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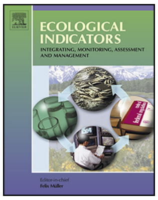

Comparative ecology of vascular plant, bryophyte and testate amoeba communities in four *Sphagnum* peatlands along an altitudinal gradient in Switzerland

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ABSTRACT

Monitoring tools are needed to assess changes in peatland biotic communities and ecosystem functions in response to on-going climate and other environmental changes. Although the responses of soil organisms and plants to ecological gradients and perturbations do not always correlate, peatland monitoring is mainly based on vegetation surveys. Testate amoebae, a group of protists, are important contributors to carbon and nitrogen cycling in organic soils and are useful bioindicators in peatland ecology and paleoecology. There is however little comparative data on the value of testate amoebae, vascular plants and bryophytes as bioindicators of micro-environmental gradients in peatlands.

We compared the relationships of testate amoebae, bryophytes, and vascular plants with soil temperature, water table depth, micro-habitats and the carbon and nitrogen content of *Sphagnum* mosses in four peatlands along a 1300 m altitudinal gradient in Switzerland. We used the full diversity of vascular plants and bryophyte but only a selection of ten easily identifiable testate amoeba morpho-taxa (i.e. species or species-complexes).

Indirect and direct gradient ordinations, multiple factor analysis (MFA) and transfer function models for inferring water table depth showed that a selection of ten testate amoeba taxa are more powerful (% variance explained in RDA) and accurate (discrimination among habitats) indicators of local conditions (micro-habitat type, water table depth and *Sphagnum* C/N ratio) than the vegetation (vascular plants and bryophytes either individually or combined and considering the full diversity).

Our study showed that a limited list of ten easily identifiable testate amoeba taxa have higher bioindication value than the full bryophytes and vascular plants. Furthermore, testate amoebae can be analyzed on samples collected at any season (accessibility allowing and if precise sampling sites are well marked) – a clear advantage for biomonitoring and can be used to infer past changes from the peat record at the same taxonomic resolution. This simple approach could therefore be very useful for biomonitoring of peatlands.

1. Introduction

On-going rapid climate change, largely attributed to the increase in greenhouse gas emissions (IPCC, 2013) has stimulated a considerable research effort on how biotic communities and related processes respond to these changes especially with respect to carbon (C) dynamics. Peatlands stock approximately the equivalent of 75% of total atmospheric C (Gorham, 1991). If actively growing they represent a significant C sink, but, when mineralization surpasses accumulation (e.g. owing to drainage or climate-related drought) they become a C source (Moore and Knowles, 1989). Climate warming or precipitation decrease may reduce C sequestration even of pristine peatlands and limit the regeneration potential of damaged peatlands (de Jong et al., 2010; Samaritani et al., 2011), thus feeding back positively on warming (Arneth et al., 2010; Belyea and Malmer, 2004). It is therefore important to assess how peatlands respond to environmental change both in their structure (e.g. communities) and function (e.g. hydrology, C-balance) (Davidson and Janssens, 2006).

A powerful approach to understanding the effects of environmental perturbation is to use bioindicators. As it is clearly not possible to study all living organisms in detail the question

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then is to determine which group of organism should be studied and what information they can provide. To answer this question comparative studies of different functional and taxonomic groups are required. Research on peatland ecology has historically focused primarily on vegetation and many assumptions on ecological gradients or functioning are made on the basis of vegetation patterns.

Some groups of peatland soil micro-organisms have been quite well studied in the last few decades (Gilbert and Mitchell, 2006), and especially the testate amoebae, a group of protists building a shell (called test). The shell allows relatively easy identification and is preserved in peat and in lake sediments thus making it possible to use them for paleoenvironmental reconstruction. Testate amoebae are well correlated to the main ecological gradients in *Sphagnum* peatlands, especially soil moisture (generally measured as water table depth), pH and nutrients (Booth and Zygmunt, 2005; Mieczan, 2009; Swindles et al., 2009). This has led to their use in the study of primary (e.g. palaeoecological records, Chambers et al., 2012; Charman, 2001; McMullen et al., 2004) and secondary succession (Talbot et al., 2010). Testate amoebae are increasingly considered as a useful tool for peatland bio-monitoring and management (Davis and Wilkinson, 2004; Mitchell et al., 2008; Turner and Swindles, 2012). Although the taxonomy of these organisms is not fully satisfactory, recent studies have also shown that they could provide valuable ecological information even at low taxonomic resolution (Mitchell et al., 2014).

Few studies have compared the community patterns of testate amoebae (or other soil microbes) and plants or their respective potential as bioindicators (see for example Hajek et al., 2011; Hajkova et al., 2011). The existing ones (in peatlands and pro-glacial succession) show some discrepancy between the community–environment relationships (Carlson et al., 2010; Lamentowicz et al., 2010) and transfer function model performance (Mitchell et al., 2013) of testate amoebae vs. bryophytes and/or vascular plants. In *Sphagnum*-peatlands, higher species–environment correlations were found for testate amoebae than for bryophytes, vascular plants or both combined (Lamentowicz et al., 2010), while transfer function performance (on raw data) was higher for bryophytes for DWT but not for pH (Mitchell et al., 2013). Here we address this question in four *Sphagnum* peatlands located along elevation gradient from the Swiss lowlands (ca. 600 m a.s.l.) to the subalpine–alpine limit (ca. 1900 m a.s.l.).

To our knowledge, there are only three published studies of testate amoeba communities along altitudinal gradients. In forests soils and mosses, between 400 m and 2500 m a.s.l. in Bulgaria Todorov (1998) showed that species richness decreased with altitude and that there was a significant difference among the biotopes. Mitchell et al. (2004) studied testate amoeba communities in a single moss species (*Hylocomiums splendens* (Hedw.) W.P. Schimp.) in the northern Italian Alps between 1000 and 2000 m a.s.l. Community structure was found to be more strongly correlated to soil chemical composition (silica vs. calcareous) than to altitude and no significant decrease in species richness was found with altitude. Krashevska et al. (2007) studied the testate amoeba communities in litter and upper soil horizons of three sites in a tropical mountain forest at 1000, 2000 and 3000 m a.s.l. in Equator. Testate amoeba density was lowest at the mid altitude and highest at 3000 m a.s.l., with an opposite pattern for specific richness. However part of these differences may be due to soil type, which varied with altitude (alumic acrisol, gley cambisol and podzol, respectively from the lowest to the highest site) as well as vegetation. Indeed, testate amoeba diversity and community structure were shown to vary in relation to factors such as plant functional richness (Ledeganck et al., 2003) and soil type (Bonnet, 1964). These factors should therefore either be accounted for in numerical analyses, or excluded using appropriate sampling design.

Our goal here was not to specifically address diversity or ecological patterns along this elevation gradient but by studying an altitudinal gradient we aimed to simulate a change in temperature (synchronic approach) while focusing on the same specific micro-habitats of *Sphagnum*-dominated peatlands. We covered a broader range of habitats at each elevation than Mitchell et al. (2004) while still remaining in comparable biotopes at each study site, unlike other two cited elevation gradient studies. We aimed to answer two main questions: (1) How do the relationships between testate amoebae, bryophytes and vascular plants and the main micro- and macro-ecological gradients compare and thus what are their respective predictive power for these variables? (2) How powerful are these three groups for discriminating among different micro-habitats along the whole humidity gradient (pool to hummock) within sites? As developing taxonomy expertise represents a true limitation for the use of protists in biomonitoring, rather than using the full community we addressed these questions using a selection of 10 morpho-taxa that can easily be identified by untrained analysts after a short learning period.

