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Small-scale variation of corticolous microalgal covers: Effects of microhabitat, season, and space

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SUMMARY

The present study focuses on temporal and microscale spatial variation of the community structure and richness of subaerial microalgae growing on the bark of European beech (*Fagus sylvatica*) trees in temperate deciduous forests. Subaerial phototrophic biofilms present common and conspicuous microalgal communities growing on a variety of natural and man-made substrata. However, in comparison with other major microalgal communities such as phytoplankton and microphytobenthos, basic patterns of their spatio-temporal variation remain largely unknown. The bark samples were collected six times each spring and autumn in a period of 3 years (2010–2013) and were cultured on agar plates, and then individual clonal strains were identified by light microscopy. A total of 55 morphotypes (considered as operational taxonomic units for subsequent analyses) were recognized, which mainly belong to the classes Trebouxiophyceae and Chlorophyceae. Interestingly, temporal variation explained the largest proportion of variation in the community structure. This variation was primarily related to seasonal fluctuations, and although the communities recorded in spring and autumn showed many overlapping taxa, a clear distinction in species composition and abundance was observed. However, the microhabitat characteristics such as bark roughness also significantly structured the microalgal community. Conversely, spatial factors such as the height of the samples above ground or distance of the samples on a trunk seemed to be of lesser importance on this scale. Thus, we concluded that the previously unrecognized seasonal changes, resulting from variation in temperature, humidity, and irradiance, as well as the non-seasonal temporal changes, possibly resulting from local colonization or extinction of individual taxa, should be considered as one of the important factors in structuring aerial microalgal communities.

Key words: aerophytic, algae, bark, biofilm, community, ecology, epiphyte, seasonality, Trebouxiophyceae.

INTRODUCTION

Spatio-temporal heterogeneity of natural communities has raised questions concerning the underlying causes of such patterns. The observed variation of the community structure reflects the scale of the observation (Wiens 1989). Thus, different patterns (and the underlying ecological processes) of community structure emerge at different scales. Notably, the scale-dependent interplay of deterministic and neutral processes have been illustrated for various natural communities of unicellular eukaryotes (De Wit & Bouvier 2006; Telford *et al.* 2006; Vyverman *et al.* 2007; Smucker & Vis 2011; Lepère *et al.* 2013).

For example, benthic microalgal communities in freshwater ecosystems on the scale of individual continents are considerably more strongly structured by the effects of historical events and speciation than by local environmental conditions (Soininen 2004, 2007; Telford *et al.* 2006). Conversely, spatial heterogeneity of communities on intermediate spatial scales (tens to hundreds of kilometers) was found to be jointly structured by environmental and distance-related factors (Martiny *et al.* 2006; Soininen & Weckström 2009). Finally, the microscale heterogeneity of microalgal communities (i.e. differences among samples taken a few centimeters apart) tends to be relatively less correlated with the environmental factors than with actual spatial distance. Both deterministic habitat-filtering based community patchiness reflecting the patchiness of microhabitats (Machová-Černá & Neustupa 2009; Černá 2010; Neustupa *et al.* 2012), biotic interactions—differential dispersal, competition (Passy 2001), and stochastic dispersion-related processes, such as priority effect with subsequent monopolization of resources (Svoboda *et al.* 2014), might affect the observed patterns.

The temporal turnover of benthic microalgal communities on a microscale has been scarcely studied. A general pattern does not arise from few existing studies. Some studies, which focused on different algal groups, described temporal (seasonal) variation of primary production or species composition (Ledger & Hildrew 1998; Aguilera *et al.* 2007; Špačková *et al.* 2009), whereas desmid communities in strongly temperate climate with great temperature fluctuations proved to be extremely stable during a 3-year study (Svoboda *et al.* 2014). The observed seasonal fluctuations of the community structure (species composition and abundance) in temperate regions may be attributed to the variation of water temperature, light intensity, nutrient content, or disturbances such as freezing periods (Talling & Parker 2002; Zalack *et al.* 2006; Roberts *et al.* 2007; Machová-Černá & Neustupa 2009).

