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Diversity and assemblage patterns of microorganisms structured by the groundwater chemistry gradient in spring fens

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Abstract – We examined the associations of microorganism assemblages with a complete mineral richness gradient spanning from extremely mineral-rich tufa-forming calcareous fens to mineral-poor acidic *Sphagnum*-fens. We also compared the distribution of two dominant taxa, testate amoebae and monogonont rotifers, among the sites differing in water chemistry and among three microhabitats sampled at each site differing in substrate and moisture conditions. Microorganism assemblages primarily changed in relation to the mineral richness gradient; moisture was the second most important factor structuring microorganism assemblages among microhabitats (*i.e.*, wet bryophytes, submerged bryophytes and waterlogged bottom sediments). Densities of testate amoebae taxa and individuals were the highest in rich *Sphagnum*-fens, indicating a unimodal pattern along the mineral richness gradient. Numbers of testate amoebae taxa decreased notably in wet bryophytes, especially in poor *Sphagnum*-fens. This pattern might result from a strong effect of *Sphagnum* acidification due to minimal or no dilution of the acidic environment by mineral-rich groundwater. As a consequence, acid tolerant and relatively xerophilous taxa chiefly dominated in wet bryophytes of poor *Sphagnum*-fens, while poor *Sphagnum*-fen bottom sediments could provide a refuge for less tolerant and hydrophilous species. In contrast to testate amoebae, monogonont rotifers preferred bryophytes in all sites, with the number of monogonont taxa distinctly increasing from calcareous fens to poor *Sphagnum*-fens. In poor *Sphagnum*-fens, monogononts were the most abundant in wet bryophytes, probably due to reduced food competition and/or predaceous pressure resulting from the limited occurrence of other groups of microorganisms by virtue of the hostile acidic conditions in wet *Sphagnum* carpets.

Key words: Springs / fens / testate amoebae / monogonont rotifers / water chemistry

Introduction

Spring fens are unique biotopes with specific abiotic conditions supporting the occurrence of a high number of rare and threatened organisms (Hájek *et al.*, 2002; Horsák and Hájek, 2003; Poulíčková *et al.*, 2005), including many habitat specialists and glacial relic species (Hájek *et al.*, 2011; Horsák *et al.*, 2012). Although many studies have dealt with spring fen invertebrate faunas (*e.g.*, Williams and Danks, 1991; Botosaneanu, 1998; Gerecke *et al.*, 2005), comprehensive research on most groups of microorganisms is still rather limited. This is despite the fact that microorganisms are known to be very diverse and abundant in peatland habitats, including fens (*e.g.*, Bateman and Davis, 1980; Pejler and Bērziņš, 1994; Gilbert and Mitchell, 2006; Opravilová and Hájek, 2006). Microorganisms, especially protozoa, participate significantly in the decomposition of organic matter and nutrient cycling in these ecosystems, and testate amoebae are even considered to have a control position in the microbial trophic network (Mieczan, 2006, 2007). Testate amoebae are also commonly the subject of ecological studies due to their well-defined ecological preferences in relation to important ecological variables in peatlands (Charman, 1997). Besides protozoa, rotifers are another very important component of microorganism assemblages in peatland ecosystems, where they also participate in nitrogen and phosphorus cycles (Francez and Dévaux, 1985; Błedzki and Ellison, 2003).

In *Sphagnum* peatlands, moisture conditions have often been identified as the primary factor and water chemistry as the secondary factor controlling testate amoebae assemblage composition at the macro- and microhabitat scales (*e.g.*, Bobrov *et al.*, 1999; Mitchell *et al.*, 2000; Mieczan, 2009; Payne, 2011). Water chemistry is also known to be one of the most important factors

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for rotifer assemblages in *Sphagnum* peatlands (Bateman and Davis, 1980; Bērziņš and Pejler, 1987). However, little is known about the microorganisms and their relationships to environmental factors in spring fens without *Sphagna* (see Bielańska-Grajner *et al.*, 2011; Hájková *et al.*, 2011; Payne, 2011).

The studied spring fens are unique in their variable chemistry of groundwater, which induces the existence of a complete mineral richness gradient (a so-called poor–rich gradient) spanning from extremely mineral-rich tufa-forming calcareous spring fens to mineral-poor acidic *Sphagnum*-fens (Malmer, 1986; Hájek *et al.*, 2002). This gradient was associated with the vegetation composition and nutrient concentrations as well as with hydrological conditions (Hájková and Hájek, 2004; Poulíčková *et al.*, 2005). Changes in environmental conditions along the gradient of mineral richness result in differences in the composition and species richness of various groups of the biota (Horsák and Hájek, 2003; Hájek *et al.*, 2006; Opravilová and Hájek, 2006; Bojková *et al.*, 2011). These environmental conditions can also affect the structure and characteristics of spring fen microhabitats and thus play a significant role in the distribution of microorganism species (Mattheeussen *et al.*, 2005; Jassey *et al.*, 2010). Hence, the spring fens studied are unique model biotopes to explore variations in microorganism assemblages along a complete environmental gradient covering all the main physicochemical types of spring fens.

In this study, we aimed to (i) analyse the relationship between microorganism assemblages involving both unicellular and microscopic multicellular organisms and the main environmental gradient, *i.e.*, the gradient of mineral richness, (ii) compare the distribution patterns of the two most dominant taxa (*i.e.*, testate amoebae and monogonont rotifers) at two spatial scales – among the sites differing in water chemistry and among three microhabitats differing in substrate and moisture conditions that were sampled at each site and (iii) evaluate the ecological preferences of the most abundant and frequent species.

Materials and methods

Study area and sites

The studied spring fens are located in the Western Carpathian flysch zone, in the borderland between the Czech Republic and Slovakia (48°56'–49°32' N; 17°44'–18°51' E). Flysch bedrock is characterized by a serial alternation of sandstone and claystone deposits with different calcium contents, which is reflected in the chemical composition of the groundwater (Rapant *et al.*, 1996). The groundwater chemistry varies from extremely rich calcareous water, which supports cold water travertine (tufa) formation, through waters rich in calcium, sodium and potassium without tufa formation, to acidic waters rich in iron, silica and sulphates but poor in all the other elements (Hájek *et al.*, 2002).

Altogether, 13 small and isolated treeless spring fen sites were selected on the basis of previous comprehensive studies of spring fen vegetation along the mineral-richness gradient (Poulíčková *et al.*, 2005; Hájek *et al.*, 2006): four calcareous fens with strong tufa formation (*i.e.*, calcareous fens), three mineral-rich fens without tufa formation (*i.e.*, rich fens), three mineral-rich *Sphagnum*-fens (*i.e.*, rich *Sphagnum*-fens) and three mineral-poor acidic *Sphagnum*-fens (*i.e.*, poor *Sphagnum*-fens). The abbreviated names (given in brackets) of these spring fen types are used hereafter.

Field sampling and explanatory variables

Field sampling was carried out in May 2006. At each site, three samples were collected: two samples of bryophyte tufts differing in water level (submerged and wet bryophyte tufts) and waterlogged bottom sediments without vegetation (*i.e.*, sapropel), sampled from small depressions. Bryophyte tufts were cut with scissors and transported in a living state to the laboratory. Bottom sediments were collected by pipette and preserved in 4% formaldehyde in the field. In the laboratory, each bryophyte sample was washed with 20 mL of distilled water and thoroughly squeezed to extract microorganisms. The efficiency of this washing procedure was assessed to be 80% (Hindák, 1978). The extract was fixed with formaldehyde. The same volume of extract (0.5 mL) was analysed from bryophyte tufts and bottom sediments. Nomenclature followed Mazei and Tsyganov (2006) for Testacea, Voigt (1957), Bartoš (1959), and Segers (1995, 1996), for Monogononta, Foissner *et al.* (1995a, 1995b) for Ciliophora, Schwank (1990) for Gastrotricha, Timm (2009) for Annelida, Beasley (1995) for Tardigrada, and Gyliarov (1975), Balogh and Mahunka (1983) and Weigmann (2006) for Acari. Note that this study was primarily aimed for monogonont rotifers due to the methodological approach used, which did not allow preserving living material required for identification of bdelloid rotifers from all samples.

