PRIMARY RESEARCH PAPER

Differential cell size structure of desmids and diatoms in the phytobenthos of peatlands

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Abstract The mean cell sizes of microalgae vary in relation to the abiotic factors, such as nutrients, temperature, or water transparency. This study focused on the community cell size of desmids and diatoms, two dominant groups of the peatland phytobenthos. Forty samples from various temperate European peatlands were investigated. The species composition and the species richness were controlled mainly by the pH levels. Purely spatial factors also significantly affected the species composition. Interestingly, diatoms were more strongly geographically restricted than desmids. The spatial control of the species composition was limited mostly to the large taxa, which indicated that dispersal limitation may be an important structuring factor for phytobenthos at a regional scale. The mean cell sizes of desmids were related to the ombro-minerotrophic gradient, pH, and Ca concentration.

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Acidic, ombrotrophic bogs typically contained small cells, whereas minerotrophic fens had larger desmids. By contrast, the diatom size structure did not depend on the ombro-minerotrophic gradient. Thus, the cell sizes of desmids in peatlands may be used as a proxy for important environmental processes, such as transition from minerotrophy to ombrotrophy, or acidification, whereas diatoms did not primarily respond to these processes and their size structure is driven by different factors, such as conductivity.

Introduction

The size range of microalgal species inhabiting marine and freshwater habitats varies by over nine orders of magnitude in terms of cell volume (Finkel et al., 2010). Consequently, the community size structure may differ profoundly among natural phytoplankton and phytobenthos assemblages. The dynamics of microalgal size structure has been investigated mostly in phytoplankton communities, and studies show that it may be driven by various factors, such as the nutrient status (Friebele et al., 1978; Irwin et al., 2006; Litchman et al., 2009); temperature (Winder et al., 2009; Morán et al., 2010), sinking resistance (Passy, 2007), depth of the mixed layer (Litchman et al., 2009), irradiance (Key et al., 2010), or water

transparency (Finkel et al., 2009). Small algae typically predominate in oligotrophic waters where, because of their higher surface-to-volume (S:V) ratios, they benefit from more efficient nutrient uptake rates per unit biovolume (Friebele et al., 1978; Passy, 2007). Larger species become more abundant in eutrophic conditions (Ruggiu et al., 1998; Irwin et al., 2006). However, the opposite relationship has also been documented, driven by the light limitation selecting the smaller freshwater phytoplankton species with more efficient light absorption and lower sinking rates in eutrophic conditions (Finkel et al., 2009).

The microphytobenthos size structure has attracted considerably less attention, although the predominance of relatively large pennate diatoms in the phytobenthos over smaller centric diatoms in the phytoplankton has many times been documented (Round et al., 1990). Passy (2007) argued that cell sizes of phytoplankton species of diatoms were probably limited by their sinking resistance, whereas size-related dispersal limitations were important for benthic species. De Nicola et al. (2006) showed that the area-specific biovolumes of periphyton increased with the nutrient enrichment of lakes, while Cattaneo et al. (1997) reported the same relationship for stream periphyton. By contrast, studies that have focused solely on benthic diatoms have reported non-significant relationships. Lavoie et al. (2006, 2010) showed that the community size structure of stream benthic diatoms did not correlate with the local nutrient status; therefore, these diatom size data were not useful for biomonitoring. Likewise, Wunsam et al. (2002) showed that the relationship between the cell sizes of benthic diatoms in streams and the trophic levels at sites was controlled by the water color, rather than the local phosphorus concentration. Furthermore, Finkel et al. (2009) found no relationship between the cell sizes of benthic diatoms in lakes and the concentrations of nutrients. Consequently, Passy (2007) argued that diatoms may considerably differ from many other groups of microalgae in terms of the dynamics of their per biovolume nutrient uptake. The biovolume of diatom cells has two distinct parts: the metabolically inactive central vacuole, and the thin layer of cytoplasm, which contains the cell organelles. The cytoplasm is located under the frustule and it is proportionate to the cell surface (Round et al., 1990). Therefore, Passy (2007) suggested that the per unit biovolume nutrient uptake is generally size-independent

in diatoms. Other biological aspects of the community size structure of benthic diatoms have also been examined. Soininen & Kokocinski (2006) found a weak positive relationship between the community cell size and the latitude for boreal stream benthic diatoms. However, this weak relationship was not supported by the species temperature optima, and it could not be readily ascribed to the temperature-size rule. Heino & Soininen (2006) found that smaller species of benthic diatoms from boreal streams were significantly more frequently distributed within their study region. This suggested that smaller species of stream phytobenthos may have larger populations and more efficient passive dispersal; therefore, spatial factors may be less limiting on their regional distribution.

In this study, we investigated the parallel community size structure of diatoms (Bacillariophyceae, Stramenopila) and desmids (Desmidiales, Viridiplantae), which are two major groups forming the microphytobenthos of freshwater peatlands. These wetland habitats, which typically have a low pH, have not been investigated with respect to the size structure of their microalgal communities. The diversity of desmids and diatoms in peatlands is usually positively related to the pH values of individual localities (Coesel, 1982; Mataloni, 1999; Nováková, 2002; Neustupa et al., 2009). The pH gradient in peatlands has often been used to distinguish between strongly acidic, ombrotrophic bogs, and more pH-neutral, minerotrophic fens (Wheeler & Proctor, 2000). This relationship, although still relevant, is less important in temperate peatlands, where minerotrophic poor fens may have very low pH values (Hájek et al., 2006; Neustupa et al., 2011a). However, the anthropogenic acidification of Central European ombrotrophic bogs, which was caused primarily by acid rains in the second half of the twentieth century, has produced extremely acidic conditions in these habitats, which now often have pH values less than 4.0. Interestingly, the pH gradient in peatlands was also found to be more or less unrelated to their trophic gradient (Bridgham et al., 1996; Wheeler & Proctor, 2000). The pH levels and the corresponding ombro-minerotrophy transition were reported to be correlated more strongly with the species composition of vascular plants (Bedford et al., 1999; Vitt, 2006) or bryophytes (Bragazza & Gerdol, 2002) than nutrient concentrations. This may be due to fluctuating and spatially variable nutrient