2. Methods

2.1. Study sites

We selected four sites in the same meso-climatic region (transition from Swiss Plateau to pre-Alps), in the northern Swiss Alps (canton Bern, Fig. 1). All sites are legally protected at least since 1987 and are categorized as open bogs in the Swiss Federal bogs and mires inventory (Ecker et al., 2008). The four study sites are located along an altitudinal gradient from 580 m a.s.l. to 1890 m a.s.l. The regional climate is temperate continental; only the lowest site (Lörmoos, Table 1) is slightly drier and warmer than the other three, being less influenced by higher rainfall associated to mountains. General climatic data was obtained from MeteoSwiss, the Swiss Federal Office of Meteorology and Climatology. The two lower sites have been impacted by peat harvesting and/or cattle grazing, but the upper two are considered to be relatively pristine (i.e. as close as can be in Switzerland).

2.2. Sampling design and fieldwork

Plots were selected in autumn 2010 in the main micro-habitats within each study site: pool, pool edge, dry hollow, lawn, hummock, and dry hummock, with five replicates per micro-habitat in most cases and three at the minimum (total: 19–25 plots per site and 84 overall). Pools were missing in Lörmoos owing to the general dryness of the site. As Lörmoos was particularly dry in autumn 2010 and no pools with standing water existed, we selected the lowest microtopography habitats and called them “hollows” to differentiate them with the pools with standing water that existed in the other sites. These hollows were indeed the wettest microsites in Lörmoos. In Rotmoos the “pool edge” micro-habitat was clearly defined, with specific vegetation, owing to the steeper slope between the lawns and the pools. Percentage cover of vascular plants and bryophytes were estimated according to the Londo scale (Londo, 1976). Nomenclature follows Lauber et al. (2007) for vascular plants and Jahns (2007) for bryophytes. *Sphagnum* species were identified, first in the field and identifications were confirmed by microscopy following Daniels and Eddy (1990).

An iron bar was inserted in each plot to obtain a time-integrated measurement of the depth of water table (DWT). The bars were left for ten to twelve months (September to November 2010–August 2011). The DWT was estimated as the vertical distance between the top of the moss carpet and the limit between the rusted and

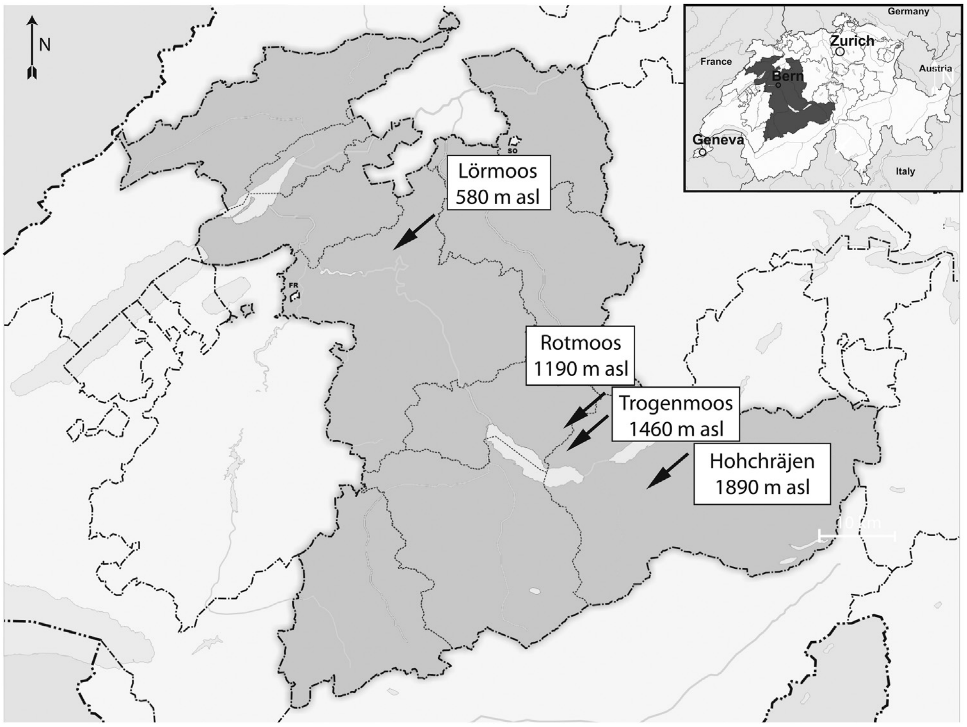

Fig. 1. Location of the four studied *Sphagnum* peatlands in Switzerland.

non-rusted parts of the bars. Though more approximate than direct regular or continuous WTD monitoring, it allowed us to estimate the maximum water level in each plot. This method was adapted from the PVC discoloration method (Booth et al., 2005) since the PVC tapes we experimented with did not discolour under anoxic conditions (Mitchell unpublished). Soil temperature was measured in three micro-habitats and three replicates per site using HOBO Pendant® Temperature/Alarm Data Logger (8K-UA-001-08, precision 0.14 °C at 25 °C). The selection of plots to be monitored was made in order to cover the whole variability inside each micro-habitat. Therefore at each site one or two micro-habitat(s) did not have any measured temperature data. The data loggers were inserted at 7.5 cm depth, corresponding to the maximal density of testate amoebae shells (Buttler et al., 1996; Mitchell and Gilbert, 2004) and programmed for one measurement every 15 min from October 2010 to August 2011, except for the highest site, which could not be accessed until May 2011.

2.3. Testate amoebae and moss elemental chemistry

Five or six *Sphagnum* mosses were collected in each plot and pooled to constitute a representative sample of each plot. The section between 5 and 10 cm under the top of the capitulum was used for testate amoeba extraction, thus avoiding the vertical micro-distribution of species in the upper centimetres (Mitchell and Gilbert, 2004). Testate amoebae were extracted using a standard sieving (200 µm) and back-sieving (15 µm) method (Booth et al., 2010) using deionised water. The shells were concentrated by centrifugation and stored in 2 mL tubes with three drops of glycerol ($C_3H_8O_3$) and one tablet of *Lycopodium clavatum* (batch No 938934, 10679 ± 426 spores per tab) standard preparation from Lund University (Sweden) according to Stockmarr (1971). *Sphagnum* C and N contents were measured by pyrolysis on the moss remaining after testate amoeba extraction and the C/N ratio calculated from these data.

Testate amoebae were identified and counted up to a minimum of 150 shells but any slide was counted entirely to avoid possible bias due to the uneven distribution of shells depending on size or shape (i.e. smaller taxa being more likely to move closer to the edges of the coverslip). To assess the bioindicator potential of testate amoeba even with minimal taxonomic knowledge, we selected ten taxa which are abundant in *Sphagnum* peatlands, known to be well correlated with humidity and pH gradients and are commonly used in paleo reconstruction (Charman et al., 2004; Mitchell, 2003; Opravilova and Hajek, 2006): acid pools taxa: *Archerella flavum*, *Amphitrema wrightianum*, *Hyalosphenia papilio*; acid lawns: *Heleopera rosea*, *Hyalosphenia elegans*; acid hummocks: *Nebela militaris*, *Assulina muscorum*, *Assulina seminulum*, *Nebela tincta* s.l. and *Nebela*

Table 1
Geographical and climatic characteristics of the four studied *Sphagnum*-dominated peatlands in Switzerland.

Site	Coordinates		Altitude [m. a.s.l.]	Temperature [°C][a]	Precipitation [mm][a]	Study area taken in account [m^2][c]
	North	East				
Lörmoos	7°17′39″	46′58′54″	580	7.9	1028	1600
Rotmoos	7°50′30″	46°47′39″	1190	4.7	≈1300–1500[b]	2200
Trogenmoos	7°51′48″	46°45′39″	1460	3.1	≈1300–1500[b]	1900
Hohchräjen	7°58′09″	46°36′41″	1890	1	1575	1800

[a] Mean annual average for 1961–1990, taken.

[b] Estimated from MeteoSwiss general cartsmaps.

[c] Estimated using the geoportal of canton Bern (http://www.apps.be.ch/geo/fr/karten.html).

