

Disentangling the effects of water chemistry and substratum structure on moss-dwelling unicellular and multicellular micro-organisms in spring-fens

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ABSTRACT

Water chemistry is known to be one of the most important factors controlling species composition of many macro-organisms in wetlands. It is unclear to what extent micro-organisms respond to water chemistry as compared to chemistry-mediated substratum structure. We explored how the assemblages of different groups of micro-organisms in bryophyte tufts of spring-fens were determined by water chemistry and substratum structure. The aim was to compare unicellular autotrophic diatoms, unicellular heterotrophic testate amoebae and multicellular heterotrophic monogonont rotifers. Assemblages of all three groups showed a strong compositional gradient correlated with water pH and conductivity, calcium concentration and dominance of Sphagnum. While a second strong gradient in species composition of diatoms and testate amoebae was explained by factors such as substratum structure and water content, that of rotifers remained unexplained. Unlike the other two groups, testate amoeba assemblages were significantly determined by phosphates. Nitrates and iron were important species composition determinants for diatoms. Rotifers differed from the other groups in that they did not respond significantly to silica, iron or nutrients. When variation caused by substratum characteristics and water chemistry were partitioned out, testate amoebae were controlled more by substratum, while rotifers and diatoms were controlled more by water chemistry. Variation explained by individual effects of substratum or water chemistry, as compared to shared effects, was much lower for rotifers than for testate amoebae and diatoms. Our results show that, in semi-terrestrial ecosystems, pH and calcium concentrations are generally the main drivers of variation in species composition of unicellular and multicellular microorganisms, mirroring well described patterns for macro-organisms, providing support for general ecological hypotheses. Other water chemistry variables differed between shell-forming and other organisms, and between autotrophic and heterotrophic organisms. Even though water chemistry variables controlled the structure of the bryophyte tufts that acted as substratum for the micro-organisms, both water chemistry and bryophyte structure effects were independently significant for diatoms and testate amoebae. On the other hand, no effects of either substratum characteristics or water chemistry were found for rotifers. This was because their species composition is not influenced by chemical factors, apart from pH and calcium, which both strongly influence the occurrence of Sphagnum.

Key words: bryophytes, diatoms, mineral-richness gradient, monogonont rotifers, testate amoebae, variation partitioning

1. INTRODUCTION

Water chemistry is known to be one of the most important factors controlling species composition and richness of many wetland macro-organisms (Vitt 2000; Hájek *et al.* 2002; Horsák & Hájek 2003; Tahvanainen 2004; Horsák 2006). Less information is available for micro-organisms, which are key organisms in wetland ecosystem functioning, especially peatlands, responding quickly to environmental changes (Gilbert *et al.* 1998a). Ecologists tend to postulate general concepts (e.g., ecological niches and gradients) on the basis of vegetation or larger animals only, despite uncertainty over whether micro-organisms, like macro-organisms, respond directly to water chemistry, or whether substratum structure is more important. Substratum type has been reported as an important factor controlling species composition of some micro-organismal assemblages (Cox 1988; Rothfritz *et al.* 1997; Dugan 2001; Cantonati &

Spitale 2009). Because of their ability to absorb and retain large quantities of water, thus protecting organisms that live within them against climatic changes (Strong 1967; Corbet 1973), bryophytes are one of the most appropriate substrata for many groups of micro- and meio-organisms (Suren 1992; Linhart *et al.* 2002; Bertrand *et al.* 2004; Vincke *et al.* 2006). Although some authors regard bryophytes as neutral substrata that do not influence micro-organismal assemblages (cf. Bertrand *et al.* 2004 for diatoms), other studies suggest some relationships between the composition of bryophyte tufts and microbial assemblages (Pouličková *et al.* 2004 for diatoms, Opelt *et al.* 2007 for bacteria, Lamentowicz *et al.* 2010 for testate amoebae). Different bryophyte species have different abilities to retain water in their cells and, consequently, provide different moisture conditions for organisms inhabiting them. *Sphagnum* is unique in having large dead cells (hyalocytes) that are specialised for water retention, and hosts

diverse microflora and microfauna (Hingley 1993). Moisture intensity has therefore been reported as another important environmental factor controlling species composition of micro-organismal communities (De Graaf 1956; Ohtani 1986; Van de Vijver *et al.* 2003; Pouličková *et al.* 2004; Lamentowicz *et al.* 2010). Apart from moisture, bryophyte chemistry can also influence autotrophic primary producers and, consequently, heterotrophic micro-organisms, such as testate amoebae (Mitchell *et al.* 2004).

Micro-organisms are characterised by small body size, high dispersal potential, large population sizes, ability to enter dormancy, and rapid reaction to changing environment (Cáceres 1997; Bohonak & Jenkins 2003, Korhonen *et al.* 2010). Due to these characteristics, the proportions of ubiquitous and cosmopolitan species are significantly higher in micro- than macro-organisms (Fontaneto *et al.* 2008; Segers & De Smet 2008). It is not clear whether the presumed dispersal capability of micro-organisms causes their tighter relationships to environmental factors. It is generally believed that short generation time and wide distribution of micro-organism species makes them good bioindicators (Mitchell *et al.* 2008). On the other hand, the significance of mass effects in high-dispersal systems (Ng *et al.* 2009) may result in a weaker response of species composition to ecological gradients. Particular taxonomic groups of micro-organisms differ in body structure (uni- *versus* multicellular), position in the trophic chain (auto- *versus* heterotrophy), ability to move and/or become dormant, which makes it difficult to draw universal conclusions based on a single taxonomic group. Unicellular and multicellular organisms were compared for their species richness patterns by Hillebrand *et al.* (2001), whose results indicated that the dispersal ability of some taxonomic groups (benthic diatoms or ciliates) is rather restricted, and ubiquity is probably not as high as was thought.