concentrations in these localities because any available ions are utilized rapidly by organisms (Kellogg & Bridgham, 2003). The stressing effects of extremely low pH values were also linked to changes in the carbon uptake mechanisms of peatland microalgae because the concentrations of HCO_3^- ions rapidly diminish in the pH levels less than 5.5 (Moss, 1973).

Consequently, in the present study we predicted that poorly buffered ombrotrophic localities will have relatively smaller algae with higher S:V ratios, which may facilitate more efficient nutrient uptake and higher growth rates, allowing them to cope with unfavorable conditions in these habitats. By contrast, comparatively larger sized species were predicted in minerotrophic fens. It was expected that these predicted trends in the community size structure would primarily be correlated with the pH values and with the estimates of ombro- versus minerotrophy of individual localities based on field observations of their water supply and hydrography. The current study tested whether pH-related environmental processes in peatlands, such as acidification, or the transition from a minerotrophic fen to an ombrotrophic bog stage would be reflected in the mean cell sizes of phytobenthic microalgae. Given the possible difference between diatoms and desmids in terms of their per unit biovolume nutrient uptake scaling, this study aimed to determine whether there was a difference in the community mean size dynamics between these two major phytobenthic groups.

The effects of geographical factors were also tested, i.e., whether the spatial distances among localities reflected large scale processes, such as history, climate, or dispersal, which may account for a significant component of the variation in species structure. This study also tested whether purely spatial effects would be more pronounced in the relatively larger species of both groups. The cell size of individual taxa should be positively correlated with their dispersal limitations (Heino & Soininen, 2006; Passy, 2007). Consequently, this factor may play a role in structuring the local phytobenthic communities in peatlands, where larger species might be more spatially structured than species with smaller cells. Finally, the species composition of desmids and diatoms was used to test whether there were congruent patterns in the ordination of sites, and whether the species richness of both groups followed similar patterns among the investigated localities.

Materials and methods

Localities and sampling

In total, 40 peatland localities were sampled during June and July 2011 (Supplementary Table 1). The site selection was aimed to include a range of different peatland habitats in temperate Europe. The study sites were positioned in four regions: Krušné Hory Mts., Northwest Bohemia, Czech Republic; Dokesko district, Northeast Bohemia, Czech Republic; West Pomerania, Poland; and Bornholm Island, Denmark. The pH ranged from 3.3 to 7.3, and the localities ranged from typical ombrotrophic raised bogs to minerotrophic fens fed by ground and surface waters. The position of the localities on the ombrotrophic to minerotrophic gradient was estimated using a three-level scale, based on a visual inspection of their hydrography and physiognomy. The samples taken in typical ombrotrophic raised bogs (characterized by a central cupola elevated above the bog margins) that are fed mostly by the rainwater were assigned with the lowest score. Conversely, the apparent minerotrophic localities positioned at the peaty margins of lakes or in the alluvium of streams that are mostly fed by the ground or surface waters were assigned with the highest score. The intermediate localities, such as mountainous bogs with an active peat cupolla and substantial precipitation located on slopes, which increase the relative amount of surface water influx, were assigned with 2. Thus, each locality was assigned a score ranging from 1, for purely ombrotrophic bogs, to 3, for typical minerotrophic fens. In each locality, approximately 10×10 cm of the epipelon was sampled from the uppermost 5 mm layer using a 100 mL plastic syringe. The pH and conductivity values were measured in the field using a combined pH/conductometer (WTW 340i, WTW GmbH, Weilheim, Germany). Total nitrogen concentrations were measured using the chemiluminiscent nitrogen dioxide (NO_2) assessment method, which involves the high-temperature catalytic conversion of ammonium, nitrite, and nitrate to nitrogen dioxide. Total phosphorus concentrations were evaluated by acid persulfate digestion. Organic and condensed inorganic forms of phosphates were converted to orthophosphates by heating with acid and persulfate. Ca and Fe concentrations were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The phytobenthos

samples were fixed in the field using Lugol's solution (3–4% final concentration). Two hundred desmid cells were identified in each sample during systematic inspections of the Lugol's solution-fixed samples at $400\times$ magnification using an Olympus BX51 light microscope equipped with a Z5060 digital microphotography equipment. Two hundred diatom cells were also identified in each sample using Naphrax (Brunel Microscopes Ltd, Wiltshire, UK) mounted permanent diatom slides at $1{,}000\times$ magnification.

Species data analysis

Two-dimensional non-metric multidimensional scaling (NMDS) was used with a Bray-Curtis distance measure to determine the species composition patterns of desmids and diatoms at individual localities. The species data were square-root transformed and Wisconsin double standardized using the *metaMDS* function in the *vegan* package of R, ver. 2.13.0. (Oksanen et al., 2011; R Development Core Team, 2011). The congruence of the two ordinations was evaluated using the function *procrustes* in the *vegan* package. This function conducts asymmetric Procrustes superimposition, which minimizes the squared differences between two ordinations (Bookstein, 1991; Peres-Neto & Jackson, 2001). The resulting Procrustes sum of squares indicated the goodness of fit between the desmids and diatoms, based on the ordinations of the localities that were illustrated graphically with a Procrustean superimposition plot showing the positions of sites in two superimposed ordinations. The non-randomness of the congruence between the two ordinations was evaluated using the permutation Procrustes test, implemented in the function *protest* of the *vegan* package. The randomization of site assignments was based on 9,999 random replicates.