Water temperature, pH, conductivity and dissolved oxygen were measured in the field using a portable instrument (WTW Multi 340i/SET) before sampling (Table 1). In autumn, water samples were collected and concentrations of soluble Ca, Mg, Al, Fe, SO_4, NO_3 and PO_4 ions were measured in an accredited laboratory. These samples were collected in the late autumn because the most stable water chemistry was found during this period (Hájek and Hekera, 2004).

Data analyses

The relationships among environmental variables were examined by principal component analysis (PCA) for 13 spring fen sites (Appendix 1). Cluster analysis based on relative Euclidean distance and Ward's method and non-metric multidimensional scaling (NMDS)

Table 1. Mean, minimum and maximum values of physico-chemical variables measured in four groups of fens and adjusted R^2 of significantly fitted variables in NMDS. Significance levels: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s., not significant; CalcFen – calcareous fens with a strong tufa formation; RichFen – mineral-rich fens without tufa formation; RichSpha – mineral-rich *Sphagnum*-fens; PoorSpha – mineral-poor acidic *Sphagnum*-fens; T – water temperature.

	Conductivity ($\mu S.cm^{-1}$)	pH	T (°C)	Dissolved oxygen ($mg.L^{-1}$)	Ca ($mg.L^{-1}$)	Mg ($mg.L^{-1}$)	PO_4^{3-} ($mg.L^{-1}$)	SO_4^{2-} ($mg.L^{-1}$)	NO_3^- ($mg.L^{-1}$)	Al ($\mu g.L^{-1}$)	Fe ($\mu g.L^{-1}$)
CalcFen											
Min	422	7.3	9.0	8.6	60.6	2.4	0.01	20.4	0.17	75	97
Mean	464	7.7	10.6	9.3	81.3	10.9	0.07	32.4	6.4	196	315
Max	525	8.2	14.0	10.4	112.0	16.5	0.17	50.2	12.0	409	816
RichFen											
Min	106	6.6	9.0	2.2	15.4	3.6	0.01	18.2	0.7	156	269
Mean	240	6.9	12.2	4.6	44.5	8.9	0.14	28.8	1.7	220	327
Max	414	7.3	16.0	7.9	71.5	15.5	0.22	49.5	2.5	263	359
RichSpha											
Min	48	5.3	9.0	6.6	6.8	1.2	0.33	3.5	0.1	145	853
Mean	64	6.4	9.3	7.6	8.4	1.9	0.48	11.4	0.5	417	2378
Max	74	7.1	10.0	8.5	9.3	2.7	0.74	18.6	1.3	590	3660
PoorSpha											
Min	42	2.8	6.3	2.5	3.7	0.7	0.01	5.7	0.1	60	46
Mean	51	4.0	10.8	4.8	4.6	1.7	0.10	6.1	0.4	257	830
Max	65	5.2	15.0	7.4	5.4	3.1	0.31	6.4	0.6	395	2090
Fitted variables (Adj. R^2)	0.857***	0.759***	n.s.	0.207**	0.761***	0.399***	n.s.	0.489***	0.435***	n.s.	n.s.

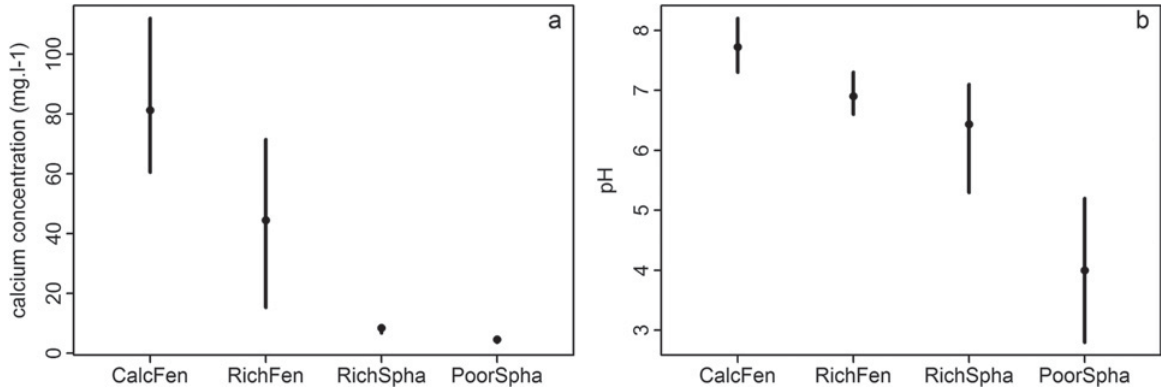

Fig. 1. Range plots with mean, minimum and maximum values showing the variation of the most important physico-chemical variables in four groups of fens. See Table 1 for details about the spring fen groups.

were used to expose the main patterns of species composition in the whole assemblages. In the cluster analysis, samples of all microhabitats within each site were pooled together to obtain more robust dataset for the comparison of assemblage composition among fens differing in mineral richness. In NMDS, samples of microhabitats were kept separate to display the distribution of all samples along the first and second NMDS dimensions with respect to both mineral types of fens and individual microhabitats. Prior to these analyses, the species density data were log-transformed as $Y = \log(n+1)$ to reduce the influence of dominants. Conductivity as a variable best representing the mineral-richness gradient (Adj. $R^2 = 0.857$, $P < 0.001$) was fitted into the diagram using thin-plate splines (command "ordisurf" in the "vegan" package). To run NMDS, a wrapper function "metaMDS" ("vegan" package) was used. This function runs NMDS from several random starts until a convergent solution is found. Based on the groups of spring fens obtained in the cluster analysis, indicator species for each group were identified using indicator species analysis (Dufrêne and Legendre, 1997) with the Monte-Carlo permutation test to test their significance ($P < 0.05$; 4999 permutations). Furthermore, we focused on the two dominant groups, testate amoebae and monogonont rotifers (hereafter referred as monogononts). To display patterns in testate amoebae and monogonont taxa richness on a common basis, we rarefied the number of taxa down to the lowest sum of recorded individuals for each fen (*i.e.*, 415 individuals for testate amoebae and eight individuals for monogononts) and individual-based species accumulation curves for four groups of fens were calculated for testate amoebae and monogonont rotifers, separately. The horizontal axis was scaled by the densities of individuals.

Range plots were used to display differences in the number of taxa and density due to low numbers of observations. The cluster analysis was computed in the PC-ORD 5 software package (McCune and Mefford, 2011) and NMDS and all range plots were performed using the R program (version 2.12.2, R Development Core Team, 2011).

Results

Changes in physicochemical variables along environmental gradients

PCA based on environmental data showed that nearly all environmental variables were highly correlated with the first PCA axis representing a complex gradient of mineral richness (Appendix 1).

Individual groups of spring fens varied in particular in their calcium concentration, conductivity, pH and SO_4 concentration (Table 1). Calcareous fens and rich fens differed distinctly from rich *Sphagnum*-fens and poor *Sphagnum*-fens in calcium concentration (Fig. 1a). Water pH values were the lowest in the poor *Sphagnum*-fens, which differed markedly from all the other groups of sites (Fig. 1b).

Taxa richness, density and habitat preferences

A total of 181 taxa and 22 935 specimens of microorganisms were collected (Appendix 2). Testate amoebae were the most taxa-rich and abundant, with 113 (64%) taxa and 19 408 (84.6%) specimens analysed. *Hyalosphenia papilio* Leidy, *Difflugia pyriformis* Perty, *Trinema lineare* Penard, *Centropyxis sylvatica* Bonnet et Thomas and *Centropyxis aculeata* Stein were the dominant testate amoebae species, which together represented 37% of the total assemblage count. Monogononts, with 27 (15%) taxa and 2414 (10.5%) specimens, were the second most abundant group. Species of Ciliophora, Gastrotricha, Annelida, Tardigrada and Acari were also found. In comparison with testate amoebae and monogononts, these groups achieved only low taxa richness and density (they together accounted for 19% and 4% of all taxa and individuals, respectively).

