shell aperture	Effect	Related to prey size and food web functioning	Jassey et al. (2013b)

due to the strong dominance of one species (*Hyalosphenia papilio*), and given that we had five replicates per treatment, having only 50 specimens in a few samples was considered as acceptable (Payne and Mitchell, 2009). Identification of morpho-species followed Charman et al. (2004) and Mitchell (2003). While this clearly underestimates diversity, it corresponds to the standard generally used in palaeoecological studies and that retains most ecological signal (Mitchell et al., 2014). Some taxa were thus lumped into broad morphological groups: *Nebela tincta* complex (as define by Kosakyan et al., 2012), *Euglypha ciliata* and *E. compressa*, and *Cyclopyxis eurystoma* and *C. kahli* to overcome likely identification biases when data from different analysts is combined (Amesbury et al., 2016; Mitchell et al., 2014). Seven samples were discarded because of disturbances in the mesocosm or technical problems during sampling or preparation, resulting in 98 final samples.

The chosen traits and their ecological meaning, as defined by Lavorel and Garnier (2002), Messier et al. (2010), Mlambo (2014) are given in Table 1. Test length was also used as a proxy for biovolume; as these two variables were strongly correlated ($R^2 = 0.69$) only biovolume was kept for further analyses. Biovolume is also ecologically more relevant as it is known to indicate the metabolic rate and the capacity of the food web to process energy (Fournier et al., 2012; Jassey et al., 2013a; Laggoun-Defarge et al., 2008; Makarieva et al., 2008; Tsyganov et al., 2012). All traits were measured on the observed material. The morphological dimensions were measured directly at $400\times$ magnification using an inverted IX-81 Olympus microscope, and the Olympus cellSens Dimension software. Biovolume was calculated according to the general shape of the shell, applying geometrical formulas as in Fournier et al. (2015).

2.3. Numerical analyses

We first assessed how the experimentally-induced changes in testate amoeba community structure translated into inferred water table depth as if these communities were used in a palaeoecological study. To this aim, we inferred the theoretical water table depth (WTD) based on the observed community structure of each sampling date with a transfer function. To build this transfer function we used a training set from the Jura Mountains (Mitchell et al., 1999), but keeping only the species common to both data sets and samples with a depth to water table lower than 40 cm, corresponding to the possible range of water levels in the mesocosm experiment. We used the weighted average (WA) model, with a leave-one-out cross validation (Juggins, 2015). The significance of similarity between communities throughout the experiment was assessed by an analysis of similarity (anosim, vegan package), with a Bray-Curtis dissimilarity measure and 999 permutations. ANOSIM is a non-parametric analyse allowing us to test the difference between and within groups for two or more groups, using the rank of dissimilarity values (Clarke, 1993).

We then computed the community weighted mean (CWM, funcomp, FD package) (Laliberté and Legendre, 2010) of selected traits (biovolume, test length – only for linear regression with biovolume –, aperture position, test compression, mixotrophy and aperture size) in each sample. The CWM represented the average of each trait value weighted by the relative abundance of each species, based on the fourth-corner approach (Dray and Legendre, 2008). To compare the response of community structure and community traits to water level through time, we finally computed a principal response curves (PRC) analysis (prc, vegan) on Hellinger transformed species dataset and standardized CWM of traits. Hellinger transformation (squared root of relative abundance) allows to reduce the impact of both rare and abundant taxa and to overcome the double zero problem (Legendre and Legendre, 2000). In addition, the Hellinger transformation produces a Euclidian matrix, thus following the requirement of the PRC. PRC is a multivariate tool based on eigenvector ordination (special case of redundancy analysis) suitable for time series with contrasted treatment (Van den Brink and Ter Braak, 1999).

Table 2

Overall relative abundance of testate amoeba taxa over the whole duration of the experiment (n = 98 samples), mean, median, standard error (se), minimal (min) and maximal (max). Taxa are ordered by decreasing mean relative abundance.

Morphotaxa	n	mean	median	se	min	max
<i>Hyalosphenia papilio</i>	98	78.08%	85.37%	2.23%	0.57%	98.61%
<i>Nebela tinctoria s.l.</i>	98	5.46%	2.05%	0.91%	0.00%	43.25%
<i>Arcella catinus</i>	98	4.36%	3.13%	0.52%	0.00%	38.36%
<i>Corythion dubium</i>	98	2.87%	0.00%	1.21%	0.00%	83.43%
<i>Centropyxis aculeata</i>	98	1.80%	0.31%	0.49%	0.00%	34.78%
<i>Phryganella acropodia</i>	98	1.50%	0.00%	0.45%	0.00%	27.87%
<i>Heleopera rosea</i>	98	1.40%	0.00%	0.36%	0.00%	25.16%
<i>Euglypha compressa/ciliata</i>	98	1.31%	0.48%	0.21%	0.00%	10.42%
<i>Assulina muscorum</i>	98	0.90%	0.00%	0.14%	0.00%	5.83%
<i>Hyalosphenia elegans</i>	98	0.74%	0.00%	0.13%	0.00%	8.23%
<i>Archerella flavum</i>	98	0.62%	0.00%	0.23%	0.00%	16.36%
<i>Physochila griseola</i>	98	0.27%	0.00%	0.13%	0.00%	8.94%
<i>Cyclopyxis eurystoma/kahli</i>	98	0.17%	0.00%	0.08%	0.00%	7.69%
<i>Trinema lineare</i>	98	0.17%	0.00%	0.06%	0.00%	4.48%
<i>Amphitrema wrightianum</i>	98	0.16%	0.00%	0.07%	0.00%	3.92%
<i>Argynnia dentistoma</i>	98	0.12%	0.00%	0.05%	0.00%	3.73%
<i>Assulina seminulum</i>	98	0.08%	0.00%	0.03%	0.00%	2.30%