Studies comparing the factors driving community composition among different taxonomic groups of micro-organisms are rarer than those comparing species richness and spatial distribution. The importance of pH and moisture has been documented for particular groups of micro-organisms (Berzins & Pejler 1987; Mieczan 2009; Cantonati & Spitale 2009), but the effects of substratum structure on different micro-organisms has not been compared. In addition, studies have often focused only on one habitat type (bogs for testate amoebae or littoral zones of lakes for rotifers). We therefore chose three different taxonomic groups of micro-organisms: diatoms, testate amoebae and monogonont rotifers, which we studied along a gradient of mineral richness, from extremely mineral-rich spring-fens to mineral-poor *Sphagnum*-fens. Diatoms are unicellular, silica-forming autotrophic organisms which can fix energy from the sun and thus form the base of the food chain (Round *et al.* 1990); testate amoebae represent a group of unicel-

lular, heterotrophic protists producing different types of shells or plates, which often contain silica (Warner 1990); monogonont rotifers are small multicellular aquatic animals characterised by the ability to withstand unfavourable periods in diapause, and by female parthenogenesis allowing rapid population growth under favourable environmental conditions (Ricci 2001; Gilbert 2004; Schröder 2005).

The main aim of our study is to compare responses of three taxonomic groups of micro-organisms, differing in trophic and body structure, to environmental variables. We studied the effects of both substratum (i.e., bryophyte species composition, tuft structure and water content) and water chemistry on species composition. We also compared major gradients in species data and the ability of particular variables to explain species composition within the groups. The following hypotheses were tested: (1) water chemistry is more important for diatoms (autotrophs) than for the other two groups (heterotrophs), because dissolved minerals and nutrients directly affect photosynthesis and autotrophic growth; (2) much of the explained variability will be shared between substratum and water chemistry, because the structure of the bryophyte tuft is influenced by water chemistry (e.g., *Sphagnum* *versus* pleurocarpous mosses, Gorham & Janssens 1992, Paullisen *et al.* 2004), but at the same time the bryophyte modulates other important variables, such as moisture and light penetration; (3) the assemblage composition of shell-building organisms (diatoms and testate amoebae) will be more strongly influenced by dissolved silica concentration than that of monogonont rotifers.

2. MATERIALS AND METHODS

2.1. Study area and sites

The study area was located on the western margin of the Western Carpathians, stretching along the border between the Czech Republic and Slovakia (48°56'-49°32'N, 17°44'-18°51'E). This region forms part of the flysch belt, in which sandstone and claystone of variable calcium contents alternate. The chemical composition of groundwater reflects the rock chemistry (Rapant 1996). Groundwater chemistry varies from calcareous waters, which support cold-water travertine (tufa) formation (in the south of the studied area), through waters rich in calcium, sodium and potassium, without tufa-formation, to acidic waters rich in iron, silica and sulphates but poor in all other elements (in the northernmost part of the studied area) (Hájek *et al.* 2002). The following four structural habitat types were distinguished along the mineral-richness gradient: (1) calcareous fens with tufa (calcium carbonate) formation, (2) extremely mineral-rich fens without tufa formation, (3) mineral-rich and moderately mineral-rich *Sphagnum*-fens, and (4) mineral-poor acid *Sphagnum*-fens. On the basis of previous studies (Pouličková *et al.* 2005), we chose 13 sites that

best corresponded to the above-mentioned fen habitat types. Four sites were selected within petrifying springs, three sites within mineral-rich fens without tufa formation, two sites within mineral-rich *Sphagnum*-fens and four within mineral-poor *Sphagnum*-fens.

2.2. Sampling methods and sample processing

Epiphytic diatoms, testate amoebae and monogonont rotifers were sampled with their substrata (bryophytes). A total of 26 samples of bryophytes were collected in May 2006. At each site, two samples of different bryophyte tufts were collected, representing clearly distinguishable mesohabitats, differing mostly in the composition of the bryophyte tuft and/or water level. Sampled bryophytes were situated in areas with good groundwater supplies and hydrochemistry was constant within the locality. Each bryophyte tuft comprised one dominant species plus a mixture of other bryophyte species. For each sample we determined the percentage abundance of individual bryophyte species, recording a total of 14 moss and one liverwort (*Pellia*) species. Bryophyte tufts were cut with scissors and transported in a live state in plastic bags to the laboratory. In the laboratory, each bryophyte sample was weighed, washed with 20 mL of distilled water and thoroughly squeezed to extract micro-organisms. The effectiveness of the washing procedure was 80% (Hindák 1978). Squeezed bryophytes were dried at 75 °C for 48 hours and then re-weighed and water content estimated as the difference between fresh and dry bryophyte matter. The extract was fixed with formaldehyde and split into two subsamples, 0.5 mL of extract being analysed from each. The first subsample was used for processing testate amoebae and rotifers, the second for diatoms. Diatom frustules were mounted in Pleurax. For quantitative evaluation, 400 diatom valves were identified on each permanent slide. Nomenclature followed Kučera & Váňa (2003) for bryophytes, Krammer & Lange-Bertalot (1986, 1988, 1991a, b) for diatoms, Aesch & Foissner (1989) for testate amoebae and Segers (1995) for monogonont rotifers.