The sizes of cells were expressed as their biovolumes, surface areas, and S:V ratios. The algorithm proposed by Neustupa et al. (2011a) was used to estimate the biovolumes and surface areas. The frontal views of desmids and the valvar views of diatoms were used to compute their area (A), perimeter (P), length (a), and width (b) using TpsDig ver. 2.16. (Rohlf, 2010). The maximum thickness of cells (c) was estimated based on the published width-to-thickness ratios of individual species, or direct measurements of the cells. The surface areas (S) and biovolumes (V) of diatom cells were estimated using $S = (2 \cdot A) + (P \cdot c)$ and $V = A \cdot c$, whereas the corresponding values for desmid cells were estimated based on a general ellipsoid ($V_{\text{ellipsoid}}$), using a, b, and c values, and the area (A_{ellipse}) and perimeter (P_{ellipse}) of an ellipse with a and b axes. The volume of a desmid cell (V) with a generally ellipsoidal layout was approximated using the formula $V/V_{\text{ellipsoid}} = A/A_{\text{ellipse}}$, i.e., $V_x = (A_x \cdot V_{\text{ellipsoid}})/A_{\text{ellipse}}$. Hence, after the algebraic simplification of trivial geometric formulas for scalene ellipsoids it gave $V_x = (2 \cdot A_x \cdot c)/3$. Then, the mean relative biovolume values for individual samples were estimated as

$$V_{\text{sample}} = \frac{\sum_{i=1}^{\text{spec}} \left(V_{\text{spec}} \cdot k_{\text{spec}} \right)}{200}$$

where V_{spec} is a mean estimated biovolume of a particular species, k_{spec} is the actual number of cells counted in this sample out of the total of 200 cells. Similarly, the surfaces of desmid cells with general ellipsoidal layouts were approximated using the formula $S = (P \cdot S_{\text{ellipsoid}})/P_{\text{ellipse}}$. Full details of these computations and the alternative computations for desmid cells with multiple radiations are described in Neustupa et al. (2011a).

Abiotic values, the mean relative desmid and diatom biovolumes, and the surface areas and S:V ratios of individual sites were log transformed so that they could be compared unequivocally using linear and partial linear correlation analyses with the pH values at the localities (defined at the log scale) in PAST, ver. 2.15. (Hammer et al., 2001). In addition, the effects of abiotic factors on the mean biovolumes of desmids and diatoms were also evaluated using the multiple regression analyses with the optimal model chosen on the basis of the Akaike's information criterion (AIC) using the *stepAIC* function of the MASS package of R, ver. 2.13.0. (Venables & Ripley, 2002). Prior to the regression analysis, both the mean biovolume data and the abiotic factors (except for the pH values) were log transformed. The abiotic factors were also standardized to zero mean and unit variance. The forward stepwise search of the optimal model, avoiding collinearity among closely related factors, was used (Burnham & Anderson, 2004).

The effects of individual abiotic factors on the species composition of samples were evaluated using a permutational multivariate analysis of variance (permutational MANOVA), which was conducted

with Hellinger-transformed desmid and diatom species data using the Bray-Curtis distance index (Anderson, 2001; Oksanen et al., 2011). The permutational MANOVA is a distribution-free function that partitions the distance matrices (typically based on species composition data) among external sources of variation. This method is considered a robust alternative to parametric MANOVA and ordination methods, such as redundancy analysis (Legendre and Anderson, Legendre & Anderson, 1999; Oksanen et al., 2011). Stepwise forward selection based on the F-ratios was used to generate the optimal model for the decomposition of the variation in species data among individual log-transformed abiotic factors. The significance of individual effects was assessed using permutation tests with 9,999 repetitions. Partition of variance in community structure attributed to purely spatial and environmental factors was performed using the redundancy analysis (RDA) based variance partition (Borcard et al., 1992). This analysis was conducted using *varpart* function of the package vegan (Oksanen et al., 2011) in R, ver. 2.13.0. (R Development Core Team, 2011). The adjusted R^2 values were used for the partitioning of variance (Peres-Neto et al., 2006). The original matrix of geographic distances among localities was converted using PAST ver. 2.15 to principal coordinates that covered the spatial variation. The *varpart* function then used the Hellinger-transformed species data and the standardized environmental factors (Oksanen et al., 2011). Significance of the testable fractions (such as pure effects of space and environmental factors) were calculated using permutation tests with 9,999 repetitions. For parallel analyses of relatively smaller and larger taxa, the species datasets of both groups were divided into two subgroups, based on the median values of the cell biovolume, and these subgroups were evaluated separately.