All analyses were carried out on R statistical software (R Core Team, 2016). Community based measures were computed using the R package vegan (Oksanen et al., 2015), while functional diversity measures were calculated using the R package FD (Laliberté et al., 2014) and mean water table depth was assessed with transfer function using R package rioja (Juggins, 2015).

3. Results

3.1. Testate amoeba diversity and community structure and inferred water table depth

A total of 17 morpho-species were recorded in the samples (Table 2). The richest samples were the dry plots at T4-T5 with a species richness of 10–11 and the poorest sample (intermediate, T5) showed only two species. *Hyalosphenia papilio* was the most abundant taxon, representing $78\% \pm 2.2\%$ of the total count on average. The two next most abundant taxa were *Nebela tinctoria s.l.* ($5.5\% \pm 0.9\%$) and *Arcella catinus* ($4.4\% \pm 0.5\%$; Table 2).

The communities were similar in all treatments at the beginning of the experiment (analysis of similarities at T0: $R = 0.05$, $p = 0.242$, Table 3) and subsequently diverged among treatments (maximum at T5, $R = 0.62$, $p = 0.001$, Table 3). *Hyalosphenia papilio* strongly dominated the communities in the wet and intermediate treatments throughout the duration of the experiment (min: 78.4% wet T5, max: 92.9% intermediate T3; Table 4). In wet plots, some taxa expanded slightly by the end of the experiment (*Amphitrema wrightianum*, *Phryganella accropodia*), but otherwise, the community structure was rather stable over the course of the experiment. By contrast, in the dry plots, *H. papilio* decreased strongly over time from 81.3% to 16.9%, and some taxa indicative for high water table, disappeared (e.g. *Archerella flavum*,

Table 3

Analysis of similarity among treatments for each sampling time (T0-T6) based on a Bray-Curtis dissimilarity index and 999 permutations.

Time	R Statistic	Sig
T0	0.05	0.242
T1	0.15	0.087
T2	0.18	0.03
T3	0.33	0.006
T4	0.51	0.009
T5	0.62	0.001
T6	0.56	0.001

Cyclopyxis eurystoma/kahli), while dry indicators increased (e.g. *Corythion dubium*, *Nebela tinctoria s.l.*, *Assulina muscorum*).

In wet plots, the inferred water table depth was constant at around –2 cm throughout the experiment, increasing to 0 cm at T6 (Fig. 1). In intermediate plots, the trend was very similar to that of wet plots, with a slight lowering of the water table depth after T3 until T6 to –4 cm. In dry plots, the inferred water table depth lowered to –20 cm by T6.

3.2. Change in traits space over time

The community weighted mean of traits illustrates how the space defined by selected traits (related to the ecological niche) was filled and how the traits space evolved over the experiment (Fig. 2). Aperture position, test material and aperture size remained similar in the wet and intermediate plots over the entire course of the experiment, while a clear shift occurred in the dry plots. After T4 a shift in test material was observed in dry plots from protein to silica and agglutinated (Fig. 2b). At T6 the community in dry plots was dominated by agglutinated or silica test, and a ventral position of aperture (Fig. 2a). The proportion of mixotrophic species stayed around 60% over the course of the experiment in the wet and intermediate plots (Fig. 2c), but decreased gradually in the dry plots from to less than 20% by T6. By contrast, test compression did not vary in the dry and intermediate plots but decreased between T3 and T5 in the wet plots (Fig. 2d), and was tended to increase again at T6. Pseudostome (aperture) size decreased in dry plots from T3 onwards (Fig. 2e). Test length and biovolume decreased slightly in dry plots from T3 onwards and remained stable in wet and intermediate plots (Fig. 2f & g).

3.3. Principal response curves

The two principal response curves (PRC) analyses on 1) Hellinger-transformed species data and 2) CWM of Gower distance and standardized traits data, with treatment and time as explanatory variables, were significant ($p < 0.01$, based on the analysis of variance of the analysis). These two analyses explained 61.9% and 61.0%, respectively of the variance: specifically, the conditional, constrained and unconstrained fraction of variance in the Species PRC was 14.2, 47.8 and 38.1, respectively, while these fractions in the CWM PRC were 17.8, 43.2 and 38.9, respectively (Fig. 3). Testate amoeba communities do not diverge significantly between the intermediate and wet plots (Fig. 3a), both being dominated by *Hyalosphenia papilio*. By contrast, the communities in dry plots gradually diverged over time mainly due to the replacement of *H. papilio* species from the *Nebela tinctoria* complex and *Corythion dubium*.