Bryophytes were divided into four functional groups: 1) *Sphagnum* species: *S. fallax*, *S. flexuosum*, *S. palustre* and *S. papillosum*; 2) crawling (pleurocarpous) species with loose tufts: *Brachythecium rivulare*, *Calliergonella cuspidata* and *Plagiomnium affine* agg. (*P. ellipticum*, *P. elatum*); 3) crawling (pleurocarpous) species with dense tufts: *Cratoneuron filicinum*, *Palustritella commutata* and *P. decipiens* and 4) erect (mostly acrocarpous) species: *Bryum pseudotriquetrum*, *Fissidens adianthoides* and *Philonotis caespitosa*. For each sample, the percentage cover of each bryophyte functional group was determined.

Conductivity (corrected for H⁺ ions and standardised to 20 °C) and pH of the water were measured *in situ* using portable instruments (WTW Multi 340i/SET). To characterize each site, samples were taken in October 2006. Water samples were collected in autumn due to

relative stability of water chemistry (Hájek & Hekera 2002) and PO₄³⁻, NO₃⁻, Ca²⁺, Mg²⁺, Si and Fe content were measured in an accredited laboratory (for details see Hájek & Hekera 2004).

2.3. Data analyses

Principal Component Analysis (PCA) was used to show relationships among explanatory variables. The species abundance data were log-transformed, as $Y = \log(n+1)$, to reduce the influence of dominants. Down-weighting of rare species was applied in all multivariate analyses. Detrended Correspondence Analysis (DCA) was used to study relationships between species composition and measured variables. Spearman's rank correlations (r_s) were used to examine possible relationships between explanatory variables and site scores on the first two ordination axes. Bonferroni corrections of the significance levels were used for multiple comparisons of environmental variables (Holm 1979). Predictive power of the bryophyte assemblage composition was analysed using site scores on the first three DCA ordination axes. The variation partitioning approach was used to determine the relative variation in testate amoebae, diatom and monogonont rotifer data, explained by two groups of variables (water chemistry and substratum characteristics) in Canonical Correspondence Analyses (CCA). The CCA method was used because of the long species data gradient ($SD \geq 4$). Because the ecological gradient spanned calcareous tufa-forming fens to extremely mineral-poor fens tending to ombrotrophy, we assumed unimodal responses for many species, which justifies using CCA rather than RDA (Lepš & Šmilauer 2003). A forward selection algorithm with the Monte Carlo permutation test (1999) was used to find significant explanatory variables. For each organismal group, the individual and shared effects of water chemistry and substratum characteristics were studied. For this analysis only variables that contributed significantly to the explanation of species data variation were selected. We used a novel variation partitioning algorithm for CCA proposed by Peres-Neto *et al.* (2006). This algorithm calculates permutation-based adjusted R^2 values and, unlike the 'classical' algorithm (Borcard *et al.* 1992), provides an unbiased estimate of the explained variation. We used 1000 permutations to obtain the adjusted R^2 . Note that the adjustment, as well as the use of the unimodal species response model (Økland 1999), markedly decreases the percentage of explained variation. Therefore, the frequent interpretation that 'unexplained variation' is caused by unmeasured environmental variables and stochasticity in biological processes should be carefully reconsidered (Økland 1999). Rather, the relative differences among organismal groups should be seen as the principal results of our analyses. Permutational multivariate analysis of variance using distance matrices (PERMANOVA; command 'adonis' from the 'vegan'