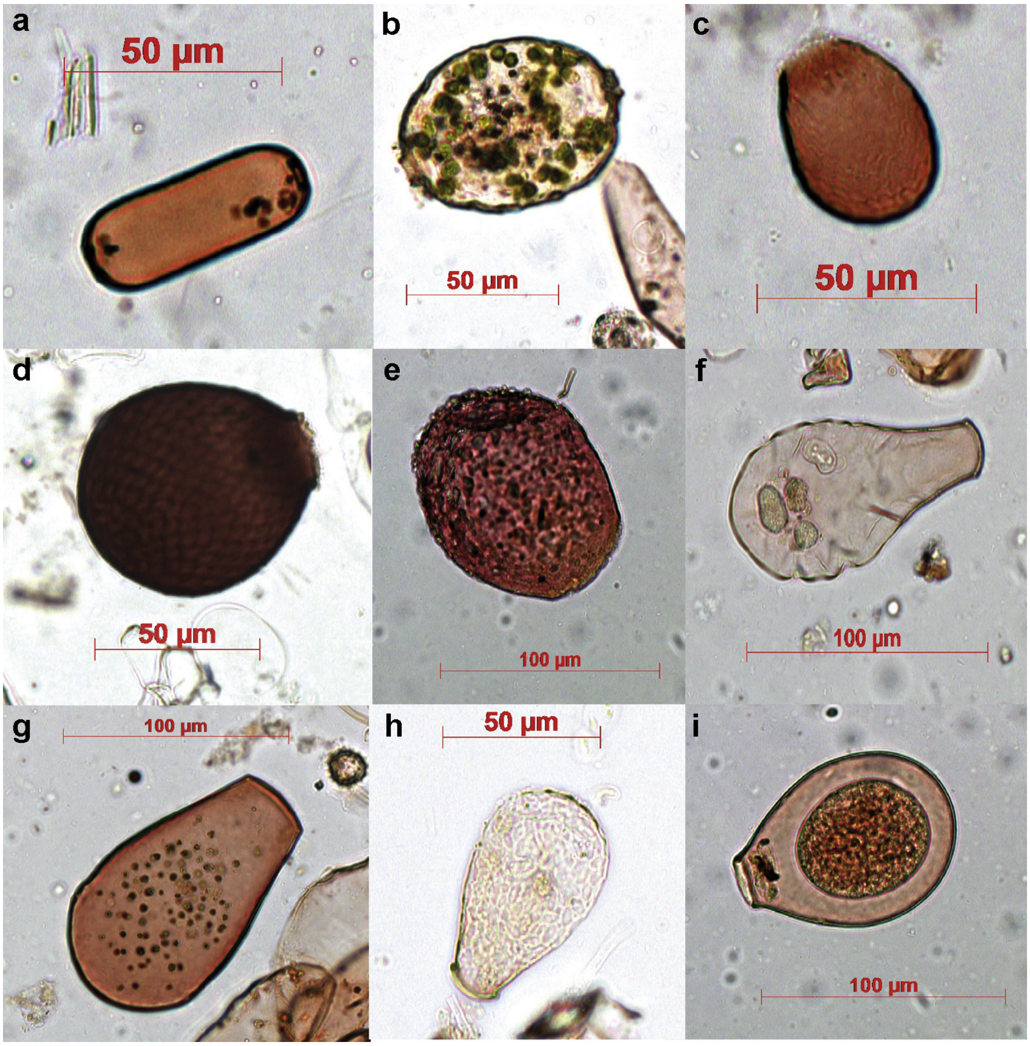

Fig. 2. Pictures of the selected testate amoeba morpho-taxa: (a) *Archerella flavum*, (b) *Amphitrema wrightianum*, (c) *Assulina muscorum*, (d) *Assulina seminulum*, (e) *Heleopera rosea*, (f) *Hyalosphenia elegans*, (g) *Hyalosphenia papilio*, (h) *Nebela militaris*, and (i) *Nebela tincta*.

collaris, Fig. 2. The taxonomic resolution we used corresponds to the approach used in many palaeoecological studies (Charman, 2001). Testate amoeba taxa that did not correspond to the ten selected taxa were also counted to reach the total of 150 shells. This enabled us to calculate the contribution of the selected taxa to the overall community.

Using a limited subset of ten taxa and a relatively coarse taxonomic resolution has, potentially, two main advantages: (1) it makes it easier for non-specialists to conduct similar analyses as only minimal training will be required to be able to identify the ten selected morpho-types and (2) it strongly reduces the risk of confusion among analysts making comparisons among studies much easier. As we tend to calibrate a tool to monitor peatlands, the choice of easily identified taxa is a strong advantage with respect to the potential use of this method by peatland managers or for high school and undergraduate student projects. The potential disadvantage however is that a given species complex may include taxa with contrasted ecological preferences in which case the accuracy of the ecological inference would be lower. This approach represents an alternative to other approaches such as using genus-level identification (Wilkinson and Davis, 2000) or using all taxa but pooling morphologically similar taxa regardless of taxonomic affiliation (Mitchell et al., 2014). Results were expressed in relative abundance (percentage of the total population) for multivariate numerical analyses.

2.4. Environmental data

The environmental data included depth to water table (DWT), C and N content and the C/N ratio of *Sphagnum*, altitude, micro-habitat, growing degree days (GDD) calculated from the temperature data using the software HOBO® Ware Pro 3.2 (actual temperature method, no cut-off, threshold: 0 °C), median temperature (T_{Med}), temperature range (T_{Range}) and the mean of daily temperature range ($\mathrm{Amp}T_{\mathrm{Med}}$) during the main vegetation period. The vegetation period was calculated from the beginning of increase of GDD until July 31st 2011 (peak biomass). Environmental data were used either globally or split in two sub-matrices: micro-environmental (DWT, C, N, C/N, $\mathrm{Amp}T_{\mathrm{m}}$) and macro-environmental (Altitude, GDD, T_{Med}, T_{Range}). Measured data for *Sphagnum* total N, C and C/N and DWT ($n = 84$) and temperature (GDD, T_{Med}, T_{Range} and $\mathrm{Amp}T_{\mathrm{Med}}$, $n = 34$) are given in Table 2.

As only 34 from the 84 plots were equipped with temperature data loggers, we completed the environmental dataset in order to work with the full vegetation and testate amoeba data. For the micro-habitats for which temperature data was available we calculated the mean of the three measured values within the micro-habitat and used these values for the two missing values. For the microhabitats lacking temperature data we replaced the missing values with predicted values based on the observed data and the estimated joint distribution of the temperature and all

Table 2
Summary of environmental variables measured at four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland. DWT: depth to water table; GDD: growing-degree day; T_{Med}: median soil temperature; T_{Range}: soil temperature range; AmpT_{m}: mean of soil daily temperature range (the last four variables were measured at 7.5 cm depth from the beginning of the growing season to July 2011). Different letters indicate significant differences (Tukey Honest Significant Differences calculated on transformed data; see text for further details).

