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Aquatic Ecology
A Multidisciplinary Journal Relating to Processes and Structures at Different Organizational Levels

ISSN 1386-2588
Volume 50
Number 4

Aquat Ecol (2016) 50:697-709
DOI 10.1007/s10452-016-9587-y

Springer

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Received: 29 January 2016 / Accepted: 21 June 2016 / Published online: 25 June 2016

Abstract The nature of the relationships between host plants and associated microalgal epiphyton in freshwater ecosystems is largely undetermined. Many studies assume that the host plant, as a biologically active substrate, must have a direct influence on associated epiphytic communities, whereas other studies favor the view that host plants are a neutral substrate with only indirect effects on epiphytic communities. Here, we addressed the question of whether host plant taxonomic identity influences epiphyton community structure in comparison with other factors (which also provided further insight into the general ecology of freshwater microphytobenthos) by examining four types of natural plant substrates at five freshwater sites in the Czech Republic. The results demonstrated that host plant type significantly affected epiphytic community structure; most notably, the genus *Sphagnum* was found to support remarkably different epiphytic communities, probably including several microalgal substrate specialists. Other host plants we examined included *Utricularia* spp., *Nymphaea* spp., and *Potamogeton natans* L., all of which were presumably neutral substrates. The differences among the host plants varied among sites, however, suggesting the presence of an interaction between these two factors. Thus, we concluded that host plants may not always provide a neutral substrate for microalgal epiphyton and, consequently, biomonitoring surveys should account for variation relating to this factor. Finally, our work showed that epiphytic diatoms (Bacillariophyceae) and desmids (Desmidiales) adopted similar group strategies, thus allowing for generalizations of patterns across entire microphytobentic communities.

Keywords Community structure · Desmids · Diatoms · Epiphyton · Host plant · Host specificity

Introduction

Epiphytic communities of microscopic algae and cyanobacteria are important components of aquatic ecosystems. Epiphytic communities form the basis of many food webs in aquatic ecosystems (James et al. 2000; Hart and Lovvorn 2003), and the complex

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Handling Editor: Dr. Miquel Lurling.

Electronic supplementary material The online version of this article (doi:10.1007/s10452-016-9587-y) contains supplementary material, which is available to authorized users.

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competitive interactions between phytoplankton, benthic microalgae (particularly epiphyton), and macrophytes (host plants) determine the ecosystem character and ecosystem response to changing environmental conditions (Sand-Jensen and Borum 1991; Havens et al. 2001). Freshwater microphytobenthos, including epiphyton, are influenced by prevailing environmental conditions, especially with regard to water pH, conductivity, and nutrients (e.g., Coesel 1982; Soininen et al. 2004; Fránková et al. 2009; Machová-Černá and Neustupa 2009; Neustupa et al. 2009), as well as light conditions (e.g., Müller 1999; Asaeda et al. 2004; Hillebrand 2005). Furthermore, spatial and, to a much lesser extent, temporal factors play important roles in determining microphytobenthic community structure (Messyasz and Kuczyńska-Kippen 2006; Machová-Černá and Neustupa 2009; Veselá 2009; Krivograd Klemenčič et al. 2010; Neustupa et al. 2012; Svoboda et al. 2014). Benthic microorganisms can also be influenced by biotic interactions, including intraspecific competition (Jones et al. 2000) and predation (Cattaneo 1983; Jones et al. 2000; Hillebrand 2005). However, the influence that the host plant has in shaping the epiphytic community still remains a matter of debate.

Host plants were found to have a positive effect on the epiphyton, with potential underlying mechanisms consisting of, for example, the release of inorganic nutrients through macrophyte surfaces enhancing epiphytic growth, particularly in oligotrophic waters (Eminson and Moss 1980; Burkholder et al. 1990) or during the early stages of epiphyton development (Albay and Akcaalan 2003). This situation could conceivably lead to mutualism between macrophytes and epiphytic algae (as in Ulanowicz 1995). In contrast, some studies reported that host plants might have a negative effect on associated epiphytic communities, through, for instance, the production of allelopathic substances (reviewed in, e.g., Gross 2003; Hilt 2006) or by attracting predators that selectively remove competitive epiphytes (Brönmark 1985; Thomas et al. 1985). Nevertheless, the neutral substrate hypothesis (Shelford 1918; supported by e.g., Siver 1977; Cattaneo and Kalff 1979; Cattaneo et al. 1998; Kuczyńska-Kippen et al. 2005; Laugaste and Reunanen 2005; Messyasz and Kuczyńska-Kippen 2006; Cejudo-Figueiras et al. 2010) postulates that host plants do not interact biologically or chemically with the epiphyton. In this case, macrophytes have only an indirect influence through plant architecture (i.e., substrate complexity) or owing to macrophyte position and movement in the water column (i.e., effect of light and nutrient inputs), among other means. However, generalizations on the effects of host plants on the epiphyton are still far to be done; moreover, such interactions may be entirely contextual, in that some macrophytes act as a neutral substrate, whereas others actively influence their epiphytic community (Gough and Woelkerling 1976; Blindow 1987).