Results

Species composition

In total, 206 species of desmids and 105 diatom species were recovered from the peatland samples (Supplementary Tables 2 and 3). The Procrustes analysis of the NMDS ordinations of desmid and diatom datasets demonstrated their non-random congruence (Procrustes correlation $r = 0.77$, $P < 0.001$;
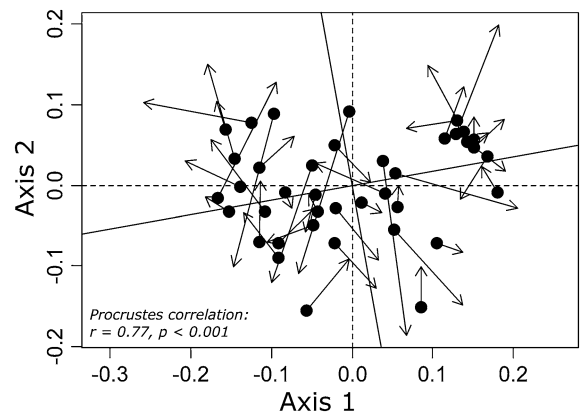

Fig. 1 NMDS ordination plot of sites based on the species composition of desmids (*arrow ends*) superimposed with the Procrustes analysis of the NMDS ordination plot based on the species composition of diatoms (*circles*)

Fig. 1). However, the relatively large residual distances among identical sites in both ordinations suggested that the community structure of desmids and diatoms did not follow exactly the same patterns relative to the abiotic and spatial factors. This was confirmed by further analyses. The species composition of desmids was most tightly controlled by the pH values of localities (Table 1). The permutational MANOVA model explained approximately 43% of the variation in the species data. Conductivity was the second most important factor, while the effect of spatial distance among localities on the species composition of desmids was also significant. The ombro-minerotrophy gradient and Ca concentrations were correlated with the pH values (Table 2), but they did not account for much of the additional variation in the desmid species data that was not related to the pH gradient (Table 1). However, the effect of the ombro-minerotrophy gradient was still marginally significant, even after accounting for the effect of pH. The other abiotic factors had no significant effects on the species composition of desmids in the samples. The species composition of diatoms was also primarily controlled by pH (Table 1). The permutational MANOVA model accounted for about 55% of the total variation. Similar to the desmids, conductivity and spatial distance had the second and third most significant effects on the species composition, which were not related to the pH gradient (Table 1). The ombro-minerotrophic gradient and Ca concentrations, i.e., two abiotic factors that were largely covered by the variation in the pH values,

Table 1 The results of individual permutational MANOVA tests partitioning variation in species composition of sites

Factor	Df	Sums of squares	F ratio	R^2	P value
Desmids, all species—abiotic factors					
pH	1	2.58	8.27	0.16	***
Conductivity	1	1.26	4.06	0.08	***
Spatial distance	2	1.32	2.12	0.08	**
Ombro-minerotrophy	1	0.52	1.67	0.03	*
Total N	1	0.47	1.50	0.03	n.s.
Fe	1	0.40	1.30	0.02	n.s.
Total P	1	0.29	0.93	0.02	n.s.
Ca	1	0.26	0.83	0.02	n.s.
Residuals	30	9.35		0.57	
Diatoms, all species—abiotic factors					
pH	1	2.81	14.25	0.22	***
Conductivity	1	1.60	8.08	0.12	***
Spatial distance	2	1.20	3.05	0.09	***
Ca	1	0.45	2.29	0.03	*
Ombro-minerotrophy	1	0.38	1.90	0.03	*
Total N	1	0.31	1.57	0.02	n.s.
Fe	1	0.27	1.35	0.02	n.s.
Total P	1	0.14	0.71	0.01	n.s.
Residuals	30	5.92		0.45	

The effects of individual factors were evaluated sequentially following the stepwise forward model selection

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s. $P > 0.05$

were also marginally significant. The effects of other factors, including the total nitrogen and phosphorus, were not significant.

The concentrations of total nitrogen and phosphorus were mutually positively correlated, although they were not significantly related to pH or conductivity (Table 2). The species richness of desmids and diatoms at the sites was mutually positively correlated (Fig. 2a). The species richness of both groups was also strongly positively correlated with the pH (Fig. 2b, c) and measures related to ombro-minerotrophy and the Ca concentration (Table 2). By contrast, the species richness of diatoms and desmids was not related to conductivity, the concentrations of nutrients, or other abiotic factors.

Table 2 The results of linear correlation analyses/partial linear correlation analyses among individual environmental factors and community parameters

	pH	Conductivity	Ombro-/minerotrophy	Total N	Total P	Ca	Fe
Conductivity	0.14[n.s.]						
Ombro-/minerotrophy	**0.74*****	−0.16[n.s.]					
Total N	−0.23[n.s.]	0.19[n.s.]	−0.03[n.s.]				
Total P	0.10[n.s.]	0.17[n.s.]	0.19[n.s.]	**0.68*****			
Ca	**0.85*****	**0.41****	**0.66*****	0.01[n.s.]	0.18[n.s.]		
Fe	−0.27[n.s.]	−0.11[n.s.]	−0.17[n.s.]	**0.46****	0.09[n.s.]	−0.05[n.s.]	
Mean cell biovolume—diatoms	0.20[n.s.]/−0.05[n.s.]	**−0.58***/−0.56*****	0.31*/−0.01[n.s.]	−0.26[n.s.]/−0.26[n.s.]	−0.32*/−0.26[n.s.]	0.12[n.s.]/0.29[n.s.]	0.26[n.s.]/0.26[n.s.]
Mean cell surface—diatoms	0.08[n.s.]/−0.07[n.s.]	**−0.67***/−0.59*****	0.25[n.s.]/0.02[n.s.]	−0.26[n.s.]/−0.04[n.s.]	−0.34*/−0.27[n.s.]	−0.03[n.s.]/0.23[n.s.]	0.23[n.s.]/0.21[n.s.]
Mean cell S/V—diatoms	0.22[n.s.]/0.09[n.s.]	**0.55***/0.36****	0.08[n.s.]/0.01[n.s.]	0.21[n.s.]/0.09[n.s.]	**0.36***/0.17[n.s.]	**0.30***/−0.04[n.s.]	−0.26[n.s.]/−0.21[n.s.]
Species richness—diatoms	**0.75***/0.46****	−0.04[n.s.]/−0.20[n.s.]	**0.58***/−0.04[n.s.]	−0.25[n.s.]/0.24*	−0.18[n.s.]/−0.43*	**0.63***/0.10[n.s.]	−0.13[n.s.]/−0.04[n.s.]
Mean cell biovolume—desmids	**0.64***/−0.01[n.s.]	0.06[n.s.]/0.09[n.s.]	**0.73***/0.44****	−0.02[n.s.]/−0.08[n.s.]	0.16[n.s.]/0.05[n.s.]	**0.65***/0.11[n.s.]	−0.08[n.s.]/0.09[n.s.]
Mean cell surface—desmids	**0.61***/−0.05[n.s.]	0.00[n.s.]/−0.02[n.s.]	**0.73***/0.42***	0.01[n.s.]/−0.01[n.s.]	0.17[n.s.]/0.02[n.s.]	**0.62***/0.16[n.s.]	−0.08[n.s.]/0.03[n.s.]
Mean cell S/V—desmids	−0.28[n.s.]/0.29[n.s.]	−0.06[n.s.]/−0.01[n.s.]	**−0.50**/−0.37***	−0.08[n.s.]/−0.04[n.s.]	−0.08[n.s.]/0.08[n.s.]	**−0.40**/−0.23[n.s.]	0.04[n.s.]/0.09[n.s.]
Species richness—desmids	**0.86***/0.33[n.s.]	−0.08[n.s.]/−0.42*	**0.76***/0.10[n.s.]	−0.26[n.s.]/−0.08[n.s.]	0.07[n.s.]/0.07[n.s.]	**0.73***/0.36***	−0.30[n.s.]/−0.28[n.s.]