The PRC based on community weighted mean of traits (Fig. 3b), shows a very similar pattern, but with more fluctuation. No clear difference is observed between the wet and intermediate plots. The dry plots diverged rapidly from the other two treatments. The traits positively correlated to the change were ventral aperture, more compressed agglutinate or silica made test, small shell and aperture sizes. By contrast, mixotrophy, test made of protein, and larger species characterised the wet and intermediate plots.

4. Discussion

We investigated the response of testate amoeba communities to experimentally-manipulated water level by comparing two approaches: community structure and functional traits responses. The two approaches showed similar response patterns in response to manipulation of water table. This indicated that mixotrophy, test compression rate, test size and pseudostome position could be used as proxies to monitor changes in water table depth.

Table 4

Relative abundance of testate amoeba taxa per treatment (wet, intermediate – inter – and dry plots) and sampling time (T0 to T6), with number of plots counted (n), mean (%) and standard error (se %). In order to show pattern, species are ordered in increasing optimal water table depth (WTD, Optima and tolerance – Tol) as inferred using a transfer function from the Jura Mountains peatlands (Mitchell et al., 1999). In dry plots, the shift of community structure towards an increasing dominance of dry adapted species appears significantly.

Taxa	WTD (cm)		Treatment	T0			T1			T2			T3			T4			T5			T6		
	Optima	Tol.		n	mean	se	n	mean	se	n	mean	se	n	mean	se	n	mean	se	n	mean	se	n	mean	se
<i>Amphitrema wrightianum</i>	9.4	4.1	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	3.1	0.4
<i>Archerella flavum</i>	10.9	8.5	Wet	5	4.6	2.2	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
<i>Cyclopyxis eurystoma/kahli</i>	11.1	9.2	Wet	5	0.5	0.3	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.4	0.4	5	0.0	0.0	5	0.0	0.4
<i>Physochila griseola</i>	15.2	7.1	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.5	0.5	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
<i>Hyalosphenia papilio</i>	16.1	10.1	Wet	5	84.4	3.9	5	92.4	3.2	5	92.6	1.9	5	89.1	4.3	3	84.9	4.5	5	78.4	4.1	5	84.4	0.3
<i>Centropyxis aculeata</i>	17.0	7.0	Wet	5	0.8	0.5	5	0.0	0.0	5	0.2	0.2	5	2.7	1.0	3	1.5	0.3	5	0.0	0.0	5	1.7	0.0
<i>Hyalosphenia elegans</i>	19.2	9.9	Wet	5	0.3	0.2	5	0.4	0.4	5	0.3	0.2	5	3.0	1.5	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
<i>Phryganella acropodia</i>	21.6	11.5	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	6.9	2.6	5	15.2	3.8	5	7.4	0.0
<i>Heleopera rosea</i>	22.3	6.9	Wet	5	1.2	0.8	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	2.7	1.0	5	0.3	0.2	5	1.0	0.0
<i>Argynnia dentistoma</i>	23.7	7.0	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.2	0.2	3	0.0	0.0	5	0.0	0.0	5	0.0	3.5
<i>Corythion dubium</i>	23.7	11.0	Wet	5	0.0	0.0	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	3	0.2	0.2	5	0.0	0.0	5	0.0	0.7
<i>Nebela tinctoria s.l.</i>	23.8	12.6	Wet	5	1.8	0.6	5	2.7	1.1	5	2.5	0.8	5	1.5	1.0	3	0.2	0.2	5	1.0	0.7	5	0.3	0.4
<i>Trinema lineare</i>	24.2	11.3	Wet	5	0.4	0.3	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	3.0
<i>Arcella catinus</i>	24.7	8.7	Wet	5	3.5	1.4	5	3.9	2.2	5	2.3	1.4	5	1.8	0.6	3	3.2	1.2	5	4.3	1.8	5	1.1	0.0
<i>Euglypha compressa/ciliata</i>	25.5	9.6	Wet	5	1.7	0.5	5	0.4	0.3	5	1.0	0.6	5	1.2	0.8	3	0.0	0.0	5	0.0	0.0	5	0.4	0.6
<i>Assulina muscorum</i>	25.5	11.4	Wet	5	0.6	0.5	5	0.0	0.0	5	1.2	1.0	5	0.0	0.0	3	0.0	0.0	5	0.7	0.7	5	0.6	1.5
<i>Assulina seminulum</i>	29.7	12.7	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
<i>Amphitrema wrightianum</i>	9.4	4.1	Inter	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