	Lörmoos 580 m a.s.l.		Rotmoos 1190 m a.s.l.		Trogenmoos 1460 m a.s.l.		Hochräjen 1890 m a.s.l.	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
N [%]	0.540^{a}	0.021	0.692^{b}	0.042	0.564^{ab}	0.049	0.549^{a}	0.05
C [%]	43.1^{a}	0.3	43.0^{a}	0.1	$42.8^{a,b}$	0.2	42.2^{b}	0.1
C/N	$81.6^{a,b}$	2.6	67.4^{a}	3.7	84.8^{b}	5.6	84.9^{b}	5.7
DWT [cm]	21.5^{a}	3	12.2^{b}	1.3	8.1^{b}	1	10.5^{b}	1.5
GDD	1908.7^{a}	33.6	1499.7^{b}	7.3	1481.3^{b}	46.8	923.4^{c}	70.3
T_{Med} [°C]	13.6^{a}	0.2	12.7^{b}	0.1	13.1^{ab}	0.3	12.7^{b}	0.2
T_{Range} [°C]	22.0^{a}	0.8	20.5^{a}	0.8	23.1^{a}	0.7	12.5^{b}	0.5
AmpT_{m} [°C]	3.3^{a}	0.7	4.0^{a}	0.6	4.8^{a}	0.5	2.8^{a}	0.2

the other environmental variables. We used a multiple imputation based on a bootstrapped EM (expectation maximization) algorithm implemented in the Amelia II (package Amelia II for R Project for Statistical Computing, 2.14.0, Honaker et al., 2011). Amelia fills data gaps using a predictive model based on principal component analyses (PCA) on all data including samples for which all variables were measured and those which had data gaps. The missing values are calculated so as maintain the multidimensional structure of the PCA. Furthermore the model takes into consideration gradients or differences among data subsets, in our case these were the local (pool/hollow to hummock) humidity gradient and the four sites. Further information on the algorithm is given in Honaker and King (2010). The model was run five times and for each missing value the average value of the five iterations was used.

2.5. Numerical analyses

A three-pronged approach was used to explore the data:

(A) We first compared the environmental variables among sites using ANOVAs and Tukey HSD (Honest Significant Differences) tests with logarithm transformed data (natural log) for N, C, DWT and AmpT_{m}; square-root transformation for GDD and T_{Range}. We assessed how the environmental variables, the testate amoeba communities and the vegetation (i.e. vascular plants + bryophytes) varied in relation to (1) micro-habitat and (2) altitude using between class analysis (BCA). BCA can be seen as a particular case of a principal component analysis with respect to instrumental variables (PCAIV), in which a single factor is used as instrumental variable. These analyses were performed using R (R Core Team, 2012) and the packages ade4 for R (Dolédec and Chessel, 1987) and vegan for R (Oksanen et al., 2007).

(B) We then modelled the correlation between each community and the environmental variables using redundancy analyses (RDA, vegan for R Project, version 2.0-5, Oksanen et al., 2007) with Hellinger-transformed community data and assessed using transfer function how each data set performed to infer DWT. We used four data sets for these analyses: (1) vegetation (i.e. vascular plants + bryophytes), (2) vascular plants, (3) bryophytes and (4) testate amoebae. The environmental data were used as explanatory variables in the RDA. We tested each explanatory variable independently as well as the global model. Transfer function were built using weighted averaging (wa), with tolerance down-weighting, using the R package “rioja” (Juggins, 2012). Prediction error was estimated by a “leave-one out” cross-validation and the performance of the models assess by the correlation between observed and predicted values and the root mean squared error of prediction (RMSEP).

(C) Finally we explored the correlative structures among the six data matrices [(1) vascular plants, (2) bryophytes, (3) testate amoebae, (4) macro-environment, (5) micro-environment, (6) site name and humidity code (1 – pool to 5 - dry hummock) projected passively in the analysis)] using multiple factor analysis (MFA, FactomineR for R Project, version 1.21, Lê et al., 2008). We quantified the correlation between each pair of matrices using the RV coefficient.

All analyses were conducted on both the extended data set (i.e. including estimated missing temperature data) as well as on the samples for which direct measurements were available.

3. Results

3.1. Macro and micro-environmental characteristics of the four sites

Sphagnum carbon content decreased from 43.1% to 42.2% from the lowest to the highest site. *Sphagnum* nitrogen content was highest at Rotmoos and similar at the other three sites. As a result, *Sphagnum* C/N ratio was significantly higher at Rotmoss than at the other three sites (Table 2, only measured data were taken in account). Average water table was lowest at the low elevation site Lörmoos (DWT = 21.5 cm) and never reached the surface at that site. The other three sites were wetter (DWT = 8.1–12.2 cm) and all included pools. GDD decreased significantly with elevation. However, T_{Med}, T_{Range} and AmpT_{m} – measured at a depth of 7.5 cm – did not show any significant altitudinal trend.

3.2. Vegetation

The vegetation of the four sites included characteristic *Sphagnum* peatland taxa such as *Calluna vulgaris*, *Carex rostrata*, *Drosera* sp., *Eriophorum vaginatum* and *Vaccinium oxycoccos* for vascular plants and *Aulacomnium palustre*, *Polytrichum strictum*, *Sphagnum magellanicum* and *S. capillifolium* for bryophytes (Table 3). *Andromeda polifolia* and *Sphagnum cuspidatum* were found only at the two mid-elevation sites. The vegetation at Lörmoos was dominated by *V. oxycoccos* while species characteristic for very wet conditions were sparse (*C. rostrata*, *Rhynchospora alba*). Rotmoos included several species characteristic for slightly minerotrophic conditions (e.g. *Menyanthes trifoliata*, *Trichophorum cespitosum* and *Sphagnum papillosum*, Delarze et al., 2008). Trogenmoos contained both rare species such as *Scheuchzeria palustris* and *Sphagnum majus*, which are characteristic for *Caricion lasiocarpae*, a rare vegetation type only occurring in some of the best preserved mires (Delarze et al., 2008), as well as *T. cespitosum* and *Sphagnum fallax*, two taxa often associated with disturbance (Grosvernier et al.,

Table 3
Percentage cover of vascular plants and bryophytes at four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland. Rare species with an overall mean < 1% are not shown.

	Lörmoos 580 m asl		Rotmoos 1190 m asl		Trogenmoos 1460 m asl		Hochräjen 1890 m asl	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Vascular plants								
Andromeda polifolia	0	0	1.91	0.57	4.04	1.28	0	0
Calluna vulgaris	1.16	0.71	8.73	2.84	3.03	1.27	0.99	0.57
Carex limosa	0	0	0.63	0.63	5.11	2.39	11.37	3.08
Carex rostrata	0.47	0.2	6.56	2.86	0.33	0.33	11.72	2.42
Drosera sp.	1.39	0.58	1.64	0.6	3.35	1	0	0
Eriophorum vaginatum	5.55	1.69	2.23	0.93	1.92	1.07	9.98	3.16
Juncus filiformis	0	0	0	0	0	0	1.29	0.67
Menyanthes trifoliata	0	0	1.82	1.26	0	0	0	0
Molinia caerulea	3.27	1.55	3.78	0.84	0.03	0.03	0	0
Rhynchospora alba	2.66	0.75	0	0	0.08	0.08	0	0
Scheuchzeria palustris	0	0	0	0	2.01	1.12	0	0
Trichophorum cespitosum	0	0	13.11	2.45	18.18	3.16	0	0
Vaccinium oxycoccos	23.23	4.25	4.56	0.88	1.56	0.51	0	0
Vaccinium uliginosum	0	0	0	0	1.21	0.68	4.14	1.55
Bryophytes								
Aulacomnium palustre	0.77	0.5	1.42	0.69	0	0	0.04	0.04
Polytrichum strictum	0.84	0.55	3.4	1.14	0.03	0.03	5.94	2.21
Sphagnum capillifolium s.l.	25.78	5.19	4.04	2.14	0	0	9.52	3.4
Sphagnum cuspidatum	0	0	3.37	1.99	5.51	3.24	0	0
Sphagnum fallax	17.92	6.25	0.26	0.26	22.85	4.88	21.24	5.23
Sphagnum magellanicum	14.16	5.48	17.6	4.33	22.91	5.74	19.29	5.04
Sphagnum majus	0	0	0	0	2.08	1.54	0	0
Sphagnum papillosum	1.49	0.87	24	4.6	0.96	0.71	0	0
Sphagnum russowii	0	0	0	0	0	0	2.17	1.5
Sphagnum subsecundum	0	0	0	0	0	0	1.16	0.96
Sphagnum tenellum	0	0	0.32	0.32	3.73	1.86	0	0

Rare species not shown: *Carex pauciflora*, *Eriophorum angustifolium*, *Nardus stricta*, *Phalaris arundinacea*, *Picea abies*, *Pinus mugo*, *P. sylvestris*, *Vaccinium myrtillus*, *Viola palustris*, *Pleurozium schreberi*, and *Polytrichum commune*.