The numbers in bold represent correlation coefficients with $P < 0.01$

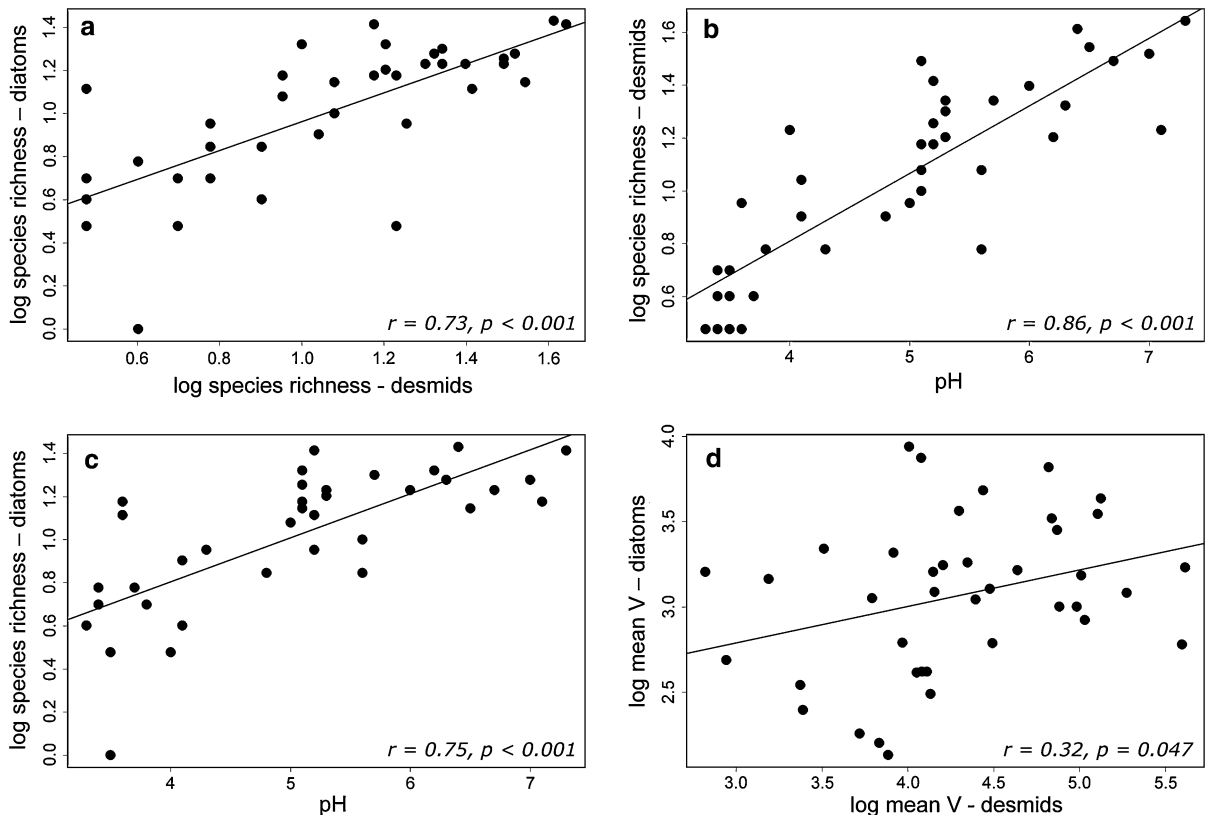*** $P < 0.001$, ** $P < 0.01$; * $P < 0.05$, n.s. $P > 0.05$

Fig. 2 Linear correlation analyses of the species richness values for diatoms and desmids in samples (**a**), pH values and species richness of desmids (**b**) and diatoms (**c**), and the mean cell biovolumes of both groups (**d**)