Based on the cluster analysis of species data, the studied fens were classified into four groups (Fig. 2): calcareous fens, rich fens, rich *Sphagnum*-fens and poor *Sphagnum*-fens. This grouping confirmed our pre-selection of sites along the gradient of mineral richness. Groups of

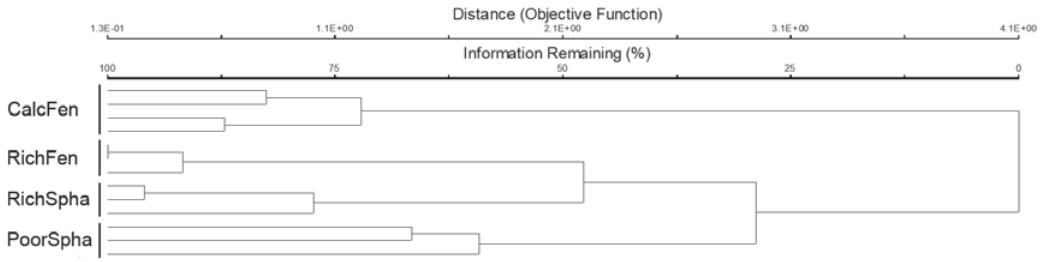

Fig. 2. Cluster analysis based on all species data. Ward's method as a group linkage method and relative Euclidean distance as a distance measure were used for the computation. See Table 1 for details about the spring fen groups.

sites differed in their density of taxa and individuals, particularly in testate amoebae and monogononts. Density of testate amoebae taxa and individuals were the highest in rich *Sphagnum*-fens, indicating a unimodal pattern along the mineral-richness gradient. However, this pattern disappeared when using rarefied numbers of taxa, which indicated a possible role of the sampling effect in the observed changes in taxa richness (Fig. 3). Analogous to the rarefied taxa richness, individual-based accumulation curves suggested a potential role of the sampling effect in taxa richness as well (Fig. 4a). Numbers of monogonont taxa increased from calcareous fens to poor *Sphagnum*-fens (Fig. 3). An increase in the number of taxa along the mineral-richness gradient was also confirmed by individual-based accumulation curves, which showed that sampling efficiency was enough in all groups of fens (Fig. 4b). An increase in the rarefied number of taxa and density along the mineral-richness gradient was also evident, but it was not as distinct as in the observed numbers of taxa (Fig. 3).

Density and species richness of testate amoebae and monogononts differed among submerged and wet bryophyte tufts and bottom sediments (Fig. 5). The numbers of testate amoebae taxa were similar in the three microhabitats within calcareous fens and rich fens, whereas they were lower in wet bryophytes in rich *Sphagnum*-fens and in both wet and submerged bryophytes in poor *Sphagnum*-fens (Fig. 5). The numbers of taxa found in bottom sediment increased from calcareous fens to poor *Sphagnum*-fens. Bottom sediments hosted the highest density of testate amoebae individuals in calcareous fens and poor *Sphagnum*-fens. In contrast, wet bryophytes supported the most individuals in rich fens. Rich *Sphagnum*-fens had similar densities of individuals in the three microhabitats. Monogononts were most abundant in bryophytes in all groups of sites. Their number of taxa increased only slightly in the bottom sediments of poor *Sphagnum*-fens (Fig. 5).

Twenty significant indicator species of testate amoebae and three indicator species of monogononts of the genus *Lecane* were found by indicator species analysis (Fig. 7). *Paraquadrula irregularis* Deflandre was the best indicator of calcareous fens being most abundant in wet bryophyte tufts. Many indicator species of rich fens often also occurred in other groups of fens, but some of them avoided all microhabitats of poor *Sphagnum*-fens (*Difflugia lucida* Penard, *Difflugia bryophila* Penard) or only wet bryophytes (*Pseudodifflugia gracilis* Schlumberger, *Tracheleuglypha dentata* Deflandre, and *Trinema enchelys* Leidy). All indicators of both *Sphagnum*-fen groups avoided calcareous fens; only two of them (*Corythion dubium* Taránek and *Euglypha ciliata* Leidy) occurred in the rich fens. Three *Lecane* species of monogononts (*Lecane acus* (Harring), *Lecane lunaris* (Ehrenberg) and *Lecane pygmaea* (Daday)) indicated conditions of wet bryophytes in poor *Sphagnum*-fens.

Considering the microhabitat preferences of indicator species, a similar pattern was found in many species of testate amoebae (*e.g.*, *Centropyxis cassis* Deflandre, *P. gracilis*, *C. sylvatica* and *Heleopera sphagni* Leidy) along the mineral-richness gradient. They often dominated in bryophytes or showed no clear preferences in calcareous and rich fens (*i.e.*, in fens without *Sphagnum*). In contrast, they prevailed in bottom sediments and nearly avoided wet bryophytes in poor *Sphagnum*-fens. A remarkable exception was *Plagiopyxis declivis* Thomas, an indicator of calcareous fens, which preferred bottom sediments in all groups of rich fens.

Relationships between microorganism assemblages and environmental gradients

The main changes in species composition among sites were related to groundwater mineral richness, represented by water conductivity as the best fitted variable in the NMDS diagram (Adj. $R^2 = 0.857$, $P < 0.001$; Fig. 6, Table 1). The other significantly fitted variables showed a linear change along the gradient of mineral richness as well (Table 1). The position of samples on the second NMDS dimension showed distinct differences among the microhabitats. Samples were arranged along the moisture gradient from the lowest moisture (wet bryophyte tufts) to the highest moisture (bottom sediments) (Fig. 6). The position of samples also showed a different species composition of bottom sediments from that of bryophyte tuft samples in the rich *Sphagnum*-fens and poor *Sphagnum*-fens.