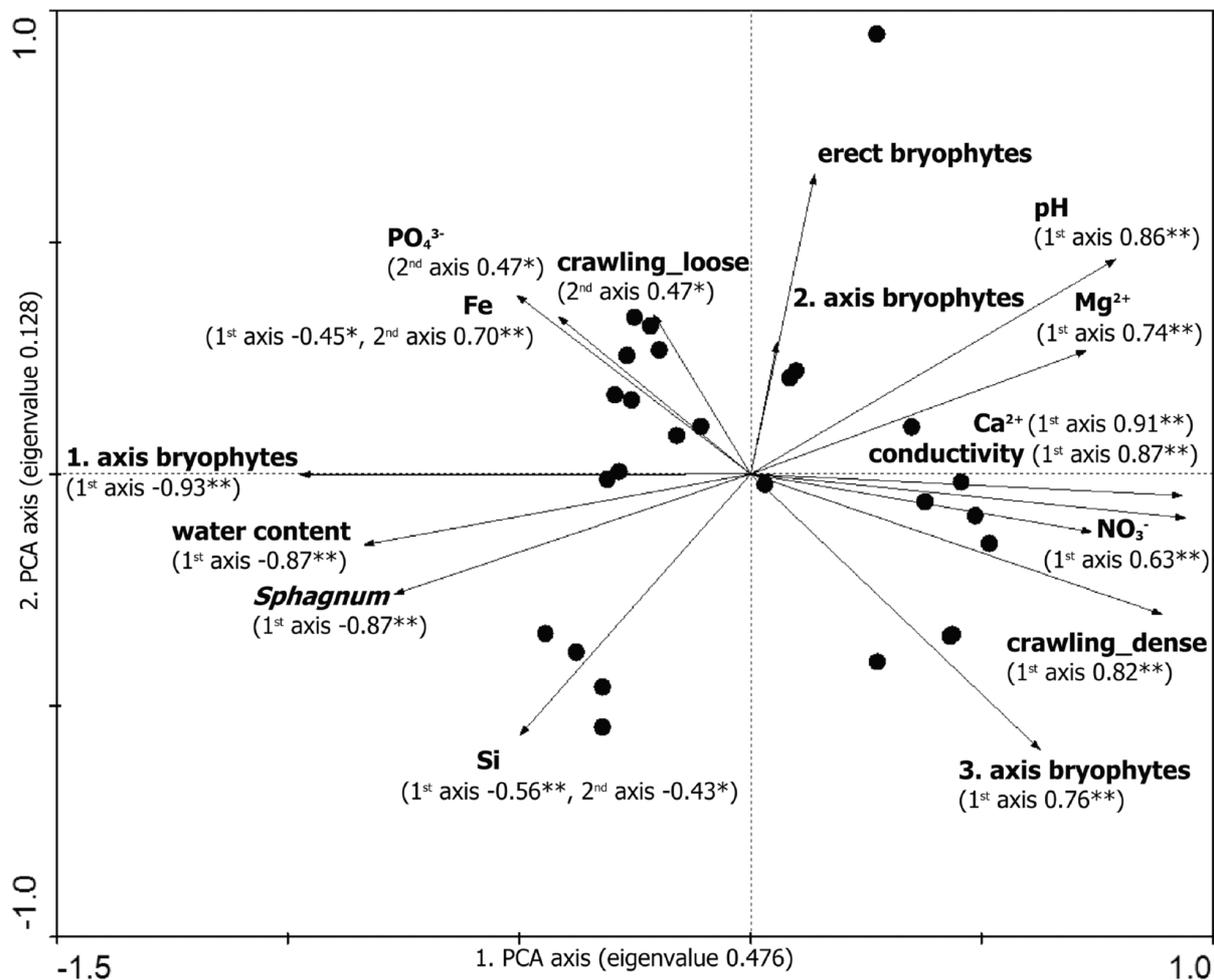


Fig. 1. PCA diagram of sixteen explanatory variables on the first two ordination axes. Eigenvalues of axes, correlations of explanatory variables with the first two PCA axes (r_s) and their significance (** $p < 0.003$; * $p < 0.05$) are given in parentheses. Only significant correlations are shown.

library; Anderson 2001; McArdle & Anderson 2001) was used to evaluate whether there were statistically significant differences in testate amoebae, diatom and monogonont rotifer assemblages between crawling dense, crawling loose and *Sphagnum* tufts. Erect tufts were not analysed because there were insufficient number of samples. The CANOCO 4.5 package (ter Braak & Šmilauer 2002) and R software were used for multi-dimensional analyses and STATISTICA 8 (Hill & Lewicki 2007) for uni-dimensional analyses.

3. RESULTS

3.1. Relationships among measured variables

Many of the explanatory variables were inter-correlated, as is illustrated by the PCA ordination diagram of sixteen environmental variables in figure 1. The first PCA axis was positively correlated with water chemistry variables (pH, conductivity, Ca²⁺, Mg²⁺, NO₃⁻), bryophyte composition of the sample (the third DCA axis) and abundance of dense crawling bryophytes. Other substratum (the first DCA bryophyte axis, water

content, *Sphagnum* dominance) and water chemistry characteristics (PO₄³⁻, Si) were negatively correlated with the first PCA axis. The second PCA axis was particularly correlated with the acidic part of the gradient (Fig. 1), positively with PO₄³⁻, Fe and abundance of loose crawling bryophytes, and negatively with Si. Abundance of erect bryophytes and the second DCA axis of bryophytes were correlated with the second PCA axis in the alkaline part of the gradient, but these correlations were not significant. The correlations suggested that the *Sphagnum*-dominated samples were characterised by rather high water content and Si concentration, whereas samples dominated by loose crawling bryophytes had higher iron and phosphate concentrations.

3.2. Variation in bryophyte assemblages

Species composition of bryophytes as a substratum was closely related to the gradient of mineral-richness (Fig. 2). Species were arranged along the first DCA axis from calciphilous species (*Bryum pseudotriquetrum*, *Palustriella commutata*), through meadow species