Size structure

The mean relative biovolumes of desmids and diatoms (Supplementary Table 4) were positively correlated, although this relationship was only marginally significant (Fig. 2d). The biovolumes of desmids were highly positively related to the pH (Fig. 3a), the Ca concentrations (Fig. 3b), and the ombro-minerotrophy gradient. The latter correlation was still highly significant after the effects of all the other abiotic factors were accounted for in the partial linear correlation analysis (Table 2). In contrast to the desmid assemblages, the biovolumes of diatoms were not linearly related to the pH gradient (Fig. 3c), although they were marginally related to the ombro-minerotrophy gradient (Table 2). However, they were significantly negatively related to the conductivity values at sites (Fig. 3d; Table 2). Interestingly, the relationship between the pH and the mean diatom biovolumes suggested a

unimodal response of diatom mean cell sizes in the community (Fig. 3c). This relationship was confirmed by the second order polynomial univariate regression analysis, which detected a highly significant relationship ($F = 9.40$, $R^2 = 0.34$, $P < 0.001$). The results of the correlation analyses of the mean relative surface areas and S:V ratios with abiotic factors were very similar to the mean biovolumes of both groups (Table 2). The multiple regression analyses of mean desmid and diatom biovolumes on the abiotic factors confirmed the dominant effect of the ombro-minerotrophy gradient on the desmid cell size data. This qualitative factor was chosen as the first explanatory variable on the basis of the AIC value (Table 3). The Ca concentrations of the localities were included as the second factor of the model that accounted for 58.0% of the total variation in the mean desmid biovolumes. The pH values were not included by the model selection procedure because of their collinearity with the above-
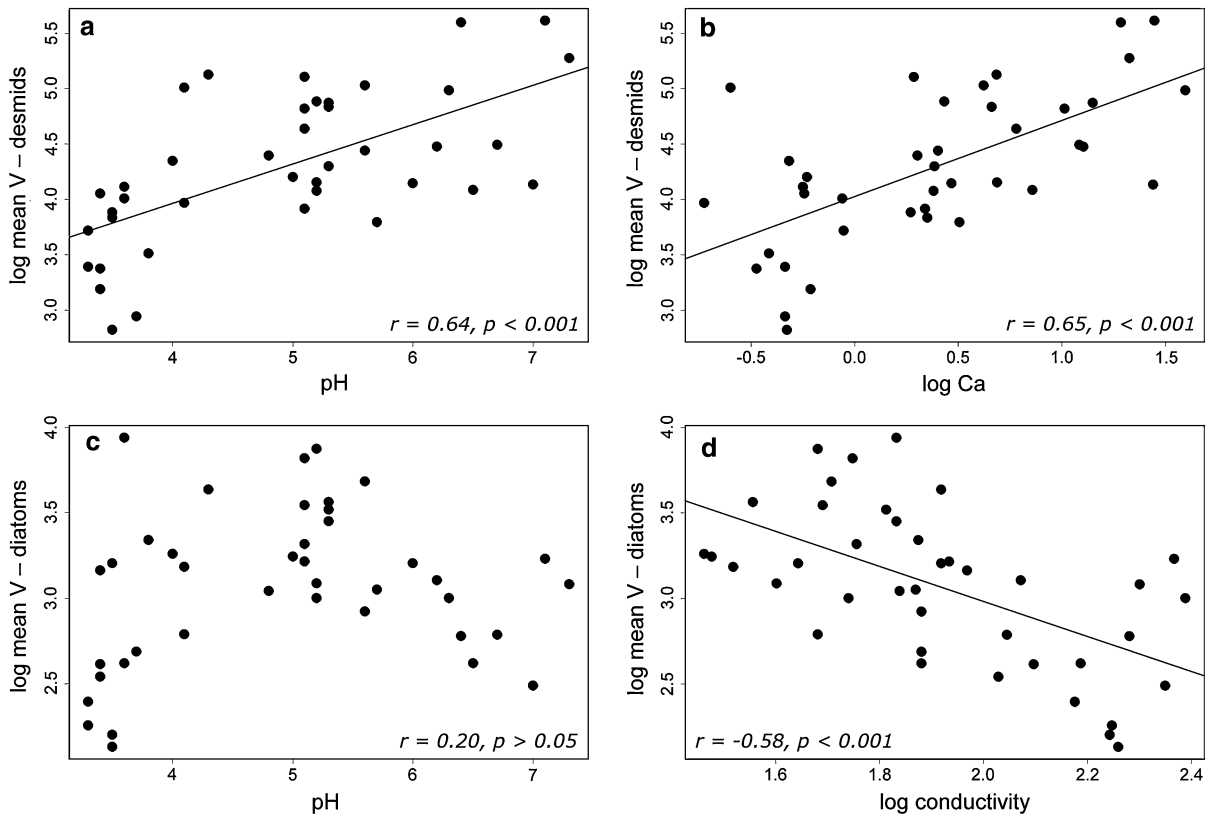
Fig. 3 Linear correlation analyses of the mean cell biovolumes of desmids versus pH (**a**) and Ca concentrations (**b**), and the mean cell biovolumes of diatoms versus pH (**c**) and conductivity (**d**)

Table 3 The results of multiple regression analyses evaluating effects of abiotic factors on the variation of mean biovolumes of desmids and diatoms at the localities

Factor	Regression coefficient	Standard error	t-statistic	P value	R^2/adjusted R^2
Desmids, mean biovolumes					
					0.58/0.56
Intercept	4.29	0.07	60.31	***	
Ombro-minerotrophy	0.36	0.10	3.78	***	
Ca	0.20	0.10	2.09	*	
Diatoms, mean biovolumes					
					0.62/0.58
Intercept	3.07	0.05	65.03	***	
Conductivity	−0.32	0.05	−6.04	***	
Ca	0.22	0.05	4.09	***	
Total P	−0.14	0.05	−2.88	**	
Fe	0.10	0.05	2.15	*	