More importantly, in most cases, the influence of the host plant as a substrate has been assessed in isolation, whereas the effects of the host plant when compared to those of other factors known to influence the freshwater microphytobenthos have rarely been investigated. Such studies (Eminson and Moss 1980; Millie and Lowe 1983; Lalonde and Downing 1991; Pals et al. 2006; Cejudo-Figueiras et al. 2010) have generally concluded that the spatial distance between localities and environmental factors was the more important determinants of the epiphytic algae than the substrate itself. Only a small fraction of relevant studies (Gough and Woelkerling 1976; Woelkerling 1976) emphasized the relative importance of the effect of host plants in conjunction with the remarkable effects of other factors.

In this study we explored the combined effects of environmental factors and host specificity on freshwater algal epiphyton. We chose diatoms (Bacillariophyceae, Stramenopila, SAR) and desmids (Desmidiales, Viridiplantae, Archaeplastida) as the two model microalgal groups, because diatoms and desmids are monophyletic and taxonomically unrelated. Moreover, both groups are often the dominant phototrophs in freshwater epiphyton (e.g., Lazarek 1982; Machová-Černá and Neustupa 2009; Krivograd Klemenčič et al. 2010), and as such are frequently used as model organisms for biomonitoring (Dixit et al. 1992; Coesel 2001, 2003). Specifically, we assessed simultaneously the effects of host plants and environmental factors at several sites to determine whether (1) there is a significant influence of host plant taxonomic identities on associated algal epiphyton, particularly in regard to community structure, over and beyond the effect of spatial variability and environmental conditions; (2) algal taxa display substrate specificity; and (3) the observed patterns are consistent between diatoms and desmids. As has already been mentioned, there are many reported cases of distinct spatial patterns of benthic algal communities; thus, we expected (1) to find noticeable differences in epiphytic community structure mainly between isolated water bodies, whereas the effect of host plants was expected to be non-significant. Consequently, we presumed that (2) there would be no substrate specialists among the microalgae identified on the macrophyte substrate. At last, we expected (3) to record similar patterns for both diatom and desmid communities.

Materials and methods

Our research focused on the comparison of the algal epiphytic communities associated with different types of natural plant substrates. To achieve this, epiphyton were sampled at five isolated sites in the Czech Republic (Table 1). All study sites consisted of stagnant oligotrophic or mesotrophic water bodies (boggy pools and ponds). Four types of macrophytes (host plants)—*Sphagnum* spp. (SP), *Utricularia* spp. (UT), *Nymphaea* spp. (NY), and *Potamogeton natans* L. (PO)—common to the sites were sampled (Table 1). The host plants could be divided into two groups based on their architecture (i.e., substrate complexity), which might also affect the associated epiphyton community structure according to the neutral substrate hypothesis. The genera *Sphagnum* and *Utricularia* have a relatively complex plant architecture characterized by dense branching and numerous smaller leaves, whereas *Nymphaea* and *Potamogeton* have a comparatively simple plant architecture characterized by smooth stems and floating leaves. At each site, epiphyton communities were sampled on three thalli of every host plant in order to get replicates. Ideally, each group of replicates (e.g., the first replicates of all sampled macrophytes at the site) was collected within a 1-m^2 area, with replicate groups separated by approximately 5 m. A total of 51 samples were collected; although some host plants were absent in three of the five sampling sites, at least three host plants were sampled at each site (Table 1). Water pH and conductivity were measured with a combined pH/conductivity meter WTW 340i (WTW GmbH, Weilheim, Germany) immediately in the field (Supplementary Table 1). These environmental variables were specifically chosen because they have been previously shown to explain a substantial degree of microphytobenthic community variation (e.g., Fránková et al. 2009; Neustupa et al. 2013). Sites S1, S2, and S3 were small, and thus only three measurements of pH and conductivity were carried out for each of these sites, whereas for the larger sites S4 and S5, where macrophytes were more spread, additional measurements were required, with nine measurements taken at site S4 and eight at site S5. Still the distance of 5 m between the groups of replicates was maintained.

Sampling was conducted at the end of August 2012 and the beginning of September 2012 (Table 1). Given that different host plants have different growth rates, which is supposed to affect epiphytic colonization rates (Millie and Lowe 1983), late summer was considered to be the best time for sampling, as by that time the shoots of macrophytes were fully grown and were covered by relatively well-developed epiphyton. Epiphyton samples were obtained by plant squeezing or careful brushing of the host plant surface; both techniques are common and highly efficient ways of sampling epiphytic communities (e.g., Asaeda et al.

Table 1 Study sites and additional information

Site	Type	GPS N (°)	GPS E (°)	Sampling date	pH	Conductivity (μS.cm^{-1})	SP	UT	NY	PO
S1	Boggy pool	50.5789	14.667397	28/8/2012	4.7 ± 0.1	89.3 ± 0.3	+	+	+	
S2	Boggy pool	50.577356	14.661661	28/8/2012	6.2 ± 0.1	193 ± 1	+	+	+	+
S3	Boggy pool	48.923533	14.839153	8/9/2012	5.4 ± 0.1	21.3 ± 0.3	+		+	+
S4	Pond	50.043147	13.440767	27/8/2012	5.8 ± 0.3	221.9 ± 16.2	+	+	+	+
S5	Pond	48.828142	14.597161	8/9/2012	6.1 ± 0.1	74.1 ± 1.1	+	+		+