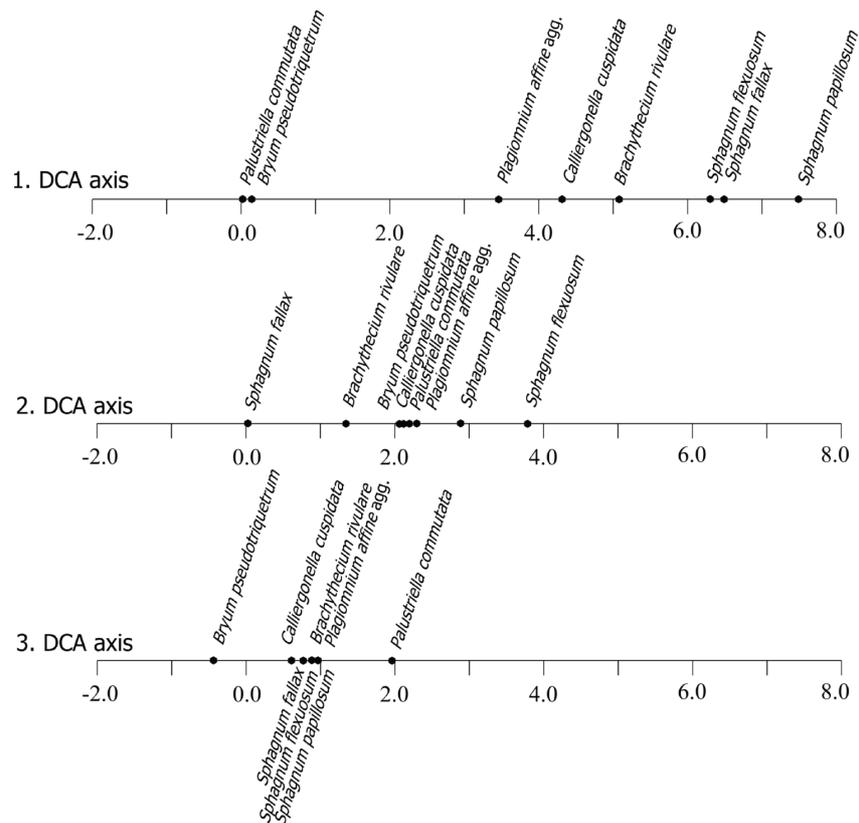


Fig. 2. Position of bryophyte species along the three DCA axes. Only species with a fit >10% are shown. The first axis explains 21.0% of species data, the second axis 17.1% and the third axis 9.6%.

inhabiting mostly moderately calcium-rich spring fens, to *Sphagnum* species in acidic, mineral-poor stands (Fig. 2). The second gradient (the second DCA axis) in bryophyte species composition was developed only in the acidic part of the first DCA axis. Species were ordered from *S. flexuosum*, occupying mineral-poor fen springs, through *S. papillosum* and meadow species (e.g., *Brachythecium rivulare*) which were co-dominants with other *Sphagnum* species, to *S. fallax*, which occupied the most acidic, often drier microhabitats. On the other hand, the third DCA axis was only displayed in the calcium-rich part of the first DCA axis. Erect species (mostly *Bryum pseudotriquetrum*) were positioned at one end of the third axis, and dense crawling species (mostly *Palustriella commutata*) at the opposite end. PERMANOVA showed that the species composition of all groups (i.e., testate amoebae, diatoms and monogonont rotifers) differed significantly ($P < 0.01$) among crawling dense, crawling loose and *Sphagnum* tufts.

3.3. Major gradients and variation in micro-organism species data

The assemblages of all three groups displayed very sharp compositional gradients, correlated with water pH and conductivity, calcium concentration and dominance of *Sphagnum* (Tab. 1). While the second major gradient in species composition of diatoms and testate amoebae was correlated with measured variables (substratum

structure and water content), that of rotifers remained unexplained. The second compositional axis of diatoms was explained by more measured variables (also by water-chemistry variables) compared to testate amoebae. When we compared the variation explained by environmental variables in particular CCA analyses, the variables connected with substratum structure explained slightly more variation in species data than the water chemistry variables (see Appendix). The most important water chemistry variables were those related to base saturation (i.e., pH, water conductivity and calcium concentration). They explained the highest percentage of variation in species data for all three groups (Tab. 2). While testate amoeba composition was also influenced by phosphates and silica, and that of diatoms by nitrates, silica and iron, no other water chemistry variable contributed to the explanation of monogonont rotifer data (Tab. 2). Within the substratum variables, abundance of *Sphagnum* was the variable which explained most variation in diatoms and testate amoebae, whereas bryophyte species composition, expressed by the first DCA axis, explained most variation in monogonont rotifers. For diatoms, water content also contributed to the explanation of species variation (Tab. 2). When both substratum and water chemistry variables were analysed together in a single CCA with forward selection (Tab. 3), substratum variables (the first DCA axis and/or abundance of *Sphagnum*) were the most important for all three groups.

Tab. 1. Correlations between explanatory variables (substratum and water chemistry) and sample scores on the first two ordination axes, based on DCA analyses performed separately for diatoms, testate amoebae and rotifers. Values of Spearman's correlation coefficient (r_s) and their significance (p) are shown; significant correlations after Bonferroni correction are in bold (** $p < 0.003$) and significance of $p = 0.05$ (*) are in italics.

	Diatoms				Testate amoebae				Monogonont Rotifers			
	1. axis DCA		2. axis DCA		1. axis DCA		2. axis DCA		1. axis DCA		2. axis DCA	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
water content	0.60	**	<i>0.49</i>	*	0.47	*	<i>-0.47</i>	*	0.81	**	-0.10	n.s.
<i>Sphagnum</i>	0.88	**	0.15	n.s.	0.88	**	-0.06	n.s.	0.78	**	-0.17	n.s.
erect bryophytes	-0.31	n.s.	<i>-0.43</i>	*	-0.36	n.s.	0.30	n.s.	<i>-0.40</i>	*	0.13	n.s.
crawling_loose tufts	-0.11	n.s.	<i>0.53</i>	*	0.02	n.s.	-0.17	n.s.	0.12	n.s.	0.36	n.s.
crawling_dense tufts	-0.74	**	-0.64	**	-0.81	**	0.19	n.s.	-0.81	**	-0.04	n.s.
1. DCA axis bryophytes	0.94	**	<i>0.42</i>	*	0.96	**	-0.17	n.s.	0.88	**	-0.04	n.s.
2. DCA axis bryophytes	-0.11	n.s.	-0.19	n.s.	-0.09	n.s.	0.28	n.s.	-0.17	n.s.	-0.24	n.s.
3. DCA axis bryophytes	-0.63	**	-0.20	n.s.	-0.63	**	-0.01	n.s.	<i>-0.60</i>	*	-0.10	n.s.
pH	-0.82	**	<i>-0.43</i>	*	-0.76	**	0.37	n.s.	-0.79	**	-0.01	n.s.
conductivity	-0.86	**	-0.38	n.s.	-0.88	**	0.14	n.s.	-0.84	**	0.09	n.s.
Ca ²⁺	-0.87	**	<i>-0.40</i>	*	-0.89	**	0.20	n.s.	-0.88	**	0.12	n.s.
Mg ²⁺	-0.80	**	-0.15	n.s.	-0.69	**	-0.02	n.s.	-0.59	**	0.15	n.s.
Si	0.56	**	0.33	n.s.	<i>0.56</i>	*	-0.36	n.s.	<i>0.56</i>	*	-0.05	n.s.
Fe	0.47	*	<i>0.51</i>	*	<i>0.45</i>	*	0.24	n.s.	<i>0.41</i>	*	-0.03	n.s.
NO ₃ ⁻	-0.70	**	-0.30	n.s.	-0.62	**	-0.12	n.s.	<i>-0.52</i>	*	-0.15	n.s.
PO ₄ ³⁻	0.34	n.s.	0.21	n.s.	0.29	n.s.	<i>0.54</i>	*	0.11	n.s.	0.09	n.s.