The optimal models were chosen using the forward stepwise selection based on the Akaike's information criterion

*** $P < 0.001$,
** $P < 0.01$, * $P < 0.05$,
n.s. $P > 0.05$

mentioned factors (Table 2). The mean biovolumes of diatoms were optimally explained by a set of variables, including conductivity values, Ca, total P, and Fe concentrations (Table 3). This multiple regression model explained about 62% of the variation in the mean diatom biovolumes at the localities.
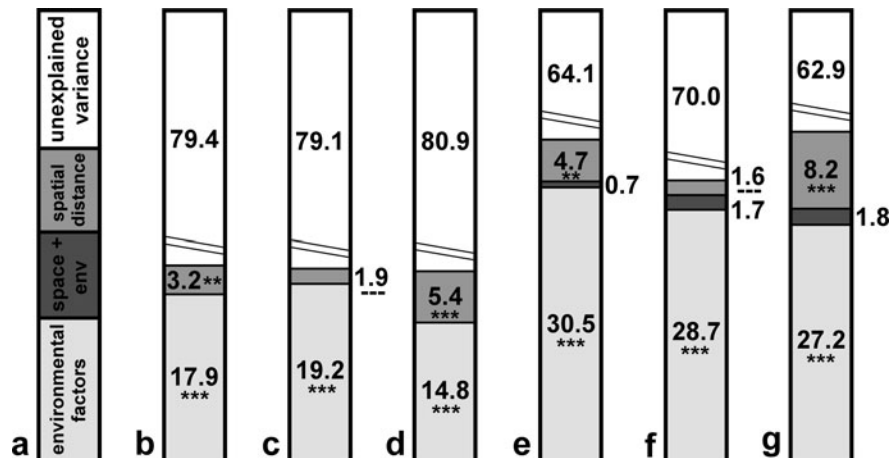
Fig. 4 Results of variance partitioning into individual fractions of environmental factors, spatial distances, combined effects of space and environment (when applicable), and unexplained variance (**a**). Individual analyses were based on complete species data of desmids (**b**), small (**c**) and large (**d**) desmid species, as well as on complete species data of diatoms (**e**) and small (**f**) and large (**g**) diatoms. The proportions of unexplained variance were cut off for better visibility of other fractions. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s. $P > 0.05$

Variance partition of the environmental and spatial factors by a series of partial RDA's illustrated that the environmental factors had strongly significant effects on the species composition of both desmids and diatoms (Fig. 4). The adjusted R^2 values of the variance explained by environmental factors were consistently lower for desmid datasets (Fig. 4b–d), than for diatoms (Fig. 4e–g). Interestingly, the purely spatial effects were much more pronounced among the large species of both groups, whereas their fractions were considerably lower and even insignificant for datasets consisting of small species (Fig. 4).

Discussion

This study illustrated the dominant effect of pH on species composition and species richness of the phytobenthos of peatlands. Similar patterns of species composition of peatland desmids and diatoms along a pH gradient have been reported in previous studies (Coesel, 1982; Mataloni, 1999; Lederer & Soukupová, 2002; Falasco & Bona, 2011). The pH was also found as the main correlate of species richness in the peatland phytobenthos (Mataloni, 1999; Neustupa et al., 2009). This pattern was explained by the low availability of nutrients in low pH conditions (Coesel, 1982) and the direct stress effects of H^+ ions on the biological membranes of unicellular organisms that inhabit

strongly acidic environments (Gross, 2000). Single measurements of the total concentrations of nutrients did not correlate with the pH levels or the ombro-minerotrophic gradient, which supported previous studies that reported a weak relationship between the pH and trophic gradients in various types of peatlands (Bridgham et al., 1996, 1998; Wheeler & Proctor, 2000). By contrast, the pH levels were strongly correlated with the estimated ombrotrophic or minerotrophic status of sites. This supported a general distinction between poorly buffered ombrotrophic bogs and minerotrophic fens based on their pH levels (Vitt, 2006). However, there were still several strongly acidic, minerotrophic poor fens that conformed to the notion that the Central European peatlands, which are located near the southern limits of the global range of these habitats, may not be unequivocally differentiated solely on the basis of their pH levels (Hájek et al., 2006). Nevertheless, we should note that our study was only based on a limited number of samples and the addition of further localities could possibly change the observed pattern of the relation of peatland hydrography to the observed pH level.

Interestingly, the mean cell size dynamics of desmids and diatoms was considerably different among the sites. The mean biovolumes of desmids were optimally explained by the ombro-minerotrophic gradient and the Ca concentrations (tightly correlated with the pH levels) in the multiple regression model,

as well as in the univariate linear correlation analyses. Conversely, the diatom mean biovolumes were not unequivocally related to these factors. The purely ombrotrophic acidified peat bogs, which typically had low pH values, had considerably smaller desmids than the more pH-neutral sites. This pattern may probably be explained by the generally unstable conditions in these poorly buffered ombrotrophic bogs. These conditions generally favor smaller species with higher growth rates and higher surface-to-volume ratios (Friebele et al., 1978; De Nicola et al., 2006). We can conclude that the data on the cell size distribution of desmids in peatlands could possibly be used as a quantitative measure of the community response to key environmental gradients in these habitats, such as the transition from a minerotrophic fen to an ombrotrophic bog stage, or acidification.