The environmental data are presented as mean ± standard error, counted from all measured values within the site ($n = 3$ for S1, S2, and S3; $n = 9$ for S4; $n = 8$ for S5)

(+) Samples taken from a particular host plant, i.e., SP *Sphagnum* spp., UT *Utricularia* spp., NY *Nymphaea* spp., PO *Potamogeton natans*

2004; Pals et al. 2006; Neustupa et al. 2011). Only the uppermost submerged parts of the host plants (to a maximum depth of 10 cm) were sampled to avoid variability caused by different positions of macrophytes in the water column (ÓNeill Morin and Kimb 1983) and by vertical zonation of the epiphytic community (Müller 1995, 1999). All samples were fixed in Lugol solution immediately following collection to prevent changes in species ratios in the epiphytic communities due to a sudden change in ambient conditions.

The study was based on community structure of epiphytic diatoms and desmids. In the laboratory, the relative abundance of algal species in each community was counted directly using an Olympus CX 31 light microscope. For each sample, 200 randomly encountered diatom cells and 200 randomly encountered desmid cells were identified to the species level, and colonies were counted up to 10 cells. Determination of 200 cells per sample has been widely used in previous studies on both desmids (e.g., Pals et al. 2006; Neustupa et al. 2012; Svoboda et al. 2014), and diatoms (Neustupa et al. 2013). Diatom and desmid species identification were performed separately, due to the slightly different methodological approaches required for each. Diatom species were determined at a magnification of 1000 × using permanent slides that were created by first annealing over a gas burner flame (Battarbee et al. 2001) and then mounting into the synthetic resin Naphrax (Brunel Microscopes Ltd. Wiltshire, UK). Identification of diatom species was performed using the standard taxonomic monographs of Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Krammer (2000, 2002, 2003), Lange-Bertalot and Metzeltin (1996), Lange-Bertalot (2001), and Lange-Bertalot et al. (2011). Desmid species were determined at 400 × magnification directly from samples preserved in Lugol solution, following the standard taxonomic monographs of Růžička (1977, 1981), Lenzenweger (1996, 1997, 1999, 2003), and Coesel and Meesters (2007).

Statistical analyses were conducted using PAST ver. 2.17c (Hammer et al. 2001) and R ver. 2.15.1 (R Development Core Team 2012) with the *vegan* package (Oksanen et al. 2013). The resulting datasets of relative abundances were prepared separately for diatoms and desmids, with each dataset consisting of 50 applicable samples. Two samples (different ones for each dataset, see Supplementary Table 1) were excluded from further analysis because of very low abundances of a particular algal group. The datasets were comprised of all identified algal cells, including rare species, and thus standardization of species data was unnecessary. Environmental data (i.e., pH and conductivity) were normalized and a principal coordinate analysis (PCO) coupled with a CLUSTER analysis based on Euclidean distance among samples was carried out in order to identify groups of sites (water bodies) with comparable environmental conditions.

Two separate distance-based permutational multivariate analyses of variance (PERMANOVA; Anderson 2001, 2005; McArdle and Anderson 2001) were performed, one for each of the two algal groups (i.e., diatoms and desmids), to test for differences in the community structure of epiphyton due to environmental conditions and in relation to host plants. Analyses were based on Bray-Curtis similarities calculated on untransformed data, and each term was tested using 4999 random permutations. The design of the analyses thus consisted of three factors, namely Environmental condition (Env; 3 levels as identified from PCO and CLUSTER analyses, fixed, crossed), Host (Ho; 4 levels, fixed), and Site (S; 1–2 levels, random, nested in Env). Significant terms relevant to the hypothesis were investigated through post hoc pairwise comparisons using PERMANOVA *t*-tests and 999 permutations. Multivariate patterns in community structure were portrayed by NMDS ordination plots of Ho × Si(Env) centroids.

The second question we addressed was whether particular algal taxa exhibit substrate specificity. In our work, only the 25 % most abundant species from each dataset were considered to be suitable for ecological analysis, as presented in Heino and Soininen (2010). In addition, we only considered species occurring at two sites at a minimum to exclude species unique to a particular sample or site. Subsequently, the substrate preferences of the 25 diatom species and 18 desmid species included in this analysis were examined by the correlation of algal abundances with the investigated genera of host plants, using a Kendall rank correlation coefficient (Kendall 1938; Newson 2002).

To this point, all analyses were performed separately for diatoms and desmids, thus comparisons of discovered trends could be drawn only indirectly. Therefore, direct comparisons of algal communities were performed by measuring the degree of their concordance with the Procrustes statistic (PROTEST; Peres-Neto and Jackson 2001). PROTEST compares multivariate datasets concerning exactly the same samples; the datasets are scaled, superimposed, and rotated in order to maximize their fit. In our study, we used 49 samples in which both diatoms and desmids were found in sufficient numbers. PROTEST using 999 permutations was performed for the distance matrices that were taken from the two-dimensional NMDS based on a Bray-Curtis similarity index. In addition, the differences between the two original matrices were plotted in the diagram, where the distance between corresponding objects represents the extent of the congruence of diatom and desmid communities within the sample.