1995). The highest site, Hochräjen, was characterized by some montane-alpine taxa such as *Juncus filiformis*, *Nardus stricta* (not showed in the table) and *Vaccinium uliginosum* (foothills).

3.3. Testate amoebae

Testate amoeba density averaged 209×10^3 individuals per gram dry weight of *Sphagnum* and increased almost fivefold ($82.3–393 \times 10^3$ ind g^{-1}) from the lower to the higher site (Table 4). The ten selected taxa accounted for 64% of the total community on average over the four sites. This percentage was lowest at Rotmoos (41.5%) and highest at Hochräjen (86%), mostly due to the high abundance of three mixotrophic species characteristic for wet habitats *A. wrightianum* (26% of the total community), *A. flavum* (17.9%) and *H. papilio* (16%) at the highest site (Table 4). Noteworthy was also the absence of *H. elegans* from the highest site (Hochräjen) while it was the most abundant taxon at the other three sites (15–30.3%). By contrast *A. wrightianum* was absent from the lowest site (Lörmoos) while it was quite abundant (ranking 1st to 3rd in proportion of the whole community) at the other three sites (Table 4).

3.4. Between class analyses

The first set of between class analyses (BCAs), revealed contrasted altitudinal patterns of environmental characteristics, vegetation and testate amoeba communities (Fig. 3, boxes 1–3). The BCA on environmental data showed the altitudinal gradient and a high similarity between the two intermediate sites. In the vegetation BCA the four sites were well separated, but the pattern did not correspond to the altitudinal gradient. The elevation gradient was also visible on the testate amoeba BCA although the lowest site partly overlapped with the two intermediate sites and the overall spread within each site was higher than for the environmental data.

In the BCAs with micro-habitats as explanatory factor the humidity gradient was well correlated to axis 1 (Fig. 3, boxes 4–6). In the vegetation BCA the hollows and pool edges were clearly separated while drier micro-habitats overlapped. Micro-habitats were better separated based on testate amoebae than on the environmental data. The unusual (rather dry) hollows of Lörmoos stood out in the BCAs on environmental as well as on vegetation data and in the testate amoeba BCA its position was close to hummocks and lawns. The pool edges (only defined in Rotmoos) also stood apart in the vegetation BCA, while this was not the case for the analyses based on environmental characteristics and testate amoeba, where pool edges were projected close to pools.

3.5. Direct gradient analyses of correlation of each community with the soil dataset and performance of transfer function models

All eleven variables tested individually in the RDA significantly explained some part of variance in the testate amoebae and vascular plant data sets while for bryophytes two variables were not significant (T_{Range} and AmpT_m). Highest correlations were found with testate amoebae for eight variables, with vascular plants with two (GDD and T_{Range}) and with bryophytes with one (T_{Med}, Table 5, upper part).

Vascular plants showed higher correlation than bryophytes for all variables related to altitude (macro-env), except for T_{Med}. This agrees with the presence or higher abundance of some vascular plant species either at high elevation (*J. filiformis* or *V. uliginosum*) or low elevation (*V. oxycoccos*). Likewise the similar pattern observed for testate amoebae agrees with the distribution of *H. elegans* (absent from the highest site) and *A. wrightianum* (absent from the lowest site).

Table 4
Average density and relative abundance of ten testate amoeba morpho-taxa at four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland.

Taxon	Lörmoos 580 m asl		Rotmoos 1190 m asl		Trogenmoos 1460 m asl		Hochräjen 1890 m asl		All sites
	Average	SE	Average	SE	Average	SE	Average	SE	Average
Density [10^3 ind/g]									
Amphitrema wrightianum	0.0	0.0	26.7	9.0	16.5	4.8	191.9	93.8	55.3
Archerella flavum	3.3	1.4	5.6	2.5	28.9	13.6	63.0	21.4	23.6
Assulina muscorum	5.8	1.4	12.8	2.1	13.9	4.0	10.7	4.5	10.9
Assulina seminulum	1.9	0.3	1.4	0.3	1.3	0.3	4.8	2.2	2.3
Heleopera rosea	0.8	0.3	1.1	0.3	0.2	0.1	3.6	2.0	1.4
Hyalosphenia elegans	24.0	4.9	20.3	4.1	23.7	6.9	0.0	0.0	17.4
Hyalosphenia papilio	1.1	0.5	1.4	0.4	6.1	1.6	47.3	10.8	12.8
Nebela collaris	3.7	1.2	3.6	0.9	1.1	0.4	6.2	1.7	3.6
Nebela militaris	2.4	1.5	1.8	0.5	1.2	0.9	1.2	0.6	1.7
Nebela tincta	5.3	1.2	5.0	1.3	1.3	0.3	9.1	3.6	5.1
Total community	82.3	13.3	191.8	30.4	184.2	30.1	392.8	109.3	209.4
% of total community	58.7		41.5		51.1		86.0		64.0
Relative abundance [% of total count]									
Amphitrema wrightianum	0.0	0.0	8.8	2.4	7.0	1.9	26.0	6.3	10.2
Archerella flavum	2.4	1.0	2.6	1.2	9.0	2.8	17.9	5.9	7.5
Assulina muscorum	8.1	1.6	7.5	0.9	5.7	0.9	2.8	0.9	6.1
Assulina seminulum	3.7	0.7	1.3	0.3	1.1	0.3	1.8	0.8	1.9
Heleopera rosea	1.2	0.4	0.8	0.2	0.3	0.2	2.3	1.2	1.1
Hyalosphenia elegans	30.3	5.9	15.0	3.0	19.3	4.5	0.0	0.0	16.3
Hyalosphenia papilio	1.0	0.4	1.0	0.3	3.2	0.7	16.0	2.3	4.9
Nebela collaris	6.6	2.1	3.5	1.2	1.1	0.4	5.2	1.7	4.0
Nebela militaris	2.5	1.4	1.4	0.4	1.1	0.8	0.7	0.2	1.4
Nebela tincta	8.0	2.0	4.7	1.2	1.3	0.4	5.8	2.2	5.0

Correlations with all micro-env variables (related more to micro-habitat variation) were highest for testate amoebae. Correlations with bryophytes were higher than with vascular plants for moss elemental chemistry variables (C, N, and C/N) while the opposite was true for DWT and AmpT_{Med}, two variables measured below the moss carpet. In the variance partitioning (based on RDA) the whole macro-env dataset explained a similar proportion of variance of the vascular plants and testate amoeba data (respectively 33.0% for vascular plants and 32.1% for testate amoeba, Table 6), but a lower proportion of the bryophyte data (16.6%). Micro-env variables explained a higher proportion of the testate amoeba than the vascular plant and bryophyte data (respectively 47.1%, 30.4% and 27.4%).

The descriptive variables were most strongly correlated with vascular plants for the site (39.1%) and with testate amoebae for micro-habitats (38.9%), in agreement with the BCA analysis.

In line with the RDA results with micro-environmental variables, the performance of DWT transfer function models was highest for testate amoebae, somewhat lower for vascular plants and whole vegetation and lowest for bryophytes (Table 6).