<i>Archerella flavum</i>	10.9	8.5	Inter	5	0.0	0.0	5	0.3	0.2	5	0.0	0.0	5	0.0	0.0	3	0.2	0.1	5	0.6	0.4	5	0.0	0.2
<i>Cyclopyxis eurystoma/kahli</i>	11.1	9.2	Inter	5	0.2	0.1	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.1	0.1	5	0.0	0.0
<i>Physochila griseola</i>	15.2	7.1	Inter	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.2	0.1	5	0.0	0.0	5	0.2	1.6
<i>Hyalosphenia papilio</i>	16.1	10.1	Inter	5	86.4	2.7	5	89.5	0.6	5	90.5	1.3	5	92.9	1.6	3	80.6	3.3	5	81.6	6.5	5	83.9	0.6
<i>Centropyxis aculeata</i>	17.0	7.0	Inter	5	0.8	0.2	5	0.1	0.1	5	0.3	0.3	5	0.2	0.2	3	0.5	0.4	5	0.0	0.0	5	0.5	0.0
<i>Hyalosphenia elegans</i>	19.2	9.9	Inter	5	0.7	0.3	5	1.9	0.6	5	0.5	0.2	5	0.0	0.0	3	1.7	1.5	5	0.4	0.3	5	0.4	0.2
<i>Phryganella acropodia</i>	21.6	11.5	Inter	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	2.6	0.7
<i>Heleopera rosea</i>	22.3	6.9	Inter	5	0.5	0.5	5	0.2	0.1	5	1.4	0.8	5	0.0	0.0	3	1.5	1.3	5	0.2	0.2	5	1.1	0.0
<i>Argynnia dentistoma</i>	23.7	7.0	Inter	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.3	0.2	3	0.0	0.0	5	0.1	0.1	5	0.0	1.1
<i>Corythion dubium</i>	23.7	11.0	Inter	5	0.2	0.2	5	0.1	0.1	5	0.2	0.2	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	0.7
<i>Nebela tinctoria s.l.</i>	23.8	12.6	Inter	5	3.6	0.6	5	2.5	0.8	5	1.3	0.4	5	0.8	0.3	3	1.6	0.9	5	0.1	0.1	5	2.0	0.2
<i>Trinema lineare</i>	24.2	11.3	Inter	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	5	0.2	0.2	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
<i>Arcella catinus</i>	24.7	8.7	Inter	5	6.5	2.0	5	2.7	0.8	5	4.2	1.3	5	4.9	1.5	3	10.5	2.8	5	15.7	6.2	5	7.1	0.0
<i>Euglypha compressa/ciliata</i>	25.5	9.6	Inter	5	0.1	0.1	5	0.7	0.2	5	1.0	0.8	5	0.1	0.1	3	0.6	0.3	5	0.0	0.0	5	0.5	0.0
<i>Assulina muscorum</i>	25.5	11.4	Inter	5	0.8	0.2	5	1.8	0.7	5	0.5	0.3	5	0.6	0.4	3	2.4	1.8	5	1.2	0.9	5	1.8	0.2
<i>Assulina seminulum</i>	29.7	12.7	Inter	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	1.1
<i>Amphitrema wrightianum</i>	9.4	4.1	Dry	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	4	0.0	0.0	5	0.0	0.0
<i>Archerella flavum</i>	10.9	8.5	Dry	5	4.5	3.1	5	1.3	1.3	5	0.3	0.3	5	0.1	0.1	3	0.1	0.1	4	0.0	0.0	5	0.0	0.0
<i>Cyclopyxis eurystoma/kahli</i>	11.1	9.2	Dry	5	0.4	0.4	5	0.4	0.2	5	1.5	1.5	5	0.0	0.0	3	0.0	0.0	4	0.0	0.0	5	0.0	0.0
<i>Physochila griseola</i>	15.2	7.1	Dry	5	0.0	0.0	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	3	5.5	2.8	4	0.0	0.0	5	1.1	0.9
<i>Hyalosphenia papilio</i>	16.1	10.1	Dry	5	81.3	5.9	5	85.7	2.6	5	77.7	6.9	5	77.6	3.9	3	42.5	12.9	4	25.2	5.4	5	16.9	6.1
<i>Centropyxis aculeata</i>	17.0	7.0	Dry	5	0.5	0.4	5	1.2	0.4	5	1.0	0.7	5	2.4	1.0	3	9.0	3.3	4	10.6	8.1	5	7.6	5.1
<i>Hyalosphenia elegans</i>	19.2	9.9	Dry	5	0.5	0.4	5	0.8	0.5	5	1.3	0.9	5	0.2	0.2	3	2.1	0.2	4	0.7	0.4	5	1.1	0.5
<i>Phryganella acropodia</i>	21.6	11.5	Dry	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	4	0.0	0.0	5	0.0	0.0
<i>Heleopera rosea</i>	22.3	6.9	Dry	5	0.7	0.3	5	0.3	0.2	5	0.9	0.6	5	0.5	0.3	3	8.8	1.9	4	2.7	2.0	5	9.0	5.1
<i>Argynnia dentistoma</i>	23.7	7.0	Dry	5	0.0	0.0	5	0.1	0.1	5	0.0	0.0	5	1.7	0.6	3	0.0	0.0	4	0.0	0.0	5	0.1	0.1
<i>Corythion dubium</i>	23.7	11.0	Dry	5	0.7	0.7	5	0.7	0.5	5	0.0	0.0	5	0.4	0.3	3	0.8	0.5	4	24.3	9.8	5	33.7	16.4
<i>Nebela tinctoria s.l.</i>	23.8	12.6	Dry	5	4.3	1.9	5	3.1	1.0	5	9.6	2.9	5	12.4	3.9	3	23.2	10.5	4	25.9	7.0	5	21.8	5.7
<i>Trinema lineare</i>	24.2	11.3	Dry	5	0.0	0.0	5	0.3	0.3	5	0.9	0.9	5	0.6	0.4	3	0.9	0.9	4	0.2	0.2	5	0.0	0.0
<i>Arcella catinus</i>	24.7	8.7	Dry	5	5.3	1.9	5	2.8	0.9	5	3.2	1.4	5	1.6	0.8	3	2.5	1.4	4	4.0	1.9	5	1.8	0.8
<i>Euglypha compressa/ciliata</i>	25.5	9.6	Dry	5	1.8	0.8	5	1.8	0.5	5	3.0	1.0	5	2.3	0.8	3	3.7	2.0	4	3.3	2.0	5	4.4	2.1
<i>Assulina muscorum</i>	25.5	11.4	Dry	5	0.0	0.0	5	1.4	0.3	5	0.4	0.4	5	0.1	0.1	3	0.7	0.3	4	2.2	1.3	5	2.3	0.7
<i>Assulina seminulum</i>	29.7	12.7	Dry	5	0.0	0.0	5	0.2	0.2	5	0.0	0.0	5	0.1	0.1	3	0.3	0.3	4	1.0	0.6	5	0.1	0.1