Results

A total of 106 diatom taxa belonging to 30 genera were found in the 51 collected samples (Supplementary Table 2). Diatom species richness per sample ranged from 2 to 36. For desmids, there were a total of 103 taxa recorded, belonging to 16 genera (Supplementary Table 3), and desmid species richness per sample ranged from 5 to 25. As all samples were collected from the oligotrophic or mesotrophic sites where more acidic conditions prevailed, the most frequent diatom genera were *Pinnularia* (25 recorded species) and *Eunotia* (22 species); the most frequent desmid genera were *Cosmarium* (33 species), *Closterium* (17 species), and *Staurastrum* (15 species).

PCO and CLUSTER analyses identified three distinct groups of sites that had comparable pH and conductivity conditions (Fig. 1); sites were clustered into groups if within-group similarity was ≥50 %. Thus, sites S2 and S4 were grouped together, as they were characterized by relatively higher values of pH and conductivity, whereas sites S3 and S5, which featured relatively higher pH but low conductivity, formed a second group. Site S1, on the other hand, exhibited intermediate values of conductivity and acidic conditions with respect to the other groups (Fig. 1). The environmental factors PERMANOVA revealed negligible effects of pH and conductivity on epiphytic community structure for both diatoms and desmids (Tables 2 and 3). In contrast, a significant Ho × S interaction was detected for both algal groups, indicating that differences in epiphytic communities among host plants varied among sites (Tables 2 and

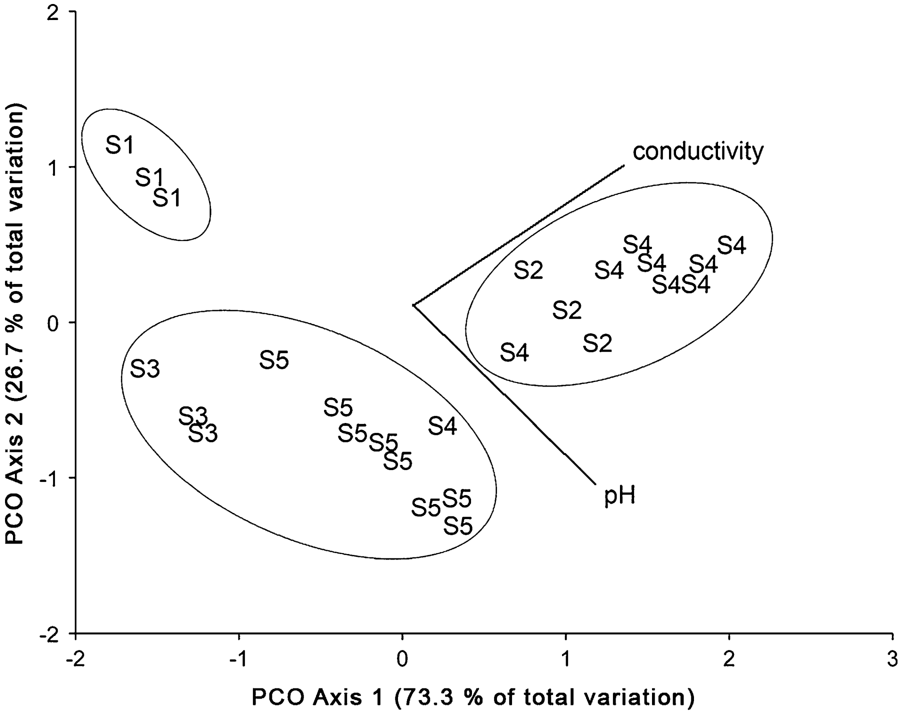


Fig. 1 Principal coordinate analysis (PCO) based on conductivity and pH. Vectors of the two environmental variables are also presented. Groups of sites (*S1*, *S2*, *S3*, *S4*, *S5*) characterized by similar pH and conductivity were identified using CLUSTER analysis. Clusters (within-group similarity ≥50 %) are enclosed within *solid lines*

Table 2 Results of PERMANOVA on diatom communities

Source of variability	df	SS	MS	Pseudo-*F*	*P*
Environmental condition = Env	2	55153	27577	1.267	0.3408
Host = Ho	3	19272	6424	1.497	0.1712
Site(Env) = S(Env)	2	44246	22123	21.256	**0.0002**
Env × Ho	5	16332	3266	0.877	0.6540
S(Env) × Ho	4	14602	3651	3.508	**0.0002**
Residual	33	34345	1041		
Pairwise comparisons					
S1	SP = UT ≠ NY				
S2	SP ≠ UT = NY = PO				
S3	SP ≠ NY = PO				
S4	SP ≠ UT = NY = PO				
S5	SP ≠ UT = PO				

The analysis is based on Bray-Curtis similarities, with each test performed using 4999 permutations of appropriate units

P values were obtained using 4999 Monte Carlo samples from the asymptotic permutation distribution; significant *P* values are shown in bold

Results of pairwise comparisons among hosts, i.e., *SP Sphagnum* spp., UT *Utricularia* spp., NY *Nymphaea* spp., PO *Potamogeton natans*, are also reported for each site

3). The site effect is clearly visible in the NMDS graphs (Fig. 2 and 3). In regard to the host plants specifically, pairwise comparisons revealed that diatom communities on the genus *Sphagnum* differed from those occurring on the other host plants (no differences were found between the other host plants) in S2, S3, S4, and S5 (Table 2). Site S1 was the only exception to this pattern, with diatom communities on *Nymphaea* differing from the remaining two host species (*Sphagnum*, *Utricularia*). Such patterns are illustrated in the NMDS plot (Fig. 2). A similar pattern also emerged for desmids, as post hoc comparisons demonstrated that communities on *Sphagnum* differed from those on the other hosts (Table 3), once more with the exception of S1 but also for S5 (Fig. 3).