Tab. 2. Results of forward selection in six separate CCAs, which identified variables that contributed significantly to the explanation of species data variation for the two groups of variables (substrate and water chemistry), separately for diatoms, testate amoebae and monogonont rotifers. Statistically significant variables were used in partitioning the effects of water chemistry and substratum in the subsequent analysis (Fig. 3). Explanatory variables are ordered according to the order in forward selection. Significant variables are in bold (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Substratum	Diatoms					
	%		Lambda		Water chemistry	
<i>Sphagnum</i>	19.7***	0.554	pH	17.8***	0.500	
1. DCA axis bryophytes	9.4***	0.264	conductivity	7.8***	0.218	
water content	5.2**	0.147	NO ₃ ⁻	6.2**	0.173	
3. DCA axis bryophytes	4.1	0.116	Ca ²⁺	6.3**	0.178	
erect bryophytes	4.1	0.115	Si	5.1*	0.144	
crawling_loose tufts	3.8	0.106	Mg ²⁺	5.1*	0.142	
crawling_dense tufts	3.7	0.104	Fe	4.3*	0.120	
2. DCA axis bryophytes	3.5	0.098	PO ₄ ³⁻	3.9	0.109	

Substratum	Testate amoebae					
	%		Lambda		Water chemistry	
<i>Sphagnum</i>	21.3***	0.377	conductivity	18.1***	0.321	
1. DCA axis bryophytes	7.5***	0.132	PO ₄ ³⁻	6.7**	0.119	
3. DCA axis bryophytes	5.6*	0.099	Si	5.8**	0.102	
erect bryophytes	5.5	0.098	pH	4.0	0.069	
water content	5.4	0.096	Fe	3.9	0.069	
2. DCA axis bryophytes	5.1	0.091	NO ₃ ⁻	3.0	0.053	
crawling_dense tufts	4.5	0.079	Ca ²⁺	2.8	0.050	
crawling_loose tufts	2.9	0.052	Mg ²⁺	2.7	0.048	

Substratum	Monogonont Rotifers					
	%		Lambda		Water chemistry	
1. DCA axis bryophytes	17.8***	0.235	Ca ²⁺	15.0***	0.204	
<i>Sphagnum</i>	11.1***	0.151	pH	6.0*	0.082	
water content	3.8	0.052	Fe	3.8	0.052	
crawling_dense tufts	3.2	0.043	conductivity	3.6	0.049	
crawling_loose tufts	2.9	0.039	Mg ²⁺	3.6	0.049	
2. DCA axis bryophytes	2.4	0.033	NO ₃ ⁻	3.2	0.043	
erect bryophytes	1.8	0.025	PO ₄ ³⁻	2.9	0.039	
3. DCA axis bryophytes	1.0	0.013	Si	2.8	0.038	

Tab. 3. The results of stepwise selection of the explanatory variables using forward selection in a total CCA of all variables for individual taxonomic groups. Lambda value, percentage of explained variation, and order in forward selection are given for each variable. Significant variables are in bold (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

	Diatoms			Testate amoebae			Monogonont Rotifers		
	Order	Lambda	%	Order	Lambda	%	Order	Lambda	%
1. DCA axis bryophytes	2.	0.264	9.4***	13.	0.039	2.2	1.	0.235	17.2***
2. DCA axis bryophytes	13.	0.076	2.7	7.	0.061	3.4	12.	0.037	2.7
3. DCA axis bryophytes	7.	0.116	4.1*	9.	0.053	3.0	8.	0.057	4.2
water content	10.	0.094	3.3	6.	0.080	4.5	11.	0.043	3.2
<i>Sphagnum</i>	1.	0.554	19.7***	1.	0.377	21.3***	2.	0.149	10.9***
erect bryophytes	14.	0.070	2.5	4.	0.106	6.0*	15.	0.026	1.9
crawling_loose tufts	15.	0.054	1.9	14.	0.028	1.6	16.	0.024	1.8
crawling_dense tufts	16.	0.053	1.9	15.	0.028	1.6	3.	0.060	4.4
pH	3.	0.164	5.8**	10.	0.046	2.6	6.	0.048	3.5
conductivity	11.	0.085	3.0	2.	0.132	7.5***	7.	0.048	3.5
Ca ²⁺	12.	0.08	2.8	11.	0.044	2.5	4.	0.050	3.7
Mg ²⁺	4.	0.151	5.4***	16.	0.028	1.6	5.	0.048	3.5
PO ₄ ³⁻	8.	0.106	3.8*	3.	0.111	6.3**	13.	0.035	2.6
NO ₃ ⁻	6.	0.133	4.7**	12.	0.044	2.5	14.	0.021	1.5
Si	5.	0.147	5.2**	5.	0.090	5.1**	9.	0.046	3.4
Fe	9.	0.098	3.5	8.	0.055	3.1	10.	0.044	3.2