4.1. Effect of the treatment on community structure and inferred water table depth

In our experiment, the response to the low water level treatment was fast: a clear response was already visible at T1, two months after T0. It is possible that microbial communities responded more rapidly to the lowered water level than in a natural site because the structure of the mesocosms did not buffer water table drawdown as would happen in natural peatland. If true, the effect of a similar drainage in natural peatland would be slower. Further experiments *in situ* would be needed to test this. Nevertheless, the Jura Mountains dataset used to calibrate the transfer function included samples from the two peatlands where the *Sphagnum* carpets (Creux de l'Epral, Jura, 990 m a.s.l.) and the “bog

microbial cocktail” (Le Cachot, Neuchâtel, 1050 m a.s.l.) were collected (Mitchell et al., 1999; Mulot et al., 2015). Accordingly, the shift in the community structure observed in the dry treatment is coherent with natural communities observed in drier microsites in *Sphagnum* peatland of the region (Mitchell et al., 1999).

The principal response curves showed the general patterns of response and allowed identifying the most responsive taxa. The lack of contrast between the intermediate and wet treatments as shown in the PRC suggests that wet indicators failed to become established in the wet plots. This could be due to several causes. As mesocosms were isolated from natural peatlands, colonisation did not compensate for local species extinction and potentially more competitive species absent at the beginning of the experiment could not reach the plots. Aiming to

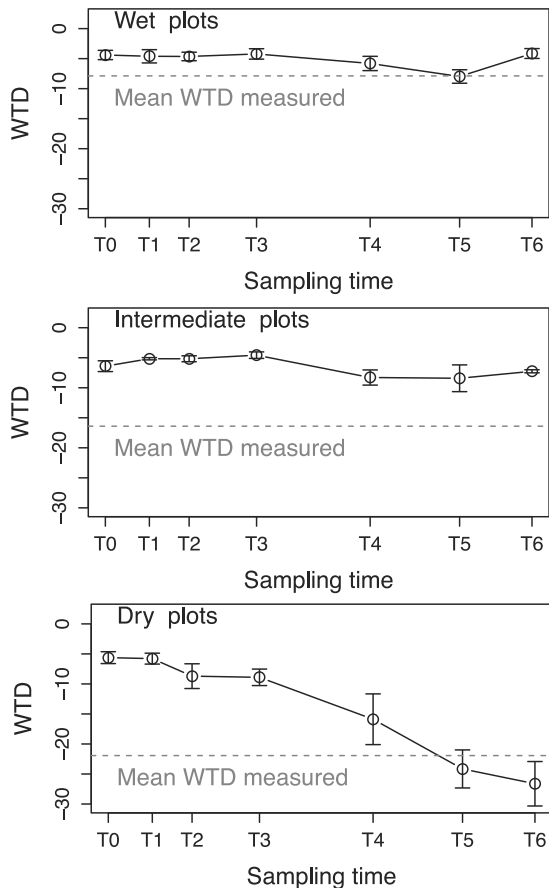


Fig. 1. Inferred water table depth (WTD) based on the testate amoeba community structure in *Sphagnum fallax* mesocosms with controlled water table depth. For each sampling point the mean inferred WTD and standard error are shown for each treatment (black dots and lines). The mean water table depth measured in each mesocosm is shown as a grey dotted line. The community structure at the beginning of the experiment was dominated by wet indicator species and the inferred water table depth was -5.5 ± 0.5 cm (se).

overcoming this bias, we seeded all plots with “bog microbial cocktail” (i.e. a mixture of water extracted from pools, lawns and hummocks), with the hope that both dry and wet indicator taxa would develop in the corresponding treatments. However, despite adding this “bog microbial cocktail”, wet indicator species were either too rare to be detected at the beginning of the experiment (e.g. *Amphitrema wrightianum*, *Physochila griseola*), or were observed in low density and then rapidly declined (e.g. *Archerella flavum*, *Cyclopyxis eurystoma/kahli*). Therefore, well-established species such as *H. papilio* could persist over time even in potentially suboptimal conditions (Fournier et al., 2016; Holyoak et al., 2005; Leibold et al., 2004). Obviously, this bog microbial cocktail addition did not suffice to provide viable populations of species from the full range of micro-environmental conditions. The reason may be that bog pool taxa are especially sensitive to disturbance and did not survive long-enough to build large populations, especially given the strong dominance of *H. papilio*. This should be further tested but if true would suggest that these wet indicators are indeed of especially high bioindication value for peatland conservation. By contrast to wet indicators, all dry indicator taxa were detectable at the beginning of the experiment, although at very low abundance. It is therefore not surprising that the effect of water level depth on testate amoeba communities, as reflected by the inferred water table as well as the PRC, was stronger and more rapid in dry plots. The mean inferred water level in dry plots decreased progressively and reached the experimental water table depth at T5. This shift was primarily due to a clear decrease of *H. papilio* and an increase in abundance of species characteristic for drier