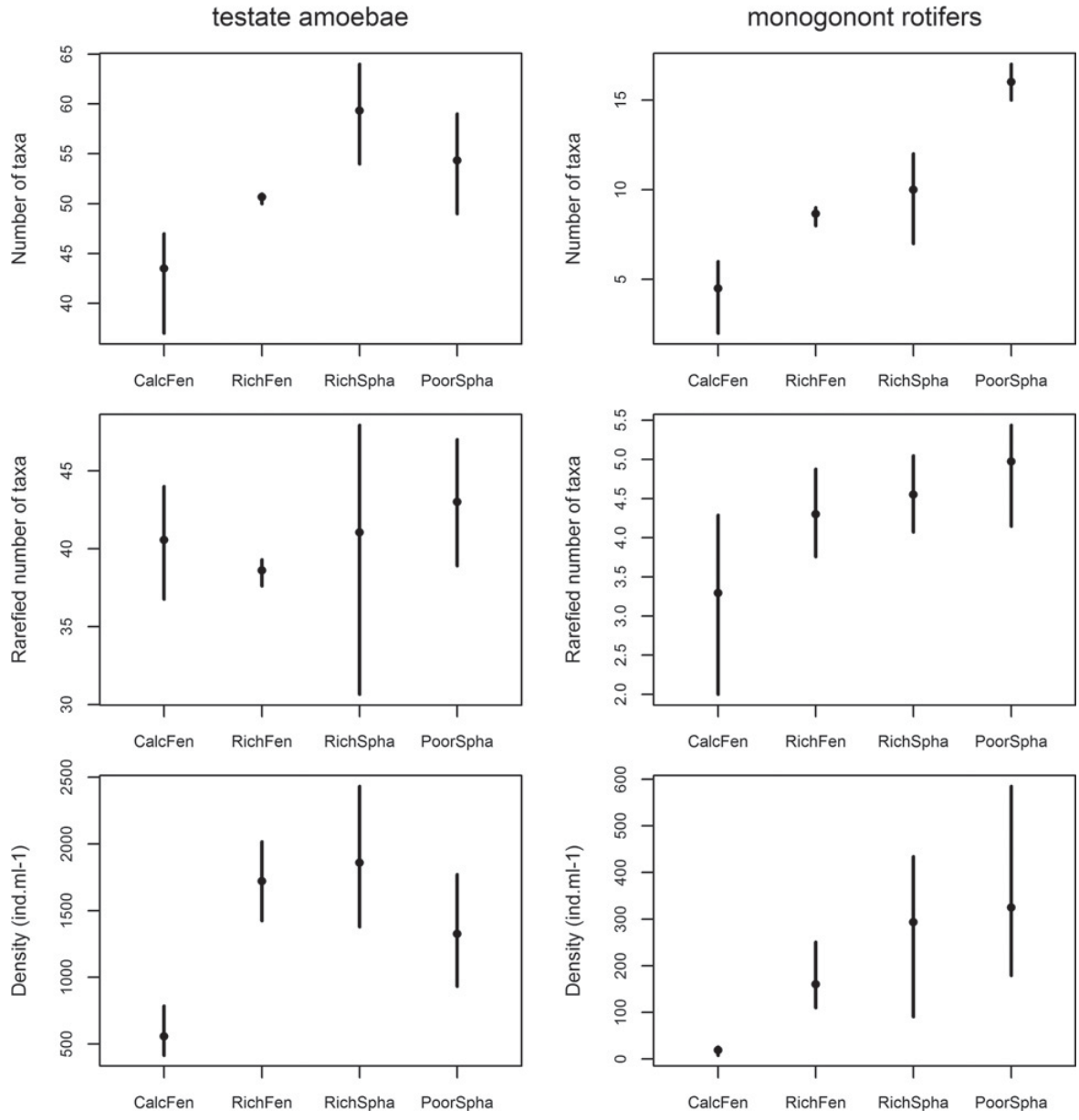

Fig. 3. Number of taxa, rarefied number of taxa and density of testate amoebae and monogonont rotifers in four groups of fens. Owing to the enormous density of *Hyalosphenia papilio* in one microhabitat sample from a rich *Sphagnum*-fen (2465 individuals) that multiply overreached all other species densities (see Appendix 2), this density was not included in the plot. See Table 1 for details about the spring fen groups.

Discussion

Relationships between microorganism assemblages and mineral-richness

In the study spring fens, the composition of microorganism assemblages varied primarily along the mineral-richness gradient (Figs. 2 and 6). Moisture was found to be the second important factor that separated assemblages among microhabitats. Contrary to our results, previous studies mostly indicated moisture conditions as the most significant factor, particularly for testate amoebae (*e.g.*, Warner, 1987; Bobrov *et al.*, 1999; Mitchell *et al.*, 2000; Schnitchen *et al.*, 2006). The explanation for this difference is probably quite simply related to the length of the mineral-richness gradient studied, as only *Sphagnum* peatlands were included in most studies. In the few studies that have covered the entire mineral-richness gradient, including calcareous fens and rich fens without tufa formation as well, a major influence of the mineral-richness gradient was evident (Opravilová and Hájek, 2006; Fránková *et al.*, 2009; Hájková *et al.*, 2011).

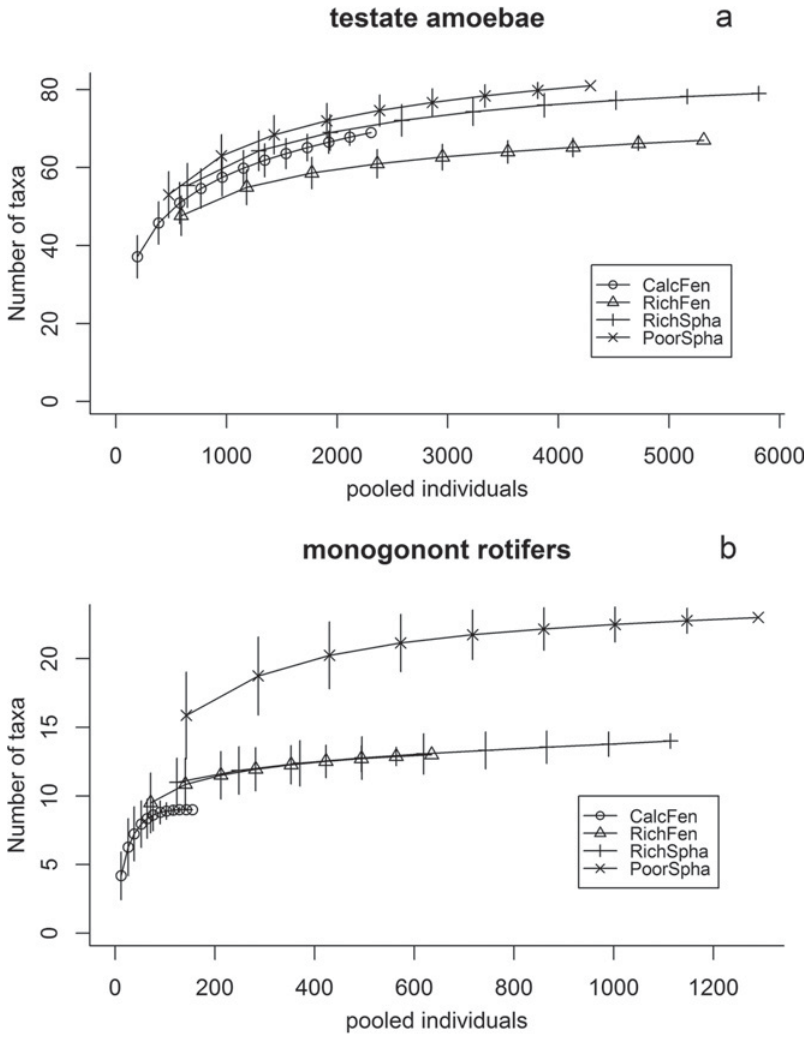

Fig. 4. Individual-based species accumulation curves for four groups of fens calculated for testate amoebae (a) and monogonont rotifers (b). The horizontal axis was scaled by the densities of individuals. The bars indicate + 2 and − 2 standard deviations. Owing to the enormous density of *Hyalosphenia papilio* in one microhabitat sample from a rich *Sphagnum*-fen (2465 individuals) that multiply overreached all other species densities (see Appendix 2), this density was not included in the plot. See Table 1 for details about the spring fen groups.

Relationships between testate amoebae assemblages and environmental gradients

In testate amoebae assemblages, the number of taxa and density differed distinctly among individual groups of fens, being the highest in rich *Sphagnum*-fens (Fig. 3) characterized by low calcium and moderately acidic pH (Table 1, Fig. 1). The preference for rich *Sphagnum*-fens was apparently related to a suitable pH that did not decrease below pH 5 (see Table 1, Fig. 1) and with a high moisture content, as the *Sphagnum* carpet is able to hold large amounts of water (Halsey *et al.*, 2000; Sullivan and Booth, 2011). A positive relationship between testate amoebae taxa richness and moisture conditions in *Sphagnum* peatlands has been documented by Warner (1987). In contrast, the lowest taxa richness and density were found in calcareous fens with tufa formation (Fig. 3). This group of fens seemed to be more sensitive to desiccation due to the low organic matter content (Hájek *et al.*, 2002; Poulíčková *et al.*, 2005) and due to the structural features of bryophyte tufts that have a lower water holding capacity than *Sphagnum* mosses (Fránková *et al.*, 2009; Sullivan and Booth, 2011). Thus, the nature of the environment in the calcareous fens apparently excludes some testate amoebae taxa and a higher moisture fluctuation might also have a possible effect. As very few studies have dealt with calcareous fen microorganisms, the precise nature of factors limiting testate amoebae density and species richness remains to be determined in future studies.