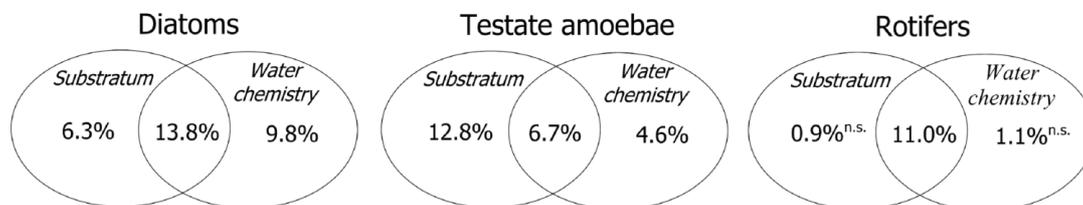


Fig. 3. Results of variation partitioning for particular taxonomic groups. Individual and shared effects of water chemistry and substratum are expressed as adjusted percentages of the total variation in species data. The significance of particular effects was tested using the Monte Carlo permutation test with 1000 runs. Non-significant effects are indicated by the abbreviation 'n.s.'. Only significant variables from both variable groups were used for the analysis (see Tab. 2).

Besides structural variables, some water-chemistry variables contributed significantly to explain diatom (pH, magnesium, phosphates, nitrates, silica) and testate amoeba (water conductivity, phosphates, silica) species composition, while structural variables alone sufficiently explained species-data variation in monogonont rotifers.

3.4. Individual effects of two different variable groups

When variations caused by substratum characteristics and water chemistry were partitioned out, the individual effect of these two variables differed for particular taxonomic groups, ranging between 0.9% and 12.8% in adjusted values (Fig. 3). Testate amoebae were controlled by substratum rather than by water chemistry (water chemistry:substratum ratio was 0.4), in contrast to diatoms for which water chemistry effects prevailed over substratum effects (water chemistry:substratum ratio 1.56). Monogonont rotifers showed virtually no individual effect of either substratum characteristics or water chemistry. Individual effects reached only 1% of explained variation and were not significant. Shared effects of both variable groups predominated over total individual effects in the rotifer data (individual:shared effect ratio was 0.18), whereas individual effects were dominant in diatom and testate amoeba data (individual:shared effect ratios were 1.16 and 2.6, respectively).

4. DISCUSSION

4.1. Differences among taxonomic groups in response to chemical and substratum variables

Assemblages of all three taxonomic groups displayed very sharp compositional gradients across the study area correlated with water pH, conductivity and calcium concentration, like vascular plants and bryophytes (Hájek *et al.* 2002), as well as molluscs (Horsák & Hájek 2003; Horsák & Cernohorsky 2008). In addition, these major gradients were linked to some substratum characteristics. Therefore, the deterministic role of pH and calcium on species composition can be considered a general rule that is valid for micro- and macro-organisms, supporting the validity of some, more general, ecological concepts, such as the evolutionary species pool with respect to pH (Pärtel 2002; Hájek *et al.* 2007).

We also discovered several differences in micro-organismal behaviour. We found that phosphates contributed significantly to explaining variation in testate amoeba assemblages. An apparent response of testacean species composition and biomass to phosphorus concentration was shown using experimental fertilization in Alaskan fens (Mitchell 2004). This might be mediated by changing the availability of prey species for testate

amoebae. A similar study by Mieczan (2009) in Poland revealed a significant effect of total phosphorus on the occurrence of testate amoebae and, like Mitchell (2004), he hypothesised indirect effects of phosphorus on testate food abundance.

Diatoms differ from the other two taxonomic groups in their response to nitrates, which partially explained their species composition. Different authors found that nitrate concentration affected the development and composition of diatom assemblages (Aboal *et al.* 1998; Bertrand *et al.* 1999; Cantonati *et al.* 2007; Soininen 2007; Cantonati & Spitale 2009) and that nitrates increased the relative importance of diatoms at the expense of other groups of micro-organisms, such as testate amoebae (Gilbert *et al.* 1998b). Nitrogen occurs as nitrate mainly in mineral-rich fens, whereas the NH_4^+ form prevails in mineral-poor fens (Hájek *et al.* 2002). This was reflected in positive correlations between water mineral-richness (pH, conductivity) and nitrates (data not shown). In our study area, mineral-rich fens were inhabited by eutrphentic species (e.g., *Navicula elginensis*, *N. veneta* and *Surirella angusta*) that were not present in mineral-poor fens. Cantonati *et al.* (2001) found that most taxa included in the higher trophic categories were also alkaliphilous. Eutrphentic diatoms are often found in springs and streams affected by organic pollution, often of anthropogenic origins (Bertrand *et al.* 1999; Cantonati & Spitale 2009). One of the tufa-forming fens was influenced by cattle grazing, which caused an increase in nitrate concentrations. This was reflected in the occurrence of eutrphentic taxa, *Navicula trivialis* and *Cocconeis placentula*.