Examining the correlations between the relative abundances of the algal species and host plant genera enabled determination of substrate specificity (significant correlations are summarized in Supplementary Table 4 for diatoms and Supplementary Table 5 for desmids). Out of 25 diatom species, only *Frustulia saxonica* Rabenhorst did not exhibit substrate preference, whereas as many as nine out of 18 desmid species did not exhibit substrate specificity, with the remaining species either positively or negatively correlated with some host plants. Considering just highly significant results (i.e., $P < 0.001$), diatom species displayed several substrate preferences, whereas among the desmids, *Staurastrum punctulatum* Brébisson alone had a strong positive relationship to *Sphagnum* ($r = 0.51$, $P < 0.001$) and a marginally significant negative correlation with the host plants *Utricularia* and *Nymphaea* (both $r = -0.22$, $P < 0.05$). Summing up all of the significant correlations for each host plant (Supplementary Tables 4 and 5) suggests that there were more negative correlations only for *Sphagnum*, and slightly more positive correlations with *Nymphaea.* Despite the very low number of significant correlations overall, these findings again support the results of PERMANOVA, particularly with regard to the distinctiveness of the epiphytic communities associated with the genus *Sphagnum*.

Previous results indicate that much the same pattern exists for both diatom and desmid communities. Nevertheless, to support such a statement, direct comparisons between diatom and desmid epiphytons were performed using Procrustes analysis, which essentially confirmed the previous indirect comparisons by demonstration of non-random congruence of diatom and desmid NMDS ordinations ($r = 0.76$, $P = 0.001$), as shown in Fig. 4. For most, the changes of sample positions were organized; for instance, the

Table 3 Results of PERMANOVA on desmid communities

Source of variability	df	SS	MS	Pseudo-*F*	*P*
Environmental condition = Env	2	58874	29437	1.342	0.3328
Host = Ho	3	10145	3382	0.756	0.6652
Site(Env) = S(Env)	2	39610	19805	24.323	**0.0002**
Env × Ho	5	12907	2581	0.576	0.8634
S(Env) × Ho	4	13811	4604	5.654	**0.0002**
Residual	31	25242	814		
Pairwise comparisons					
S1	SP = UT = NY				
S2	SP ≠ UT = NY = PO				
S3	SP ≠ NY = PO				
S4	SP ≠ UT = NY = PO				
S5	SP = UT = PO				

The analysis is based on Bray-Curtis similarities, with each test performed using 4999 permutations of appropriate units

P values were obtained using 4999 Monte Carlo samples from the asymptotic permutation distribution; significant *P* values are shown in bold

Results of pairwise comparisons among hosts, i.e., SP *Sphagnum* spp., UT *Utricularia* spp., NY *Nymphaea* spp., PO *Potamogeton natans*, are also reported for each site

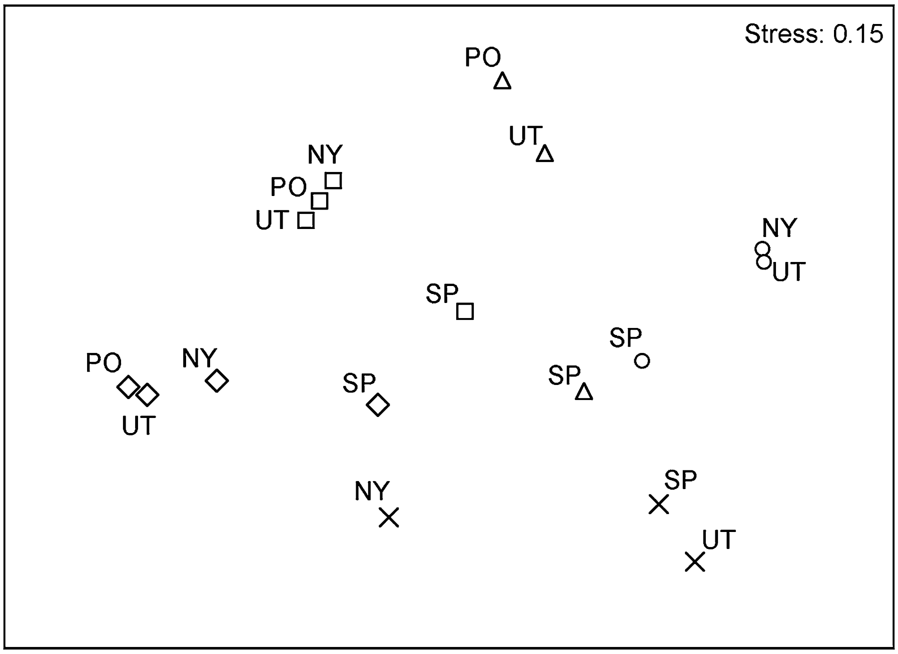


Fig. 2 NMDS ordination plot of S(Env) × Ho centroids for diatom communities. The Kruskal stress values reflect the reliability of NMDS. *Cross* S1, *diamond* S2, *circle* S3, *square* S4, *triangle* S5; SP *Sphagnum* spp., UT *Utricularia* spp., NY *Nymphaea* spp., PO *Potamogeton natans*

distinct groupings of samples remained more or less together for both diatoms (circles in Fig. 4) and desmids (arrow ends in Fig. 4). That is, grouped samples either stayed in the same place or moved in the same direction. Outlying samples, with clearly distinct community structures, remained separated for both algal groups. Thus, the general trends of both algal groups were similar.