Our data also showed a relationship between testate amoebae and mineral richness, which may explain some microhabitat preferences. Decreasing numbers of testate amoebae taxa were recorded in wet bryophytes in rich *Sphagnum*-fens and in both wet and submerged bryophytes in poor *Sphagnum*-fens, while testate taxa richness increased in the bottom sediments of *Sphagnum*-fen samples (Fig. 5). This pattern might have resulted from

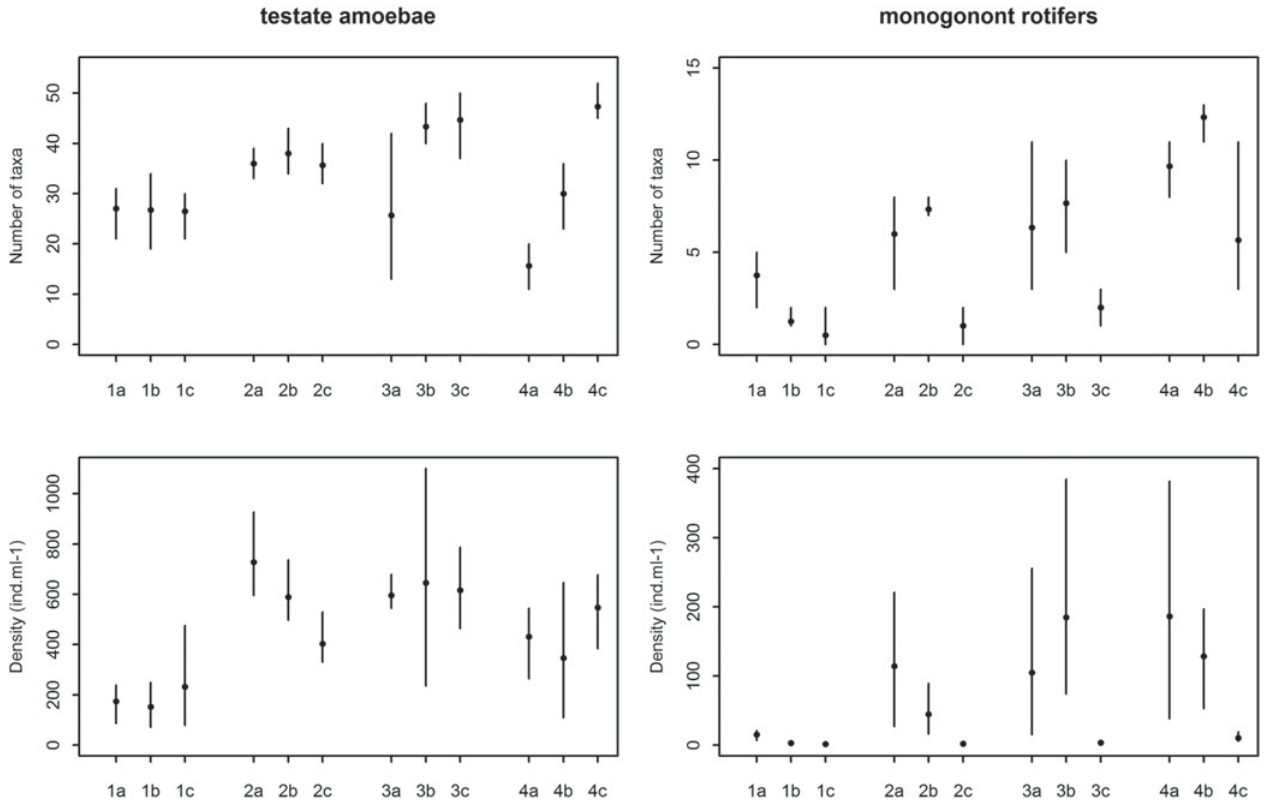

Fig. 5. The distribution of the numbers of taxa and densities of individuals of testate amoebae and monogonont rotifers in wet bryophyte tufts (a), submerged bryophyte tufts (b), and bottom sediments (c) in four groups of spring fens. Owing to the enormous density of *Hyalosphenia papilio* in one microhabitat sample from a rich *Sphagnum*-fen (2465 individuals) that multiply overreached all other species densities (see Appendix 2), this density was not included in the plot. 1 – calcareous fens with strong tufa formation, 2 – mineral-rich fens without tufa formation, 3 – mineral-rich *Sphagnum*-fens, 4 – mineral-poor acidic *Sphagnum*-fens.

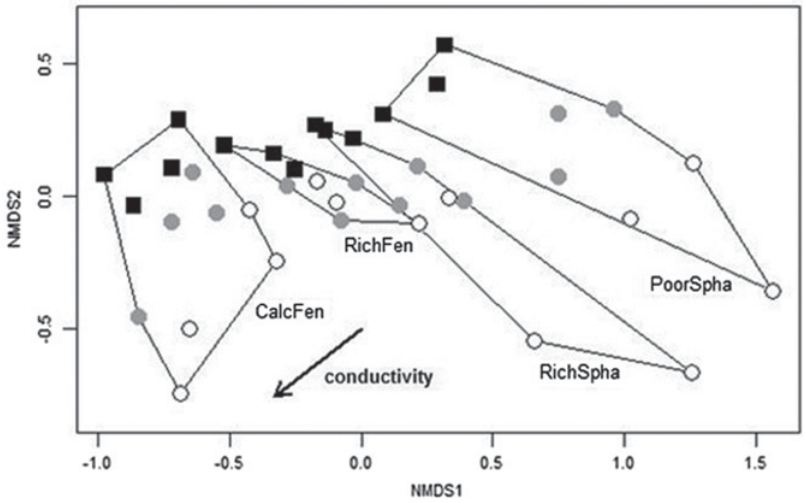

Fig. 6. NMDS ordination diagram based on all species data showing position of samples along the first two dimensions. Conductivity as a variable best representing the mineral-richness gradient (Adj. $R^2 = 0.857$, $P < 0.001$) was fitted into the diagram using thinplate splines; a linear change is indicated by an arrow. Samples belonging to individual groups of fens are separated by envelopes; samples from individual microhabitats are distinguished by the colour and the shape of the symbol. ○ – wet bryophyte tufts, ● – submerged bryophyte tufts, ■ – bottom sediments. See Table 1 for details about the spring fen groups.

the more prominent decrease in pH in bryophyte samples mainly caused by *Sphagnum* activity, compared with bottom sediment samples in the *Sphagnum*-fen sites. Such differences might also be responsible for the separation of bottom sediments in the NMDS ordination diagram (Fig. 6). *Sphagnum* mosses, through several physical and