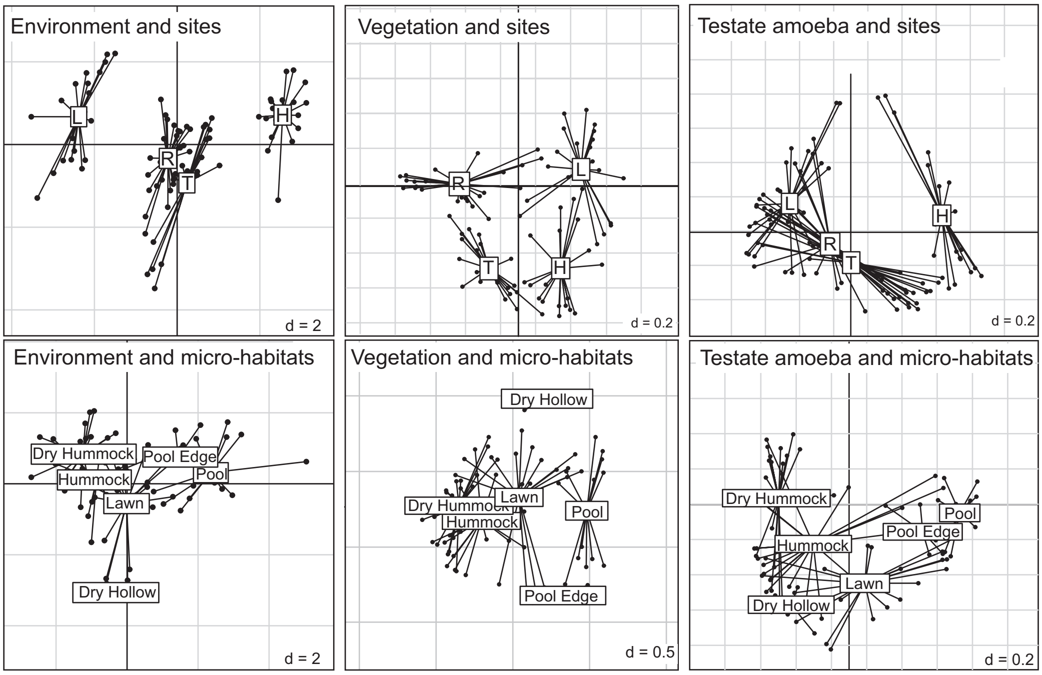

Fig. 3. Between class analysis of environmental data (left), vegetation (centre), and testate amoeba communities (right) from four *Sphagnum*-dominated peatlands in relation to sites (top row) and micro-habitats (bottom row). L: Lörmoos (alt: 580 m); R: Rotmoos (1190 m); T: Trogenmoos (1460 m); H: Hochräjen (1890 m).

Table 5

Summary results of redundancy analyses (RDAs) on vascular plant, bryophyte and testate amoeba community data, macro- and micro-environmental variables from four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland. Percentage of variance explained by individual macro- and micro environmental variables and associated p-value from RDAs on vegetation (vascular plants + bryophytes), vascular plants, bryophytes and testate amoeba data. Highest scores for each variable are underlined and highest scores per data set are bolded. Upper table: whole dataset with calculated missing values ($n = 84$), lower table: variance explained with sample directly measured ($n = 34$).

Whole dataset ($n = 84$)	Bryophytes + Vasc.		Vascular plants		Bryophytes		Testate amoebae	
	%	*p*-Value	%	*p*-Value	%	*p*-Value	%	*p*-Value
Macro-environment								
Altitude	9.3	0.005	**17.6**	0.005	4.6	0.005	19.0	0.005
GDD	7.5	0.005	14.1	0.005	3.9	0.010	13.8	0.005
T_{Range}	5.5	0.005	10.5	0.005	1.2	0.360	8.0	0.005
T_{Med}	5.5	0.005	5.4	0.005	6.0	0.005	4.0	0.010
All macro-env.	**22.8**	**0.005**	**33.0**	**0.005**	**16.6**	**0.005**	**32.1**	**0.005**
Micro-environment								
DWT	13.1	0.005	14.4	0.005	11.6	0.005	**26.0**	0.005
C	9.3	0.005	7.3	0.005	**12.2**	0.005	17.5	0.005
N	6.0	0.005	3.8	0.005	6.9	0.005	10.3	0.005
C/N	5.0	0.005	3.0	0.024	5.8	0.005	8.7	0.005
AmpT_m	4.6	0.005	5.4	0.005	2.5	0.071	5.7	0.005
All micro-env.	**28.9**	**0.005**	**30.4**	**0.005**	**27.4**	**0.005**	**47.1**	**0.005**
Descriptive variables								
Micro-habitat	28.6	0.005	27.4	0.005	27.8	0.005	38.9	0.005
Site	28.1	0.005	39.1	0.005	22.6	0.005	26.6	0.005

Raw dataset ($n = 34$)	Bryophytes + Vasc.		Vascular plants		Bryophytes		Testate amoebae	
	%	*p*-Value	%	*p*-Value	%	*p*-Value	%	*p*-Value
Macro-environment								
Altitude	12.2	0.005	**20.6**	0.005	6.3	0.077	23.5	0.005
GDD	9.3	0.015	16.2	0.005	5.1	0.140	16.2	0.005
T_{Range}	9.4	0.005	13.5	0.005	3.3	0.400	9.6	0.015
T_{Med}	6.0	0.033	7.3	0.017	6.5	0.059	4.1	0.240
All macro-env.	**29.5**	**0.005**	**42.5**	**0.005**	**18.7**	**0.046**	**39.1**	**0.005**
Micro-environment								
DWT	14.2	0.005	14.7	0.005	**11.6**	0.010	**24.3**	0.005
C	6.7	0.015	6.6	0.024	8.2	0.024	15.7	0.005
N	6.5	0.013	4.0	0.260	7.3	0.036	10.4	0.013
C/N	6.0	0.028	2.9	0.350	7.6	0.017	9.0	0.027
AmpT_m	6.2	0.025	8.0	0.013	3.2	0.370	9.1	0.020
All micro-env.	**33.9**	**0.005**	**35.6**	**0.005**	**28.7**	**0.005**	**50.7**	**0.005**
Descriptive variables								
Micro-habitat	31.7	0.005	31.9	0.005	27.6	0.005	45.0	0.005
Site	33.3	0.005	44.4	0.005	27.1	0.005	32.4	0.005

3.6. Combined analysis of testate amoeba, vegetation and environmental data

The position of samples in the multiple factor analysis (MFA, Fig. 4) showed quite clearly the altitudinal and humidity gradients. All Hochräjen plots were well separated from the three other sites, which partly overlapped in the ordination space. Rotmoos and Trogenmoos overlapped with respect to the altitudinal gradient except for the wettest micro-habitats. The position of samples from the lowest site suggested drier conditions than for the other three sites and the micro-habitat described as hollow appeared to be more similar to the lawns than to the pools of the other sites.

In the projection of species and variables (Fig. 5), pools were associated to relatively high N content and higher median soil temperature. Hummocks and dry hummocks were correlated with low water table (i.e. high DWT) and high C/N ratio. The altitudinal gradient was correlated with T_{Range} and GDD. Altitude was best correlated with two testate amoeba taxa, *H. papilio* for the higher site and *H. elegans* for the lowest one, together with two vascular plants, *V. oxycoccos* and *Molinia caerulea*. The pools of the higher site were characterized by *A. wrightianum* and *A. flavum* for the testate amoebae, *Carex limosa* and *C. rostrata* for the vascular plants and *S. fallax* for the mosses. The same micro-habitats in Trogenmoos and Rotmoos were correlated with *A. muscorum* for the testate amoebae, *T. cespitosum* for the vascular plants and *S. papillosum* and *S. cuspidatum* for the mosses. The position of the Lörmoos hollow samples clearly suggested that they were drier than pools at the other sites.

Table 6

Performance of testate amoebae-based transfer function for DWT based on data from four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland: leave-one out cross-validated tolerance down-weighted [inverse (WA.inv.tol) and classical (WA.cla.tol)] weighted average models.