The cell size dynamics of diatoms clearly differed and it was only weakly related to the ombro-minerotrophy gradient in the linear correlation analyses. Interestingly, the obvious difference between desmids and diatoms in terms of the relationship between mean size and the pH gradient was not apparent in the more acidic part of the scale. The pH levels were significantly unimodally related to the mean biovolumes of diatoms, and there was an obvious positive relationship between these variables at pH levels of 3.3–5.3. Consequently, diatoms had the largest mean biovolumes at pH of ca 5.2–5.3, whereas the mean biovolumes of desmids increased at sites with higher pH values. The decreasing size of diatoms relative to the pH at minerotrophic sites where the pH was >5.3 was most likely caused by factors not considered in this study. The nutrient concentrations and pH values were not mutually related in sites with higher pH values, and there was no significant relationship between the nutrient levels and the mean diatom biovolumes. The decrease in diatom sizes may be related to biotic factors, such as increased competition in less extreme habitats with a higher pH. This pattern could have been evaluated based on changes in the absolute quantities of diatoms, desmids, and other phytobenthic groups in the samples, but the current study was focused on the relative amounts of individual taxa in the desmid and diatom phytobenthic assemblages; therefore, the present data were not appropriate for such an analysis.

Alternatively, the less direct relationship between ombro-minerotrophy and the mean cell sizes of diatoms compared to desmids may have been caused by their different nutrient uptake scaling. Passy (2007) argued that smaller diatoms may not have significantly better per unit biovolume maximum nutrient uptake rates than larger taxa because their cytoplasm is effectively constrained to a thin layer located beneath the plasmatic membrane. Therefore, the results of the current study may also provide indirect support for this hypothesis. The difference between desmids and diatoms in their mean community biovolume dynamics, or, in other words, the lack of clear relation between pH levels of the localities and the mean biovolumes of diatoms could also be related to the differences in infraspecific size variation between both groups. The dimensions of individual desmid taxa are fairly constant within comparatively narrow limits (Coesel & Meesters, 2007) and are typically considerably lower than the differences among species. Conversely, cell sizes of diatom species vary considerably as a result of the gradual size decrease during their vegetative cell division (Round et al., 1990). Therefore, natural diatom populations must cope with regular fluctuations in their S:V ratios. Consequently, this can make the size structure of the diatom communities in peatlands generally less susceptible to the actual pH levels or hydrography of the individual localities.

Interestingly, the mean biovolumes of diatoms were significantly linearly related to the conductivity. This relationship was also significant for the mean surface areas and confirmed also by the multiple regression analysis of the mean diatom biovolumes at the localities. The conductivity values at the study sites ranged from 29 to 245 $\mu S\ cm^{-1}$, and this gradient was comparable to those commonly reported in various peatland habitats (Coesel, 1982; Mataloni, 1999; Neustupa et al., 2012). Snoeijs et al. (2002) reported significant effects of salinity on the mean cell sizes of benthic diatoms in the Baltic Sea. However, the profound salinity gradient of the Baltic Sea, where localities had 10–100 times higher salinity values than our samples, probably prevents a direct comparison with the current study of peatlands. The relationship between the mean community cell size and conductivity in the current study may reflect a more general and previously unexplored pattern in diatom size dynamics in peatlands, which should be investigated further. It should be noted, however, that both the multiple regression models explaining the variation in

the mean biovolumes of desmids and diatoms using the abiotic factors left relatively high proportions of the variability unexplained. These unexplained fractions may possibly relate to some other important abiotic factors that were not accounted in this study, such as the dissolved organic carbon (DOC). Alternatively, this variation can also be related to the purely neutral factors that principally cannot be explained by local physico-chemical variables.

The spatial structure significantly affected the community structure of desmids and diatoms at the study sites. Interestingly, the geographic distances were slightly more pronounced in diatoms, suggesting that, compared with desmids, their community structure was relatively more strongly structured by large scale processes, such as dispersal, climate, or history. Similar significant effects on the community structure at the regional level were reported in several studies of benthic diatoms from streams (Soininen et al., 2004; Heino et al., 2010; Smucker & Vis, 2011; Virtanen & Soininen, 2012). Individual geographically constrained distribution areas were also recently detected for several large desmid taxa in the genus *Micrasterias* (Neustupa et al., 2011b). In this study, the large species of desmids and diatoms were clearly more geographically restricted than taxa with smaller cell biovolumes. The significant effect of spatial distances among localities, which was not correlated with environmental data, although more important for large diatoms, was still highly significant in large desmids. By contrast, the datasets of small species lacked any significant spatial structure that was not accounted for by environmental factors. This pattern may suggest that the spatial pattern in the species data was actually related to dispersal limitations, which should be considered as a structuring factor for phytobenthic communities in peatlands. At a regional scale, dispersal limitations are probably more important for large species, which may have less effective passive dispersal (Heino & Soininen, 2006; Passy, 2007; Vanormelingen et al., 2008a). Overall, our results showed that local environmental parameters are important for structuring the phytobenthic assemblages of peatlands, but they may mask the important effects of dispersal-related processes at regional scales, which are related to the cell sizes of individual taxa. However, we should also note that species concepts of microalgae, including desmids and diatoms, are notoriously unstable, and numerous recent studies have detected cryptic or pseudocryptic diversity within traditional morphospecies (Vanormelingen et al., 2008b; Evans et al., 2009; Poulíčková et al., 2010). Small desmid and diatom species typically have fewer conspicuous morphological discriminatory characters. Thus, there may be more cryptic species in the relatively small taxa compared with larger species. This may lead to an underestimation of species diversity among small taxa in ecological studies, including this one, based on morphological species concepts. Thus, the lack of significant geographic structure among the small taxa in the current study may be explained by the low reliability of taxonomic concepts in these species. To the best of our knowledge, there have been no rigorous analyses of the level of cryptic species differentiation relative to the cell size of individual traditional taxa. However, such data would be very useful for estimating the size-related dispersal limits of microalgae.

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