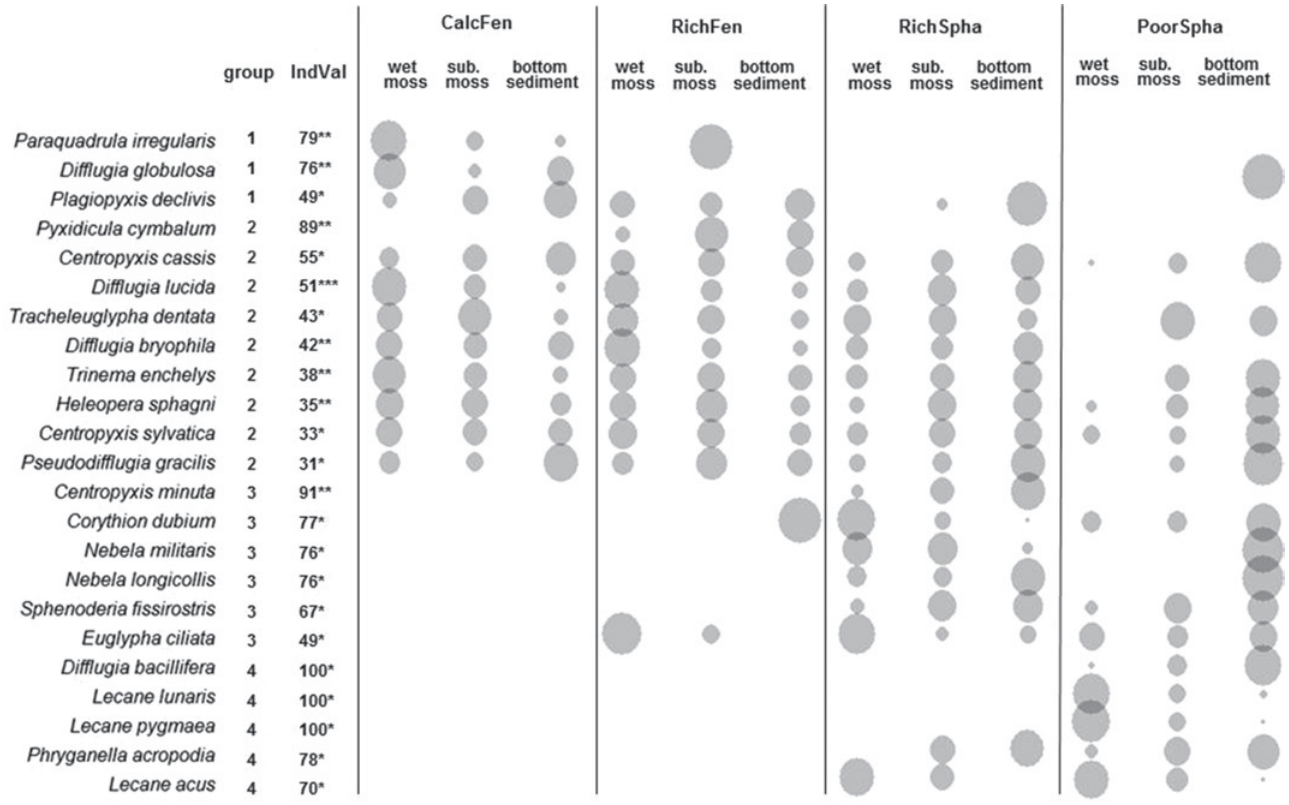

Fig. 7. Indicator species and their indicator values (Monte Carlo test, $P < 0.05$, 4999 permutations) in four groups of fens. The representation of indicator species in wet bryophyte tufts, submerged bryophyte tufts and bottom sediments was displayed in the form of spots, the size of which corresponds to the percentage representation of species densities in each group of fens. If the indicator species was represented only by one individual in the group of fens, it was not displayed by a spot. Significance levels: $***P < 0.001$; $**P < 0.01$; $*P < 0.05$. 1 – calcareous fens with strong tufa formation, 2 – mineral-rich fens without tufa formation, 3 – mineral-rich *Sphagnum*-fens, 4 – mineral-poor acidic *Sphagnum*-fens, IndVal – indicator value. See Table 1 for details about the spring fen groups.

physiological characteristics, can have a strong acidifying effect on their environment (Clymo, 1963, 1984; van Breemen, 1995). Payne (2010) reported that testate amoebae are unable to cope with acid-stress environments, possibly through H^+ interference with enzyme or membrane function. In addition, Heal (1964) pointed out that the reproduction of *Difflugia tuberculata* (Wallich) is halted below pH 4.5. In poor *Sphagnum*-fens, many testate amoebae species avoided wet bryophytes in particular (*e.g.*, *T. dentata*, *T. enchelys* and *P. gracilis*; Fig. 7). Although these species commonly inhabited wet bryophytes in other groups of fens, they dominated in the bottom sediments in poor *Sphagnum*-fens. Wet bryophyte samples in the poor *Sphagnum*-fens were obviously the most affected by *Sphagnum* acidification due to minimal or no dilution of the acidic environment by groundwater rich in minerals. The vertical chemical gradient created by *Sphagnum* mosses (from the upper part of the acrotelm without groundwater influence to the bottom part of the acrotelm exposed to minerotrophic conditions) is well-known (Karlin and Bliss, 1984; Mitchell *et al.*, 2000). As a consequence, chiefly acid tolerant, relatively xerophilous and eurytopic taxa dominated in wet bryophytes of poor *Sphagnum*-fens (*e.g.*, *Nebela bohemica* Taránek, *H. papilio*, *Assulina seminulum* Leidy; see Bobrov *et al.*, 1999; Opravilová and Hájek, 2006; Payne, 2011), while poor *Sphagnum*-fen bottom sediments could provide a refuge for less tolerant and hydrophilous species.

Altogether twenty testate amoebae species were found to be indicators for four groups of fens. The best indicator of calcareous fens, *P. irregularis*, dominated in wet bryophyte tufts. This species, characterized by its calcareous shell, was previously defined as characteristic of this type of fen by Opravilová and Hájek (2006). *P. declivis*, another indicator of calcareous fens, was the only indicator species that preferred bottom sediments in all mineral-rich groups of fens. The genus *Plagiopyxis* is considered to be soil-specific (Mitchell *et al.*, 2004), which was probably the main reason for its major association with bottom sediments. Results consistent with previously published data were also found for *Difflugia bacillifera* Penard and *Phryganella acropodia* Hopkinson, which were most abundant in bottom sediments of poor *Sphagnum*-fens (Fig. 7). *P. acropodia* is known to be a soil species (Couteaux, 1975, 1976; Smith and Headland, 1983) and *D. bacillifera* has been reported as characteristic of waterlogged habitats (Charman *et al.*, 2007; Lamentowicz *et al.*, 2008). However, we could not discuss

the relationship of both species to water chemistry due to a lack of published data.

In some cases, ecological preferences observed in our data set differed from those found in other studies. *H. sphagni* was classified as an indicator of rich fens, which contradicts the observation of Warner and Charman (1994) who reported this species in poor *Sphagnum*-fens. Furthermore, although *C. dubium* is often regarded as a ubiquitous and tolerant species (*e.g.*, Mitchell *et al.*, 1999; Mitchell *et al.*, 2004) and *Nebela militaris* Penard is considered to be characteristic of poor acidic *Sphagnum*-fens (Opravilová and Hájek, 2006; Lamentowicz *et al.*, 2008), they were significant indicators of rich *Sphagnum*-fens in our data. In addition, the association of *Sphenoderia fissirostris* Penard with wet bryophyte lawns in poor acidic *Sphagnum*-fens documented by Opravilová and Hájek (2006) was not entirely supported in our study. *S. fissirostris* was found to be an indicator for rich *Sphagnum*-fens, preferring both submerged bryophytes and bottom sediments (Fig. 7). Strong preferences for rich *Sphagnum*-fens were also found for *Nebela longicollis* Penard, which dominated in bottom sediments. However, information about the habitat preferences of this species is very scarce in other studies, which makes any comparisons difficult and questionable. More data from a broad range of habitats along the gradient of the major factors responsible for species distribution are needed.

Relationships between monogonont assemblages and environmental gradients

In contrast to testate amoebae, the number of monogonont taxa distinctly increased from calcareous fens to poor *Sphagnum*-fens (Fig. 3). An increase along the mineral-richness gradient was also evident in the rarefied number of taxa and density, although it was less pronounced. Some studies, however, have shown a decrease in rotifer taxa richness towards acidic conditions (Bērziņš and Pejler, 1987; Nogrady *et al.*, 1993). Such discrepancies might have been caused by not including bogs in our study. The acidic conditions in bogs limit the number of rotifer species to those that are tolerant to low pH values (Nogrady *et al.*, 1993), compared to poor *Sphagnum*-fen conditions, which seemed not to be limiting for many species, if any. We found that monogononts preferred bryophyte tufts in all groups of sites (Fig. 5). In poor *Sphagnum*-fens, they were most abundant in wet bryophytes. Rotifer preferences for wet *Sphagnum* in acidic peatlands have already been documented by Bērziņš and Pejler (1987). For instance, *L. acus*, significantly associated with wet bryophytes of poor *Sphagnum*-fens in our samples, is known to prefer very low pH (Bērziņš and Pejler, 1987; Pejler and Bērziņš, 1994). We assumed that monogononts might prefer wet *Sphagnum* due to reduced food competition with other microorganisms, *e.g.*, with testate amoebae. The feeding preferences of testate amoebae are quite similar to those of rotifers; both groups can feed on algae, bacteria and some protozoa (*e.g.*, Duggan *et al.*, 2001; Payne *et al.*, 2010). Furthermore, testate amoebae can behave not only as rotifer competitors, but also as their predators (Gilbert *et al.*, 2003; Jassey *et al.*, 2012). Thus, the reduced pressure from these predators resulting from the hostile acidic conditions in wet *Sphagnum* in poor *Sphagnum*-fens could play a role as well, raising the question on the importance of biotic interactions for structuring such microorganism assemblages.