situations such as *Nebela tinctoria* s.l., *Corythion dubium* and *Euglypha compressa/ciliata* (Bobrov et al., 2002; Booth, 2001; Lamentowicz and Mitchell, 2005; Mieczan, 2009). With a water level of -25 cm still within its theoretical range of tolerance (16 ± 10 cm), *H. papilio* maintained its population until T3, before decreasing abruptly in favour of *Nebela tinctoria* first and then (after T4) *Corythion dubium*. These two morpho-taxa together accounted for more than half of the testate amoeba community at T5 and T6. Therefore, even in suboptimal conditions, *H. papilio* dominated the community structure during eight months (including one winter) before better-adapted taxa were able to take over at the beginning of the second growing season.

The transfer function allowed us to assess how well the theoretical water table depth inferred from the testate amoeba community structure in the mesocosms matched the observed water table. At the beginning of the experiment, communities were largely dominated by *Hyalosphenia papilio*. The optimal water level for *H. papilio* as calculated in the transfer function was $-16 \text{ cm} \pm 10 \text{ cm}$ suggesting that intermediate plots (-15 cm) were best suitable for this species and that dry and wet plots would be less– but both equally so– favourable to this species. This is in line with previous studies that have regularly identified *H. papilio* as an indicator of moderately moist conditions (Booth and Meyers, 2010; Lamentowicz and Mitchell, 2005). However, the theoretical optimum water table for this species may be lower than the true optimum because calculations were based on one-off water table measurements taken in summer (Mitchell et al., 1999), as in most transfer function studies (but see Sullivan and Booth, 2011), while the average water table over the growing season is most likely higher due to higher rainfall and/or lower evaporation in spring and autumn. In support to this view, the proportion of *H. papilio* found at the beginning and at the end of the experiment was exactly the same in wet plots (84.4%) and only slightly lower (but not significantly so) in intermediate plots (T0: 86.4%, T6: 83.9%). This indeed suggests that the optimum average water level for this species is between -4 and -15 cm. The observed change in testate amoeba community structure could be related to the growth of both bacteria and small micro eukaryotes stimulated by oxygenation of the peat (Bragazza et al., 2016; Jassey et al., 2013b). This could explain the shift in testate amoeba community towards species belonging to another food web level (i.e. bacterivores). Such a community shift has been interpreted as being indicative for a change in C cycling at the surface of *Sphagnum* peatlands leading to the release of C to the atmosphere and faster nutrient cycling (Gilbert et al., 2003; Jassey et al., 2013a, 2012). The water level in the dry plots, ca. -25 cm, is indeed known to strongly disturb peatland functioning (Dieleman et al., 2014; Jassey et al., 2013a; Mitsch and Gosselink, 2000) and peatland restoration generally aims to raise the water table above this level (Quinty et al., 2003).

4.2. Water table changes and functional traits

Our experimental design allowed us to investigate the impact of water table on testate amoeba functional traits. High water level selected for larger species, and favoured mixotrophs (Fig. 3), as it was shown by Jassey et al. (2012, 2015). Mixotrophic testate amoeba represent up to 70% of the microbial biomass in *Sphagnum* peatlands (Jassey et al., 2013b) and were shown to contribute to C sequestration in peatlands (Jassey et al., 2015). Mixotrophy is a feeding strategy well adapted for oligotrophic aquatic conditions that characterize the pools of *Sphagnum* peatlands (Gomaa et al., 2014; Jassey et al., 2013a). Mixotrophy was thus expected to be favoured by high water level.

In our study, mixotrophy was associated with test made of protein (i.e. *H. papilio*, *Archerella flavum*). Proteinaceous tests are translucent, allowing sunlight to reach photosymbionts. Protein test secretion is still a poorly known process (Netzel, 1983; Nomura et al., 2014) but it is likely that this requires more energy and thus a higher metabolic activity than building an agglutinated test using material from the environment. The relationship between mixotrophy and size is hence