In agreement with our hypothesis, silica was one of the statistically significant variables explaining variation in the shell-building organisms (diatoms and testate amoebae), whereas it did not contribute to explaining any variation in the monogonont rotifer assemblages (Tabs 2 and 3). Silica is a major limiting nutrient for diatom growth and hence is a controlling factor in primary productivity (Martin-Jézéquel *et al.* 2000). Although Cantonati *et al.* (2006) reported that silica concentrations in springs almost never reached limiting values ($<0.1 \text{ mg L}^{-1}$), our study showed a significant influence of silica concentrations on diatom species composition, even if silica concentration was rather high (minimum value 5.5 mg L^{-1}). The possible effect of silica availability on diatoms, even in such a silica-rich environment, is supported by our observations of poorly silicified diatom frustules, but this phenomenon requires further study.

4.2. Relative importance of water chemistry and substratum

For diatoms, we found the highest proportion of variation was explained by water chemistry rather than substratum characteristics. This agrees with our initial hypothesis that autotrophic organisms will be more

influenced by dissolved mineral concentrations than heterotrophic organisms. Water chemistry, especially pH and conductivity, is considered to be the most important environmental factor influencing diatom assemblages (Cantonati 1998; Pouličková *et al.* 2004; Cantonati *et al.* 2001, 2006; Fránková *et al.* 2009). The variation of rotifer assemblages was almost completely explained by substratum characteristics and water chemistry, with no significant individual effect, contrary to diatoms and testate amoebae. This means that the monogonont rotifer species composition is not influenced by any chemical factors, other than pH and calcium, which are the same factors that influence their substratum (*Sphagnum* occurrence, composition of bryophyte tufts). This agreed with our prediction on possible differences between uni- and multicellular organisms, but more research into other multicellular organisms is needed before general conclusions can be drawn.

The contrasting behaviour of monogonont rotifers could be caused by several factors. Rotifers do not need silica for shells, unlike the other two groups. Furthermore, rotifer species composition did not significantly respond (directly) to inorganic nutrients (phosphates, nitrates). Similar results have been reported from lakes, where rotifers responded to shifts in trophic state (from oligotrophic to mesotrophic or eutrophic) by markedly increased abundance rather than changing species composition (Ejsmont-Karabin & Hillbricht-Ilkowskys 1994; May & O'Hare 2005). However, experimental nutrient enrichment in peatlands did not change rotifer biomass (Gilbert *et al.* 1998b). Rotifers are able to survive unfavourable conditions in diapause (Gilbert 2004; Schröder 2005; etc.), which could enable them to withstand perturbations such as water level decreases. In previous reports (Bartoš 1946; Koniar 1955), the occurrence of both bdelloid and monogonont rotifers on bryophytes was considered to be dependent on moisture and the ability to survive drying out, while testate amoebae were considered even more sensitive to moisture (Bartoš 1946; De Graaf 1956). More recently, it was shown that bryophyte-dwelling testate amoebae and diatoms were dependent on moisture (e.g., Mitchell *et al.* 1999; Van de Vijver *et al.* 2003; Pouličková *et al.* 2004), whereas no other ecological studies focusing on rotifers dwelling in bryophytes have been performed. In our study, the water content of bryophyte tufts explained a relatively high proportion of variation in species data of all three groups, but the water content effect was not independent of other environmental variables (see Tab. 3).

4.3. Host specificity

Even though our research was not designed to test host specificity directly, our results suggest some relationships between species composition of microbial assemblages and taxonomic identity of dominant bryophytes. Correlations between microbial species compo-

sition and substratum characteristics were found for all studied groups (Tab. 1). Dominance of *Sphagnum* and the first DCA axis of bryophyte composition also explained more variation than water chemistry variables, which is in accordance with the ability of *Sphagnum* to retain high amounts of water in dry periods, to acidify mire water, and thus create a unique microhabitat for microscopic organisms (Hingley 1993). The effect of substratum characteristics was however largely shared with the effect of water chemistry, suggesting that both water chemistry and substratum influenced microbial communities. Bryophytes respond to changes in environmental conditions in a very complex way and this could explain why bryophyte species composition explained a higher proportion of variation in microbial assemblages than particular environmental variables (see also Opravilová & Hájek 2006). Bryophytes also modulate other, not easily measured environmental variables, such as light, which could be a very important factor influencing microbial species composition. Mieczan (2009) reported the different vertical distribution of testate amoebae in various bryophyte species. It seems that different bryophytes can create very specific micro-environments. This could be the reason why some studies have reported correlations between microbial and bryophyte species composition, independent of other measured variables (e.g., Pouličková *et al.* 2004, Lamentowicz *et al.* 2010). In our study, the individual effect of substratum composition (*Sphagnum* dominance, the first and third DCA axis) sharply exceeded the effect of water chemistry for explaining testate amoeba composition.

5. CONCLUSIONS

Fen assemblages of all three taxonomic groups displayed very sharp compositional gradients, correlated with mineral richness and base saturation, which supports some general ecological concepts postulated based on macro-organisms. In addition to water chemistry, species composition of bryophyte substratum had high predictive value for species composition of assemblages of all taxonomic groups. Particular groups of micro-organisms, however, differed in their response to other water chemistry variables and whether the effects of water chemistry and substratum characteristics on species composition are shared. Species composition of silica-forming organisms (diatoms, testate amoebae) responded to silica concentration, that of testate amoebae to phosphates and that of diatoms to nitrates. Autotrophic organisms were more influenced by water chemistry (independent of substratum structure and composition) than heterotrophic ones. Because our conclusions are based on a rather small data set with respect to the number of unique fens, their general validity needs to be verified by other studies in different regions, and by more extensive studies over larger areas and more groups of micro-organisms.

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