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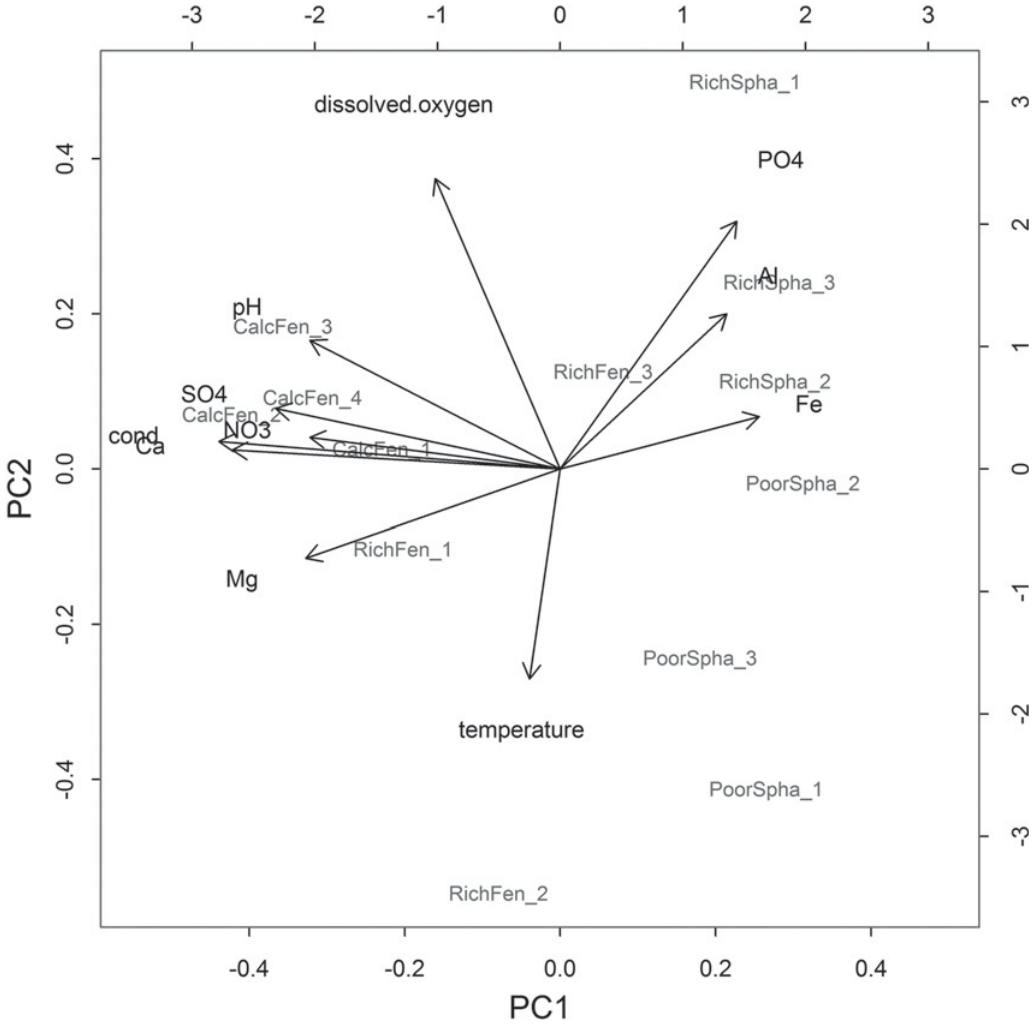
Appendix

Appendix 1. PCA ordination diagram showing the relationships among the environmental variables along the first and second PCA axes (proportion of variance: PC1 = 44% and PC2 = 18%). Numbers from 1 to 4 differentiate individual spring fen sites in each group of fens. See Table 1 for details about the spring fen groups.



Appendix 2. The list of taxa with sum of densities in each group of spring fens. See Table 1 for details about the habitat groups.

		CalcFen	RichFen	RichSpha	PoorSpha
Testacea					
	Amphitrema wrightianum Archer, 1869	0	0	0	26
	Arcella artocrea Leidy, 1879	2	0	2	0
	Arcella catinus Penard, 1890	1	32	34	46
	Arcella discoides Ehrenberg, 1843	1	103	40	105
	Arcella gibbosa Penard, 1890	0	0	0	2
	Arcella hemisphaerica Perty, 1852	0	0	5	25
	Arcella rotundata Playfair, 1918	2	8	3	1
	Arcella vulgaris Ehrenberg, 1830	0	0	0	1
	Archerella flavum Archer, 1879	0	0	0	361
	Assulina muscorum Greef, 1888	0	0	5	0
	Assulina seminulum (Ehrenberg, 1848) Leidy, 1879	1	1	193	15
	Campascus minutus Penard, 1899	2	7	12	0
	Centropyxis aculeata (Ehrenberg, 1832) Stein, 1857	270	416	367	109
	Centropyxis cassis (Wallich, 1864) Deflandre, 1929	99	171	109	44
	Centropyxis constricta (Ehrenberg, 1841) Deflandre, 1929	104	116	0	0
	Centropyxis ecornis (Ehrenberg, 1841) Leidy, 1879	0	0	5	0
	Centropyxis minuta Deflandre, 1929	1	0	13	0
	Centropyxis orbicularis Deflandre, 1929	4	8	3	5
	Centropyxis platystoma Penard, 1890	24	15	17	5
	Centropyxis spinosa (Cash, 1909) Deflandre, 1929	37	80	7	33
	Centropyxis sylvatica (Deflandre, 1929) Bonnet et Thomas, 1955	176	638	334	35
	Corythion dubium Taránek, 1881	0	2	143	5
	Cryptodifflugia penardi (Penard, 1902) Grospietsch, 1964	3	0	271	6
	Cryptodifflugia sacculus Penard, 1902	0	0	0	3
	Cyclopyxis arcelloides (Penard, 1902) Deflandre, 1929	19	34	29	10
	Cyclopyxis eurystoma Deflandre, 1929	38	70	19	14
	Cyphoderia ampulla (Ehrenberg, 1840) Leidy, 1879	62	363	212	132
	Cyphoderia laevis Penard, 1908	14	5	0	0
	Cyphoderia trochus Penard, 1899	0	2	0	1
	Difflugia acuminata Ehrenberg, 1838	0	0	0	2
	Difflugia avellana Penard, 1890	8	11	37	7
	Difflugia bacillifera Penard, 1890	0	0	0	164
	Difflugia bryophila Penard, 1902	144	584	121	1
	Difflugia cylindrus Thomas, 1953	4	0	3	0
	Difflugia difficilis Thomas, 1954	6	17	1	27
	Difflugia elegans Penard, 1890	0	0	0	4
	Difflugia fallax Penard, 1890	25	15	10	8
	Difflugia gassowski (Gassowsky, 1936) Ogden, 1983	3	0	0	0
	Difflugia glans Penard, 1902	49	38	26	0
	Difflugia globularis Wallich, 1864	0	0	0	46
	Difflugia globulosa Dujardin, 1837	11	0	0	2
	Difflugia gramen Penard, 1902	0	1	0	0
	Difflugia labiosa Wailes, 1919	1	0	0	0
	Difflugia lebes Penard, 1899	0	0	0	2
	Difflugia lemani Blanc, 1892	1	0	16	14
	Difflugia linearis (Penard, 1890) Gauthier-Lièvre et Thomas, 1958	25	17	12	16
	Difflugia lithophila (Penard, 1902) Gauthier-Lièvre et Thomas, 1958	0	1	2	0
	Difflugia lucida Penard, 1890	19	70	9	0
	Difflugia molesta Penard, 1902	1	0	0	0
	Difflugia mammilaris Penard, 1893	3	0	0	0
	Difflugia manicata Penard, 1902	1	2	0	0
	Difflugia minuta Rampi, 1950	0	24	14	1
	Difflugia penardi Hopkinson, 1909	14	23	13	6
	Difflugia pristis Penard, 1902	3	1	13	0
	Difflugia pulex Penard, 1902	0	0	2	0
	Difflugia pyriformis Perty, 1849	185	656	810	108
	Difflugia rubescens Penard, 1891	0	17	219	406
	Difflugia sphincta Jung, 1942	0	0	1	0
	Difflugia spp.	4	16	2	4
	Euglypha acanthophora (Ehrenberg, 1841) Perty, 1849	0	57	41	18