Fig. 3 NMDS ordination plot of S(Env) × Ho centroids for desmid community. The Kruskal stress values reflect the reliability of NMDS. *Cross* S1, *diamond* S2, *circle* S3, *square* S4, *triangle* S5; SP *Sphagnum* spp., UT *Utricularia* spp., NY *Nymphaea* spp., PO *Potamogeton natans*

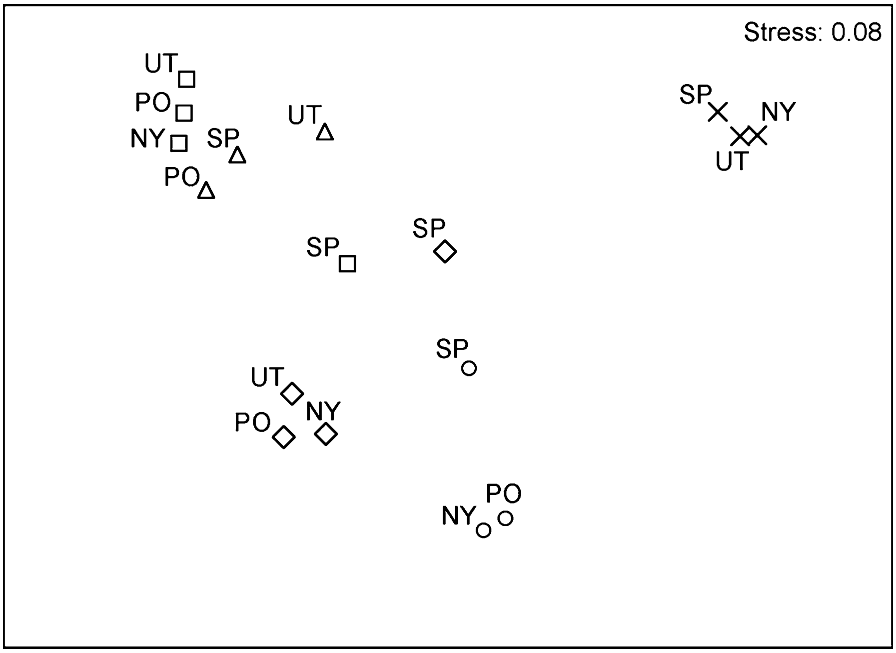
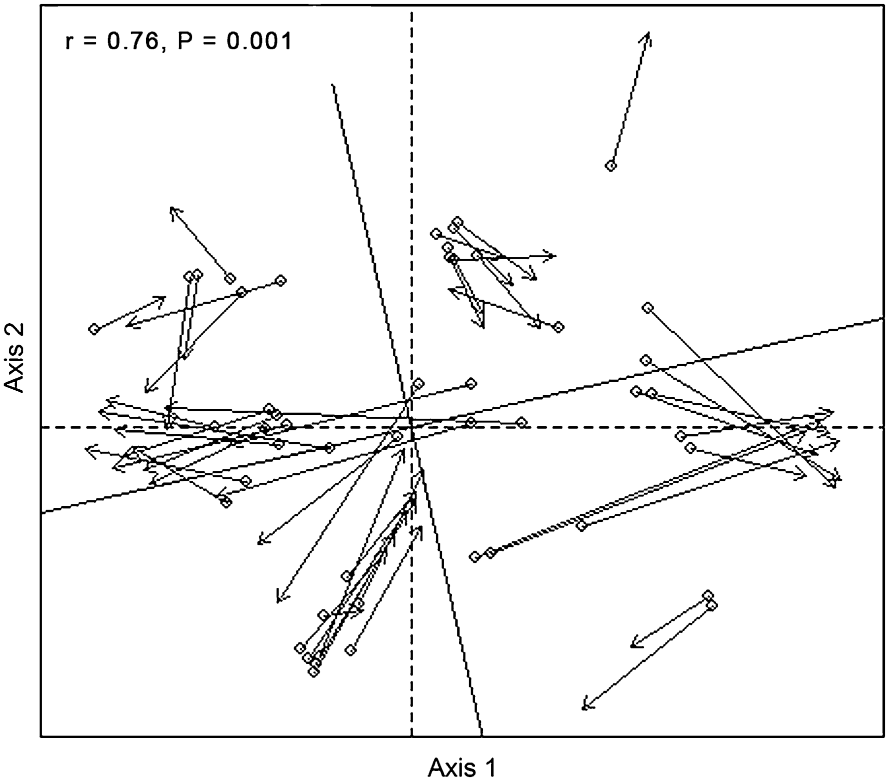