	Whole vegetation		Vascular plants		Bryophytes		Testate amoebae	
	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2
WA.inv.tol	7.00	0.46	6.66	0.51	7.84	0.32	**5.91**	0.61
WA.cla.tol	7.60	0.47	6.91	0.52	11.88	0.33	6.77	**0.62**

RMSEP: root mean square error of prediction; R^2: correlation between observed and predicted values.

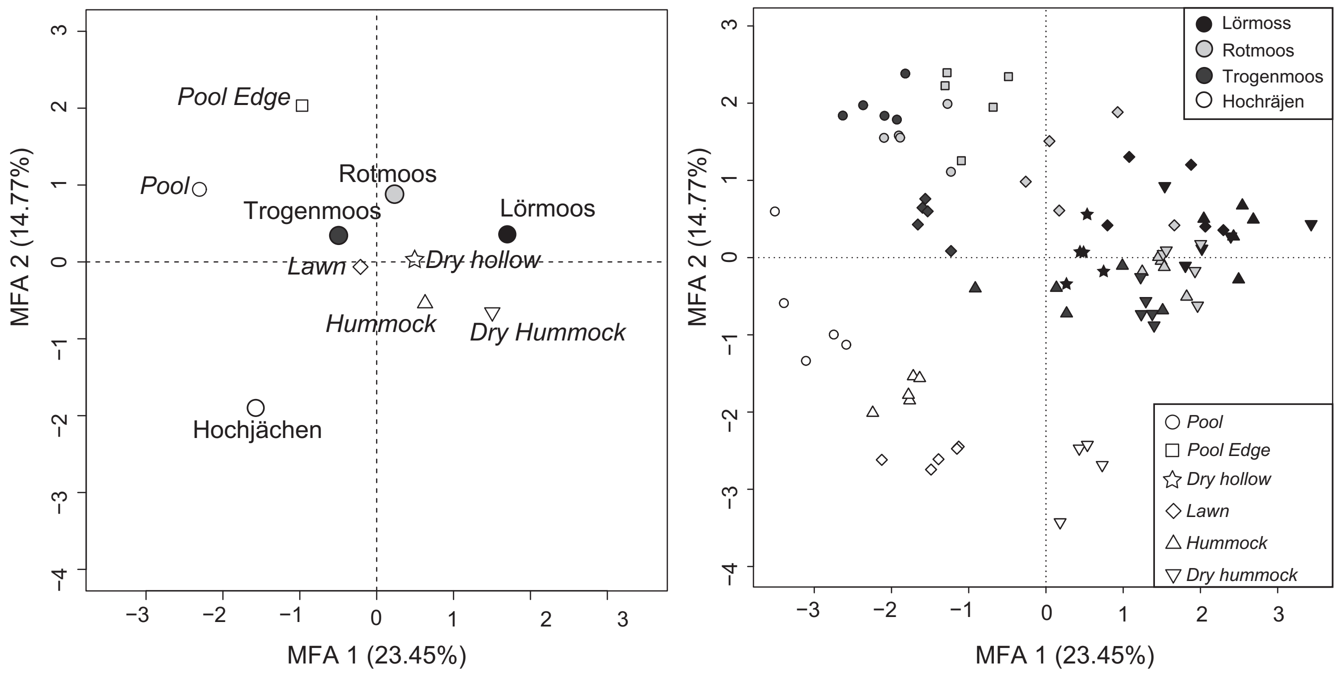

Fig. 4. Multiple factor analysis (MFA) of vascular plant, bryophyte and testate amoeba community data, macro- and micro-environmental variables from four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland. Left: projection of centroids of qualitative variables (site names and micro-habitats) projected passively on the MFA. Right: individual factor map, showing the position of samples in the MFA. Humidity gradient go from top left part of the graph to bottom right angle and altitude gradient go from bottom left part to top right part.

The RV coefficients of the MFA (Table 7), which correspond to a squared Pearson correlation coefficient showed that micro-environmental variables were more strongly correlated to testate amoebae while macro-environmental variables were more strongly correlated to vascular plants. The analysis of the reduced data set yielded similar results (details not shown).

4. Discussion

4.1. Macro and micro-environmental characteristics of the four sites

We aimed to select four comparable sites while covering a range of climatic conditions found along the elevation gradient and other factors potentially influencing peatland ecosystems that may also vary along this gradient. Indeed, several factors are associated to altitudinal gradients including differences in the degree of human impact, precipitation rates, duration of snow cover and pollution levels (Körner, 2007). The soil chemistry data measured at the four sites were within the range expected for *Sphagnum*-dominated peatlands (Bragazza and Gerdol, 2002; Waughman, 1980) and with the exception of the lowest site all micro-sites could be found at each site. Inter-site differences reflect climatic gradients (e.g. drier lower site), ombrotrophy–minerotrophy gradient (somewhat higher minerotrophic influence at the Rotmoos site), and history of human impact (drainage at the lowest site). As such these four sites therefore provide a reasonable range of general characteristics to allow the comparative study of species–environment relationships for vascular plants, bryophytes and testate amoebae.

4.2. Community–environment relationships–micro-habitats and DWT

As expected, community structure of vascular plants, bryophytes and testate amoebae all reflected well the pool-hummock gradient as shown by significant correlations to micro-habitat type (explaining 27.4–38.9% of the species data in the RDA) and the depth to the water table (explaining 11.6–26% of the data). However, a striking result of this study is that ten testate amoeba taxa were more strongly correlated than vascular plants, bryophytes, or both combined to micro-habitats (38.9% vs. 27.4–28.6%) and to the humidity gradient (26% vs. 11.6–14.4%). With the exception of temperature-related variables and sites the correlation of all variables were about two times higher with the ten selected testate amoeba taxa than with all bryophytes, all vascular plants or both groups combined. The performance of transfer functions also logically performed better with the testate amoeba data than the vascular plants and bryophytes. Furthermore

Table 7
RV coefficients from the multifactor analysis (MFA) on vascular plants, bryophytes, testate amoebae, micro-environmental variables, macro-environmental variables from four *Sphagnum*-peatlands along a 1300 m elevation gradient in Switzerland. The descriptive variables were passively projected in the analysis. Upper half matrix: *p*-values; lower half matrix: RV coefficients. MFA indicates the correlation and *p*-value with the overall combined ordination.

	Vasc. plants	Bryophytes	Amoebae	Micro-env	Macro-env	MFA
Vascular plants	1	<0.001	<0.001	<0.001	<0.001	0.769
Bryophytes	0.248	1	<0.001	<0.001	0.001	0.594
Amoebae	0.543	0.257	1	<0.001	<0.001	0.747
Micro-env	0.324	0.263	0.431	1	<0.001	0.661
Macro-env	0.375	0.115	0.296	0.164	1	0.542
MFA	0.769	0.594	0.747	0.661	0.542	1

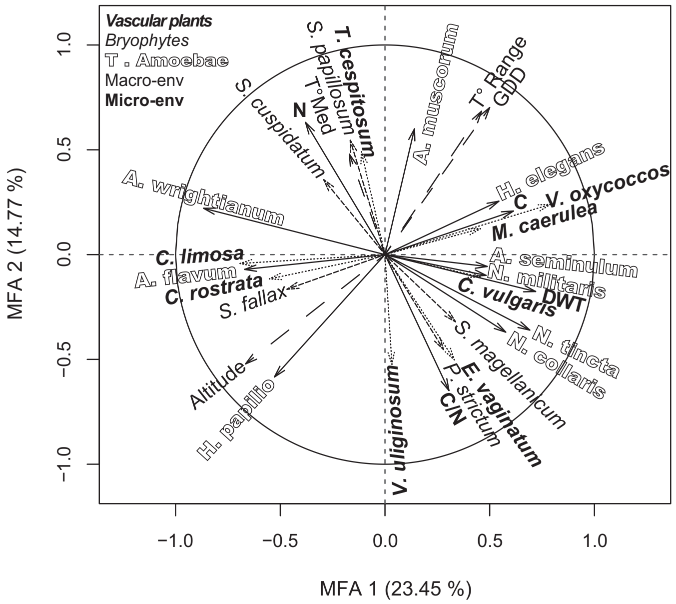

Fig. 5. Correlation circle map of the MFA of vascular plant, bryophyte and testate amoeba community data, macro- and micro-environmental variables from four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland (see Fig. 4). For clarity, only variables with a score over $\cos^2 = 0.2$ are represented. T Amoebae: testate amoebae; Micro-env: micro-environmental variables (N, C, C/N, AmpT_m); Macro-env: macro-environmental variable (Alt, GDD, T_{Med}, T_{Range}).

the performance of the models is comparable to that of models based on the full diversity of testate amoebae.