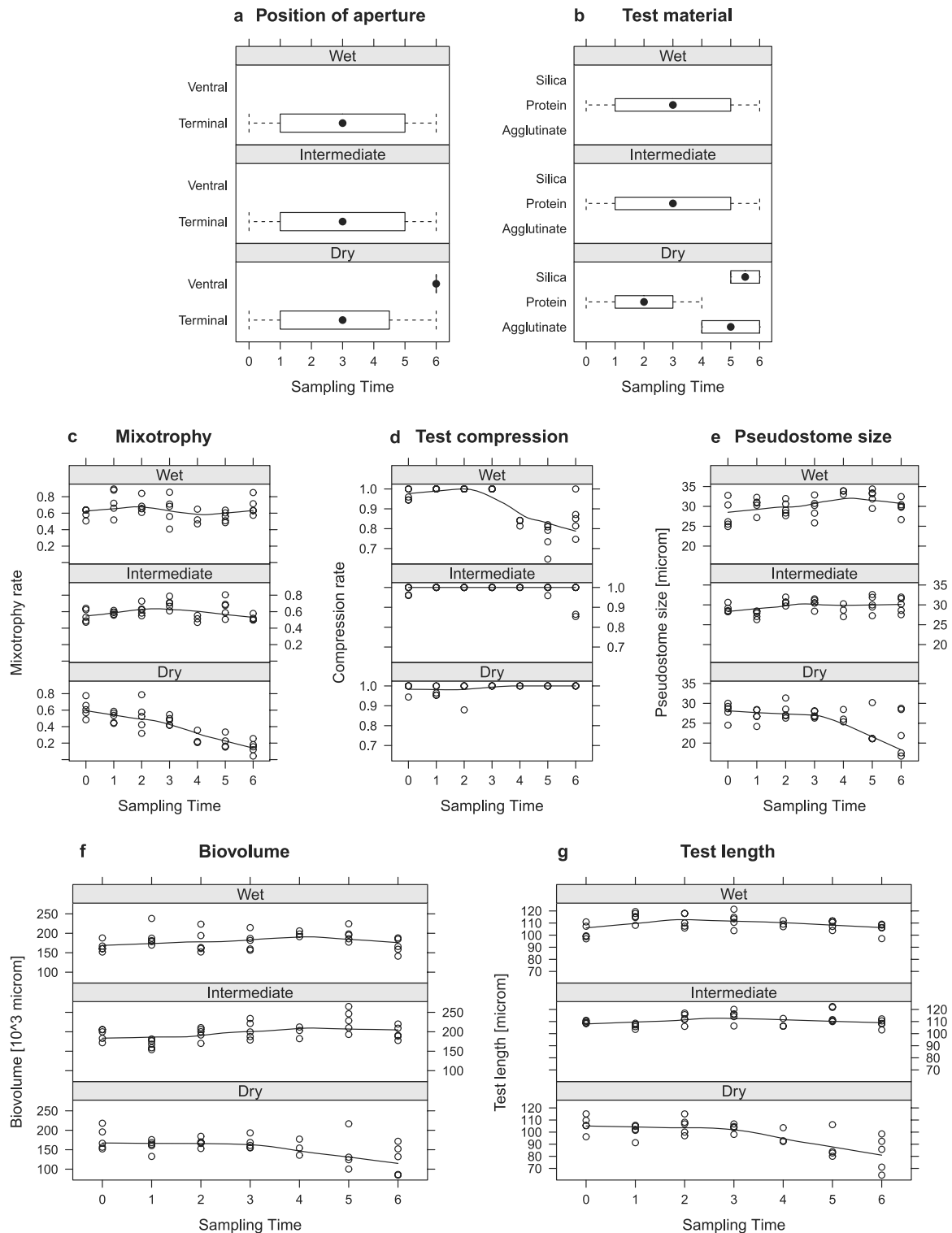


Fig. 2. Temporal changes in community weighted mean of traits (CWM) from testate amoeba communities in *Sphagnum fallax* mesocosms with controlled water table depth: a–b dominant traits for each treatment, c–g temporal patterns of individual traits with fitted smooth regression lines.

logical, as biovolume is correlated with metabolism (Makariev et al., 2008) and the number of mitochondria (Kosakyan et al., 2015). Biovolume is positively correlated with water level and thus prey size can be expected to increase under wetter conditions. This in turn would explain the selection in wet mesocosms of testate amoeba species with larger pseudostome which were shown to occupy higher trophic levels as inferred from carbon and nitrogen stable isotope analyses (Jassey et al., 2013b, 2012). The drawback is that larger species are dependent on

constant moisture to survive, be it due to their dependency on specific prey or to their likely lower physiological tolerance to drought. It should however be noted that not all peatland mixotrophic testate amoebae build proteinaceous shells (e.g. *Amphitrema* ssp., *Heleopera sphagni*, *Placocista spinosa*) and Amphitrematidae have small apertures.

Ventral (plagiostome) or hidden (cryptostome) apertures as well as test compression, are considered as adaptations to drought, allowing testate amoebae to remain active even in a thinner water film (Fournier