Fig. 4 Graphic visualization of the Procrustes analysis using 999 permutations. The NMDS ordination plot reflects the superimposition of diatom (*circles*) and desmid (*arrow ends*) samples. The distance between corresponding objects represents the extent of the congruence of diatom and desmid communities within one sample. In general, this graph shows the similarity of the group strategies



Discussion

Although it has long been assumed that host plants influence the composition of its associated epiphytic community through biological or chemical means, whether host plants have any significant effect relative to other factors that substantially affect freshwater algal benthos remains unresolved. In spite of our hypothesis, our research indicated that host plants had an effect on the community structure of their algal epiphyton; however, the variation observed among the sites suggested that effect of host plant may be context-specific. Such an outcome is highly relevant for biomonitoring. Siver (1977) and Cejudo-Figueiras et al. (2010) claimed that epiphyton might be used for biomonitoring programs regardless of substrate type; our results contradict this assumption by demonstrating that there might be substantial differences between host plants within a single site. For instance, in most of our sites, epiphytic assemblages associated with the genus *Sphagnum* significantly differed from those of other host plants, whereas in some sites, host plants supported similar epiphyton communities.

From the studies with similar approach that investigated the effects of several factors on epiphytic community structure at once, our results concur with those of Gough and Woelkerling (1976) and Woelkerling (1976), who recorded an effect of host plant alongside a remarkable spatial effect. Pals et al. (2006) also observed significant differences between several types of substrates within a site, but found much greater variation between epiphytic communities among different sites. On the other hand, Eminson and Moss (1980), Millie and Lowe (1983), and Cejudo-Figueiras et al. (2010) strongly emphasized the differences between sites and concluded that the host-plant effect was almost negligible, although those studies did not include the genus *Sphagnum*.

In our analysis, the genus *Sphagnum* seemed to support different epiphytic communities than did the other host plants, a pattern that was more or less consistent among sites and between algal groups. *Sphagnum* is a well-known macrophyte genus that can alter the surrounding physicochemical environment through acidification, a process in which carbon cations are released from *Sphagnum* cells and calcic, magnesium, or potassium cations are absorbed from the surrounding environment (Clymo 1964; reviewed in Andrus 1986). As a result, higher concentrations of acidophilic algae can be expected to occur in the immediate vicinity of *Sphagnum*. This was partly supported by our analysis of algal substrate specificity. Although the sum of significant positive correlations of algal species with *Sphagnum* did not differ from the other investigated host plants, *Sphagnum* appeared to be the substrate with higher frequency of negative correlations, an indication that some algal taxa cannot tolerate the relatively more acidic environment created by *Sphagnum* species. Moreover, and unlike the other investigated host plants, *Sphagnum* is a perennial macrophyte. Although we sampled well-developed epiphytic communities, the longer development time may be important; this aspect should be further explored through comparisons with other perennial host plants.

For both diatoms and desmids, site S1 was an exception; there, *Nymphaea* and not *Sphagnum* supported different epiphytic communities. This might be explained by the very low pH of this site, probably leading to the homogenization of algal epiphyton by selecting those species that can tolerate such acidity, algal species that may in addition be generalists when it comes to substrates. However, there must have been other factors determining the epiphytic community in the case of *Nymphaea* at this site. For example, in contrast to the complex architecture of *Sphagnum* and *Utricularia*, *Nymphaea* likely favor the occurrence of species that are adapted to maintain at relatively smooth and simple substrates.

Previous studies that investigated epiphyton within a single site often concluded that host plant significantly influenced the structure of the epiphyton community (e.g., Blindow 1987; Cattaneo et al. 1998; Laugaste and Reunanen 2005). However, Siver (1977) and Cattaneo and Kalff (1979) reported no influence of macrophytes within a single site, indicating that the pattern is inconsistent among sites, as we also found. An explanation for this inconsistency was suggested by Eminson and Moss (1980), who showed that macrophytes had a greater influence on associated epiphyton in oligotrophic waters, most likely because epiphyton has access to additional nutrients released from the host plant. However, our analysis did not reveal any interaction between host plants and the prevailing environmental conditions of the oligotrophic and mesotrophic sites. Thus, it seems more probable that some macrophytes can directly influence their associated epiphyton, whereas others act as a neutral substrate (Gough and Woelkerling 1976; Blindow 1987).