It may appear surprising that bryophytes are not better related to micro-environmental variables. Indeed, they could be expected to respond in a similar way than testate amoebae to humidity and nutrients gradients. Some variables not taken in account like niche competition, sensitivity to microclimatic variations, interstitial water conductivity or nutrients may be more important for bryophytes than for testate amoebae and could explain the relative weakness of the relationships observed here (Bragazza, 1997; Buttler et al., 1998; Malmer et al., 1992; Mitchell et al., 2000). However our results agree with a recent comparative ecological study of testate amoebae, bryophytes and vascular plants in fens and bogs, which showed that testate amoeba communities were more strongly correlated to all measured variables (conductivity, pH, magnesium, calcium and DWT) than bryophytes or vascular plants or both combined (Lamentowicz et al., 2010).

The absence of *A. wrightianum* at the lowest site is in line with its association with bog pools, which were absent from that site. *A. flavum*, a species characteristic for wet and oligotrophic lawns, was rare at the two lower sites, in line with average drier (Lörmoos) or more minerotrophic (Rotmoos) conditions. Both taxa were abundant at the upper two sites, and especially at the highest one. However, these two taxa may respond to site history (e.g. peat harvesting and subsequent revitalization, Buttler et al., 1996; Laggoun-Défarge et al., 2008) as much if not more than to differences in climate, soil moisture or nutrient content.

4.3. Community patterns in relation to sites

The four sites were best defined by the vegetation data (explaining 39.1% of variance in the RDA vs. 22.6% and 26.6% for bryophytes and testate amoebae respectively). This difference seems partly due to the presence of characteristic subalpine plant species at the highest site (e.g. *J. filiformis*, *N. stricta*), to differences in trophic status among sites (*M. trifoliata* indicating more minerotrophic conditions at Rotmoos), and to the absence of some taxa at some sites in part due to the absence of pools at the lowest site: *C. limosa* and *C. rostrata* both require very wet conditions that were rare or absent at the lowest, driest site (Lauber et al., 2007). These patterns may, however, also relate to the history of the sites. For example *V. oxycoccos* was shown to be favoured by bog revitalization measures (Graf et al., 2007) and this is in line with its high abundance at the lowest site.

4.4. Community patterns in relation to elevation and temperature

Testate amoeba communities were correlated to altitude and GDD gradient in the same range as vascular plants, but bryophytes were only weakly correlated to these two variables. The correlation of vascular plants along the altitude gradient fits with the known distribution of plants along elevation gradients and could thus be expected despite the fact that this study was focused on a very specific ecosystem and a limited potential list of plant species. By contrast, such a result was not expected for testate amoebae, which are usually primarily correlated to micro-environmental conditions such as DWT (Booth et al., 2008).

All other factors being equal, species such as *Carex limosa*, *C. rostrata*, *A. flavum* and *A. wrightianum* which are associated with wetter conditions can be expected to be more abundant at higher elevation where the summer hydric stress is shorter and less intense than at lower elevation. A recent study showed that testate amoeba communities are correlated not only to DWT but also to moisture variability (Sullivan and Booth, 2011). Moisture variability is likely to be lower at higher elevation and this could partly explain altitudinal patterns. Such differences may also have implications for the functioning of microbial food webs (Lamentowicz et al., 2013).

A recent study using a similar approach (i.e. four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland, two of which – the highest and lowest sites – were identical as in this study) showed that altitude explained a third of the variance in testate amoeba community data while DWT only explained a marginal fraction (Lamentowicz et al., 2013). This result was explained by the fact that only a single habitat (lawns) was sampled and thus the DWT gradient was very short. In our study we sampled the full DWT gradient within each site and DWT emerged as the strongest variable explaining testate amoeba community structure. Altitude explained a smaller fraction (19% vs. 33%) of variance than in the study of Lamentowicz et al. (2013). This difference between the two studies may be due to the different taxonomic resolution, to the fact that altitude explains a different proportion of variance depending on the microhabitat, or to the fact that only two of the four sites were shared between the two studies.

The distribution pattern of the two *Hyalosphenia* species in our study and the one of Lamentowicz et al. (2013) suggests a possible response to elevation with *H. elegans* being limited to warmer conditions and *H. papilio* to colder sites. However, the absence of *H. elegans* in Hochräjen, the highest site, is most likely not due to severe weather conditions as it was found near the Arctic Circle (Beyens and Chardez, 1995; Payne et al., 2006) and it was abundant in a *S. magellanicum* sample from the Northwest Territories, Canada (Mitchell, unpublished data). A possible alternative explanation is that *H. elegans* tolerates moisture fluctuations somewhat better than *H. papilio* (Sullivan and Booth, 2011). The absence of *H. elegans* from the highest site could also be due to slightly more minerotrophic conditions at this site (e.g. runoff influencing hydrochemistry during snowmelt). In line with this, *H. elegans* was shown to respond very strongly (100%) to N & P enrichment (Mitchell, 2004). However, this species did not show a clear response to N addition (Mitchell and Gilbert, 2004).

With only four sites it is clearly impossible to generalize such results. Further studies, ideally including a higher number of sites and experimental manipulations of climate DWT and other environmental factors are clearly needed to explore in more depth the relationship between *Sphagnum* peatland testate amoeba communities and altitude.

4.5. Implications for bioindication

Although vegetation analysis is a well-established method for the peatland monitoring, our study showed that the analysis of testate amoebae provides more accurate information on micro-environmental conditions than vascular plants and/or bryophytes. Furthermore we show that it is not necessary to know all testate amoeba taxa to obtain valuable ecological information; a carefully selected list of a limited number of easily identifiable taxa suffices for bioindication purposes.

The generation time of testate amoebae ranges from a few days to weeks depending on taxa and environmental conditions is much faster than that of bryophytes and vascular plants (Schönborn, 1986). Communities are rather stable under natural conditions between seasons (Warner et al., 2007), but they can change over a period of several months when conditions change (Marcisz et al., 2014). Thus testate amoebae respond to environmental changes at an appropriate speed for being used in monitoring programmes.

An additional advantage of testate amoeba analysis is that samples can be collected at all seasons (providing sites are accessible and sampling sites are permanently marked – e.g. with long sticks – to be found beneath the snow). Sampling is much faster than the time required for vegetation relevés and thus more site can be studied in a given field campaign. The time needed for microscopic analyses is reasonable once the species are known (and learning this is quick for the selected taxa). Testate amoeba analysis for peatland bioindication purposes is therefore a potentially very powerful and economically interesting approach.

5. Conclusion

Although testate amoebae are regularly used for paleoenvironmental reconstruction, their usefulness in biomonitoring is not yet fully recognized beyond academic research. Our study shows how a selection of only ten, easily identifiable testate amoeba morpho-taxa can provide valuable ecological information on micro-environmental conditions in *Sphagnum*-dominated peatlands. An untrained analyst can learn to identify these ten taxa in a few hours and only a simple microscope is needed for the analyses. This method therefore has the potential to become part of the standard toolkit of peatland managers.

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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.2015.01.043.

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