Appendix 2. *(Contd.)*

		CalcFen	RichFen	RichSpha	PoorSpha
	Euglypha ciliata (Ehrenberg, 1848) Leidy, 1878	1	6	95	34
	Euglypha compressa Carter, 1864	0	0	0	2
	Euglypha cristata Leidy, 1879	1	26	17	11
	Euglypha filifera Penard, 1890	0	0	4	21
	Euglypha laevis (Ehrenberg, 1832) Perty, 1849	25	7	7	7
	Euglypha rotunda Wailes, 1915	38	100	138	27
	Euglypha strigosa (Ehrenbeg, 1871) Leidy, 1878	0	0	0	4
	Heleopera petricola Leidy, 1879	9	1	0	1
	Heleopera rosea Penard, 1890	0	17	83	32
	Heleopera sphagni Leidy, 1874	42	87	34	15
	Heleopera sylvatica Penard, 1890	0	5	11	7
	Hyalosphenia elegans Leidy, 1879	6	1	1	14
	Hyalosphenia papilio Leidy, 1879	0	0	2544	454
	Lesquereusia epistomium Penard, 1893	0	0	0	1
	Lesquereusia spiralis (Ehrenberg, 1840) Bütschli, 1888	0	0	0	43
	Nadinella tenella Penard, 1899	0	0	2	0
	Nebela bohemica Taránek, 1882	0	4	272	418
	Nebela carinata (Archer, 1867) Leidy, 1879	0	1	0	32
	Nebela collaris (Ehrenberg, 1848) Leidy, 1879	9	0	46	17
	Argynnia dentistoma Penard, 1890	29	148	271	298
	Nebela lageniformis Penard, 1890	4	2	6	0
	Nebela longicollis Penard, 1890	0	0	39	4
	Nebela militaris Penard, 1890	1	0	34	2
	Nebela penardiana Deflandre, 1936	0	39	34	78
	Nebela tincta (Leidy, 1879) Awerintzew, 1906	0	0	34	8
	Nebela tubulosa Penard, 1890	0	0	5	28
	Paraquadrula irregularis (Archer, 1877) Deflandre, 1932	110	4	0	0
	Phryganella acropodia (Hertwig et Lesser, 1874) Hopkinson, 1909	1	0	6	66
	Phryganella nidulus Penard, 1902	0	0	0	2
	Phryganella paradoxa Penard, 1902	3	0	0	0
	Plagiopyxis callida Penard, 1910	13	14	2	0
	Plagiopyxis declivis Thomas, 1958	76	38	15	0
	Plagiopyxis intermedia Bonnet, 1959	3	4	1	0
	Plagiopyxis labiata Penard, 1910	5	0	1	0
	Plagiopyxis oblonga Bonnet et Thomas, 1955	31	15	4	3
	Plagiopyxis minuta Bonnet, 1959	0	5	0	0
	Pontigulasia incisa Rhumbler, 1896	5	0	0	0
	Pseudodifflugia fulva Archer, 1870	23	5	0	0
	Pseudodifflugia gracilis Schlumberger, 1845	48	103	54	32
	Pseudodifflugia horrida Penard, 1902	0	0	0	1
	Pyxidicula cymbalum Penard, 1902	1	9	0	0
	Quadrulella symmetrica (Wallich, 1863) Schulze, 1875	7	161	305	162
	Sphenoderia fissirostris Penard, 1890	0	0	26	10
	Sphenoderia lenta Schlumberger, 1845	0	35	17	76
	Sphenoderia splendida Playfair, 1917	0	2	3	0
	Tracheleuglypha dentata (Penard, 1890) Deflandre, 1928	9	96	46	5
	Trigonopyxis microstoma Hoogenraad et Groot, 1948	0	0	1	0
	Trinema complanatum Penard, 1890	2	0	2	11
	Trinema enchelys (Ehrenberg, 1838) Leidy, 1878	74	285	37	20
	Trinema lineare Penard, 1890	269	256	626	182
	Wailesella eboracensis (Wailes, 1911)	0	0	0	6
	Zivkovicia compressa (Carter, 1864) Ogden, 1987	1	13	3	19
	Zivkovicia spectabilis (Penard, 1902) Ogden, 1987	16	44	35	3
Ciliophora					
	Cothurnia annulata Stokes, 1885	0	1	0	0
	Cothurnia sp.	1	0	1	0
	Epistylis sp.	2	0	2	0
	Euplotes sp.	0	0	3	3
	Heterolepidoderma sp.	1	4	2	0
	Podophrya sp.	0	0	0	1
	Pyxicola carteri Kent, 1882	0	1	0	0

Appendix 2. *(Contd.)*

		CalcFen	RichFen	RichSpha	PoorSpha
	Thuricola sp.	0	0	0	1
	Vorticella sp.	11	7	1	3
	Cyrtophorida	2	1	1	0
	Hymenostomata	11	101	6	204
	Ciliophora	23	11	160	0
Gastrotricha					
	Chaetonotus succinctus Voigt, 1902	0	0	0	2
	Chaetonotus spp.	5	5	4	3
Rotifera					
Bdelloidea	Bdelloidea	267	1073	2045	1634
Monogononta	*Cephalodella gibba* (Ehrenberg, 1832)	0	2	4	19
	Cephalodella spp.	13	17	111	46
	Colurella adriatica Ehrenberg, 1831	20	7	0	1
	Colurella colurus (Ehrenberg, 1830)	16	0	0	6
	Colurella hindenburgi Steinecke, 1917	0	24	30	70
	Colurella obtusa (Gosse, 1886)	4	24	135	30
	Colurella sp.	6	9	0	0
	Encentrum sp.	0	1	0	1
	Lecane acus (Harring, 1913)	0	0	35	361
	Lecane arcuata (Bryce, 1891)	8	48	42	23
	Lecane arcula Harring, 1914	0	0	24	23
	Lecane closterocerca (Schmarda, 1859)	4	141	153	20
	Lecane flexilis (Gosse, 1886)	0	0	0	3
	Lecane hamata (Stokes,1896)	0	0	0	2
	Lecane lunaris (Ehrenberg, 1832)	0	0	0	37
	Lecane pygmaea (Daday, 1897)	0	0	0	85
	Lecane stichaea Harring, 1913	0	0	0	6
	Lecane subulata (Harring and Myers, 1926)	0	0	1	0
	Lecane subtilis Harring and Myers, 1926	0	0	1	6
	Lecane tenuiseta Harring, 1914	0	0	6	0
	Lepadella acuminata (Ehrenberg, 1834)	0	90	237	157
	Lepadella patella (O.F. Müller, 1786)	3	98	68	55
	Lepadella parvula (Bryce, 1893)	3	0	0	5
	Proales theodora (Gosse, 1887)	0	3	0	0
	Testudinella sp.	0	0	0	7
	Trichocerca bidens (Lucks, 1912)	0	0	0	4
	Monogononta	0	18	33	8
Annelida					
	Chaetogater diastrophus (Gruithuisen, 1828)	0	0	0	9
Tardigrada					
	Diphascon higginsi (Binda, 1971)*	0	0	3	0
	Diphascon cf. *humicus* Bertolani, Guidetti and Rebecchi, 1995	0	0	3	2
	Diphascon scoticum scoticum (Murray, 1905)	4	6	1	0
	Hypsibius convergens Urbanowicz, 1925	1	1	2	2
	Hypsibius dujardini (Doyere, 1840)	25	0	63	58
	Hypsibius microps Thulin, 1928	1	0	0	0
	Isohypsibius cf. *prosostomus* Thulin, 1928	0	0	1	0
	Macrobiotus furciger/pilatoi complex	0	0	4	0
	Murrayon pullari (Murray, 1907)	0	0	2	11
	Platicrista cf. *horribilis* Kaczmarek and Michalczyk, 2003	0	0	0	1
	Tardigrada	2	1	44	33
Acari					
	Acari	0	16	62	116
	Acipteriidae	0	0	1	0
	Gamasida	0	0	1	0
	Hydrachnellae (Actinedida)	0	0	1	0
	Tarsonemidae	0	1	0	0
	Trachytes sp.	0	0	1	0
Oribatida	*Ceratoppia* cf. *sexpilosa* Willmann, 1938	0	0	0	1
	Hydrozetes lacustris (Michael, 1882)	0	0	0	2
	Liebstadia similis (Michael, 1888)	0	1	0	0

Appendix 2. *(Contd.)*

	CalcFen	RichFen	RichSpha	PoorSpha
Limnozetes sphagni (Michael, 1880)	0	0	0	1
Malaconothrus gracilis van der Hammen, 1952	0	1	9	0
Platynothrus peltifer (Koch, 1839)	0	1	1	3
Tectocepheus velatus (Michael, 1880)	0	0	2	0
Trimalaconothrus foveolatus Willmann, 1931	0	2	2	25

*First record in the Czech Republic.