In terms of substrate specificity of epiphytic algae, many authors (Eminson and Moss 1980; Blindow 1987; Messyasz and Kuczyńska-Kippen 2006; Cejudo-Figueiras et al. 2010) have come to the somewhat predictable conclusion that some species demonstrate substrate specificity and some do not. Siver (1977), on the other hand, detected no substrate specificity among microalgae at all. Our work showed that there was no exceptional number of substrate specialists recorded. Comparisons of our results with published data (Supplementary Table 6) suggested that substrate specificity seems unlikely for the majority of algal taxa; for example, the diatom *Achnanthidium minutissimum* (Kützing) Czarnecki occurs in many types of microhabitats and usually at very high abundance (e.g., Eminson and Moss 1980; Blindow 1987; Poulíčková et al. 2004; Townsend and Gell 2005; Cejudo-Figueiras et al. 2010), and therefore, no substrate specificity of *Achnanthidium minutissimum* could be assumed. The same applies for the majority of other diatom and desmid species, since published studies report that they are positively correlated with other types of microhabitat or occur there in higher abundances (summarized in Supplementary Table 6), with the exceptions of *Eunotia bilunaris* (Ehrenberg) Schaarschmidt, *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst, *Eunotia paludosa* Grunow, and *Staurastrum punctulatum* Brébisson. These species are probably specific to *Sphagnum*, but only in the context of a host plant, as they are also common in sediment microhabitats (Pals et al. 2006; Machová-Černá and Neustupa 2009; Veselá 2009). Nevertheless, evaluating sediment communities is always quite problematic, as they include many species and dead diatom frustules from other microhabitats (Soininen and Eloranta 2004; Veselá 2009). The specific substrate preferences of *Encyonopsis* cf. *delicatissima* (Hustedt) Krammer, *Eunotia implicata* Nörpel, Lange-Bertalot & Alles, and *Pinnularia pseudogibba* Krammer (Supplementary Table 4) are uncertain, as no references to these were found in the literature. Generally, we concluded that the substrate specificity analysis reinforced the finding that the genus *Sphagnum* supports different algal epiphyton than do the other host plants, most likely due to the acidic conditions created by *Sphagnum* species as opposed to other macrophyte characteristics.

Research focusing on algal diversity in freshwater ecosystems usually emphasizes the strong effects of pH and conductivity on the community structure of benthic microalgae (e.g., Mataloni 1999; Štěpánková et al. 2008; Neustupa et al. 2009). Both pH and conductivity are surely important, but they often correlate with other factors that possibly enhance or obscure the pure effects of these environmental factors. The negligible pure effect of pH and conductivity found in our study was not entirely unexpected, given that the environmental parameters correlated with differences between sites. More importantly, the sampling sites were chosen to be as similar as possible, specifically either oligotrophic and mesotrophic sites, in order to achieve the required overlap of host plants chosen for the study. Thus, it is possible that the effects of the environmental parameters would be more pronounced if we had included sites with higher nutritional status or with remarkably different limnological characteristics, as described by Eminson and Moss (1980), Lalonde and Downing (1991), and Cejudo-Figueiras et al. (2010).

Given that diatoms and desmids represent monophyletic and unrelated algal groups, and because they represent the dominant of microbenthos in many freshwater ecosystems, the group strategies uncovered here must be considered suitable for wider-scale generalization. Based on the results of the indirect comparisons and the Procrustes analysis, which compared diatom and desmid communities directly, we concluded that the strategies adopted by the two groups of algae were more or less identical. Such congruence between benthic diatoms and desmids was previously reported by Neustupa et al. (2013). The exception, that should be considered, are the flagellates, which are capable of migrating over relatively long distances, enabling them to actively seek out and disperse to locations with more favorable conditions (Happey-Wood 1988; Hall and Pearl 2011). However, diatoms and desmids are also motile, and moreover it is still unknown how frequently flagellates actually migrate between different microhabitats. Thus, it is possible that even benthic flagellates follow similar trends to those exhibited by diatoms and desmids.

It must be stressed that our research focused on the community structure of epiphyton. The relative influence of different factors on epiphyton could be different if other characteristics of epiphyton (e.g., biomass, chlorophyll *a* content, or absolute densities of algae) were to be included (as in Lalonde and Downing 1991; Kuczyńska-Kippen et al. 2005; Laugaste and Reunanen 2005). In addition, it would be worth to investigate temporal variability of epiphyton, as well as the phylogenetic, size and shape structure of algal epiphyton. Even for less ambitious projects, however, we recommend replicating the methodology used in this study; that is, sampling epiphyton on natural plant substrates growing in distinct water bodies at the same time.

Conclusions

Our work explored the simultaneous effects of several factors on the community structure of freshwater algal epiphyton. The results demonstrated that host plant taxonomic identity had a significant effect on the community structure of freshwater algal epiphyton, yet there must be some interaction effect between host plants and spatial variation among sites, since the differences related to the host plants were variable among sites. On the whole, the genus *Sphagnum* appears to have a substantial influence on the epiphyton community, whereas the neutral substrate theory appeared to hold for the majority of macrophytes, particularly *Utricularia* spp., *Nymphaea* spp., and *Potamogeton natans* in case of our study. The seldom differences between epiphyton associated with other host plants may well be site-specific and influenced by other, undetermined factors; epiphyton should therefore be used for biomonitoring programs with regard to substrate, at least when it comes to *Sphagnum*. The pure effect of environmental parameters (pH and conductivity) appeared to have negligible effects, but most probably this is applied only for the case of our study. Finally, the value of the research lies in the comparison between epiphytic diatom and desmid communities. The results indicated that the patterns for both algal groups were virtually identical thus enabling generalizations to be made across the entire microphytobentic community.

Acknowledgments We are very grateful to Jana Kulichová, Jan Šťastný, Irena Šímová, and Karl Inne Ugland for their help with the research and for their constructive suggestions about the manuscript. We thank the anonymous reviewers and associate editor for their comments and recommendations, which greatly improved the manuscript. We also thank Editage Editing Service for the language and style corrections.

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