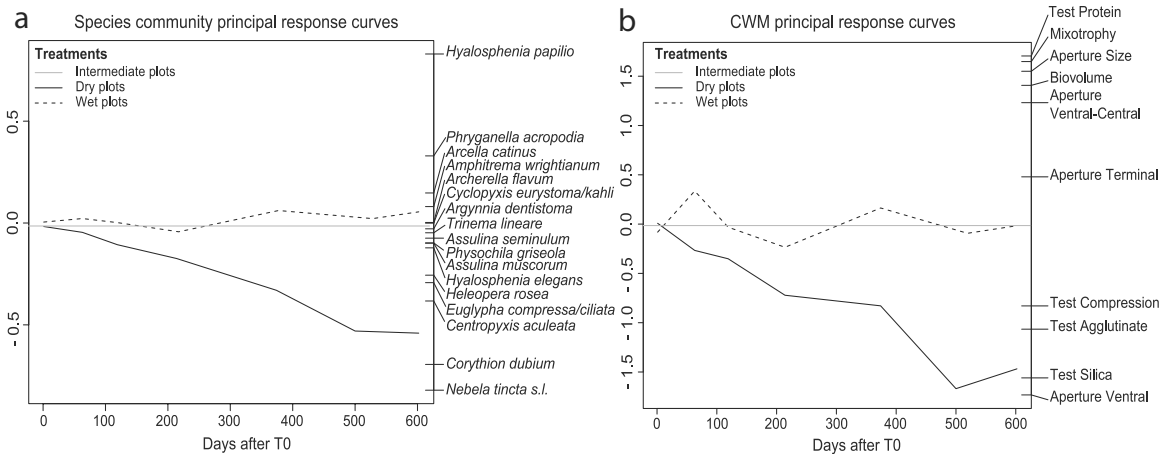


Fig. 3. Principal Response Curves (PRC) on testate amoeba community data from *Sphagnum fallax* mesocosms with controlled water table depth for (a) the species (Hellinger transformed), and (b) the CWM of traits (Gower distance and standardized) as response, and the treatment (WTD) and time as explanatory variables. Intermediate water level plots were defined as “control” treatment, the two other lines represented the variation of communities in comparison with intermediate plots. The measured water table depth (WTD) from the top of the *Sphagnum* layer in the wet, intermediate and dry treatments were respectively -4 cm, -15 cm, and -25 cm.

et al., 2015; Gilbert et al., 2003; Lamentowicz et al., 2015). In support for this view, in dry plots ventral aperture emerged as a significant trait at T6. Furthermore, community structure shifted towards the dominance of silica and agglutinate test composition, a common trait in mineral soils (Seppey et al., 2016; Szelecz et al., 2014; Tsyganov et al., 2012). On the contrary, in wet plots, where a compressed test does not represent a specific advantage, the proportion of species with a compressed test declined. Our results thus show that the traits mixotrophy, position of pseudostome and test size are informative to monitor changes in water table depth in peatland and could be used for monitoring purposes.

4.3. Future use of functional traits in ecological studies of testate amoebae

In wet to intermediate plots, only one species (*Hyalosphenia papilio*) was dominant over the entire duration of the experiment. This species was also present in drier mesocosms until T4 due to its wide tolerance to water table fluctuation while *Nebela tinctoria* s.l., *Corythion dubium* and *Euglypha compressa/ciliata* indicate drier conditions. Despite their ecological preferences, interpreting the abundance patterns of species in terms of ecological function remains difficult as we still lack detailed information on the functional role of individual species. By contrast, traits like mixotrophy, pseudostome position and biovolume, which are clearly controlled water table, can more easily be directly linked to ecological functions, and as such provide a more useful signal than the community structure. Thus, although some information is lost using functional traits, this approach can inform more directly about the status of an ecosystem (Fournier et al., 2015, 2012). It also overcomes the potential risk of erroneous interpretation to do misidentification, which currently represents a limitation of ecological and palaeoecological studies of testate amoebae (Heger et al., 2009; Mitchell et al., 2014; Payne et al., 2011).

An additional advantage of the traits approach is that it allowed overcoming biogeographical limitations. Indeed, as testate amoebae are increasingly being shown to have limited geographical distribution (Smith et al., 2008; Swindles et al., 2014), and common species such as *Hyalosphenia papilio* and *Nebela tinctoria* are now known to each represent 8–12 independent genetic species (Heger et al., 2013; Kosakyan et al., 2013; Singer et al., 2015). A strict taxonomical approach would lead to high community turnover along geographical gradients that may not correspond to changes in functioning. Using a functional traits approach allows focusing specifically on the functionally relevant signal in the community data.

Our aim was to assess the relevance of functional traits for

understanding water table fluctuation in *Sphagnum* peatlands. An advantage of the trait approach is that non-specialists of testate amoeba taxonomy may use the functional response based on morphologic characterization of the observed specimen or a relatively crude morphotype approach. Functional diversity analysis is therefore both practical and informative.

5. Conclusion

We studied the response of testate amoeba communities to experimentally manipulated water table, with the aim to better understand the impact of climatic changes on *Sphagnum* peatlands. The response of testate amoeba communities to experimentally manipulated water table depth was in line with the known ecological preferences of species documented in observational studies and coherent with the impacts on vegetation and decomposition.

The shift in selected traits also reflect the change in humidity content of the top-layer *Sphagnum* (not quantified but clear from visual observations). The reduced dominance of mixotrophic species and the shift to smaller taxa suggests functional changes in communities with an alteration of the microbial food web (i.e. increase in bacterivory vs. eukaryvory) and impact on C cycling (i.e. faster C turnover and reduced C fixation by mixotrophs). This is in line with recent evidence for a key role played by mixotrophic testate amoebae in the functioning of *Sphagnum* peatlands (Jassey et al., 2015). As these changes are relatively easy to measure, using well selected functional traits on testate amoeba community could give peatlands manager practical and resource-efficient tools to monitor the evolution or recovery of protected peatlands. The next steps will be to further experiment the effects of water table changes (e.g. drought followed by a recovery), combining mesocosms experiments and field studies and to further assess how selected response and effect traits can inform on the evolution of natural or semi-natural ecosystems facing environmental changes.

Acknowledgments

This project was supported by grant No. PSPB-013/2010 from Switzerland through the Swiss Contribution to the enlarged European Union and additional funds from the University of Neuchâtel.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2017.10.017>.

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