Benthic organisms thrive on an interface of solid and fluid environments, and the inherent two-dimensionality of this system apparently has vast effects on the establishment (colonization) and persistence (biotic and abiotic interactions) of microalgal communities on a small scale. Consequently, life on an interface affects the interrelationship of deterministic and stochastic processes shaping the community at a given scale.

The present study focused on a small-scale spatio-temporal community variation of other, much less known and

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understood, but in respect of two-dimensionality possibly even more pronounced system. The subaerial microalgal communities form ubiquitous biofilm covers on natural and man-made substrata (Hoffmann 1989; Gärtner 1994; Freystein & Reisser 2010; Büdel 2011). A life in this ancient terrestrial niche—interface of solid substrate and the atmosphere—has led to many morphological and functional adaptations (Lüttge & Büdel 2010; Karsten & Holzinger 2014; Karsten *et al.* 2016) of microalgae to desiccation, extreme temperature fluctuations, intense irradiation, and low nutrient availability (Barkman 1958; Gorbushina & Broughton 2009; Gustavs *et al.* 2010). Spatio-temporal dynamics of subaerial microalgal communities across scales is poorly understood despite their omnipresence, surprising morphological and molecular diversity, significant role in ecosystem functioning, and their bioindicating potential (Poikolainen *et al.* 1998; Freystein *et al.* 2008; Marmor & Degtjarenko 2014).

However, previous studies on subaerial microalgal communities have clarified some general patterns of their spatial distribution. On larger spatial scales (such as among regions), the geographic distance, correlated with the differences in temperature and humidity, seems to be the most important factor for the distribution of major microalgal taxa (Rindi & Guiry 2004; Neustupa & Štifterová 2013). On an intermediate scale (i.e., meters to tens of kilometers within a single region), the physicochemical factors related to different substrata, such as pH, nutrients, or water-holding capacity, seem to determine the community composition of the microalgal biofilms (Hoffmann 1989; Lukešová & Hoffmann 1996; Mataloni & Tell 2002; Freystein *et al.* 2008; Neustupa & Štifterová 2013; Kulichová *et al.* 2014; Marmor & Degtjarenko 2014; Nováková & Neustupa 2015; Štifterová & Neustupa 2015). Other important factors that correlate with microalgal distribution in subaerial microhabitats include local irradiance, sample orientation, or age of substrata (Hedenås *et al.* 2007; Neustupa & Škaloud 2008, 2010; Neustupa & Štifterová 2013). The observed prominence of the environmental factors on this scale, presumably coupled with frequent dispersal of microalgal propagules (Sharma *et al.* 2007), suggests the probable strong effects of deterministic processes, such as species sorting along the environmental gradients (Jones & McMahon 2009; Štifterová & Neustupa 2015). Previous studies that have shown considerably lower heterogeneity of biofilm community structure on this spatial scale were typically focused on a single type of a homogeneous substrate and, thus, the observed homogeneity of phototrophic microcommunities might have reflected the local homogeneity of environmental conditions (Mataloni *et al.* 2000; Cutler *et al.* 2013).

Very few studies concerning the small-scale (i.e., millimeters to centimeters) spatial variation of microalgal biofilms have been conducted, so far. They mostly concerned vertical distribution of microalgae in soil microbial communities (Garcia-Pichel *et al.* 2003; Hu *et al.* 2003). However, Grondin and Johansen (1993) studied the horizontal spatial heterogeneity of soil algal densities along the transect spanning 24 m with distances among samples 0.013, 0.03, 1, 12, and 24 m. They found significant variation of algal density among spots, but the coefficients of variation for each distance class were similar, which demonstrate that algal patchiness can be as significant on a scale less than 0.013 m as it is on a scale of 24 m.

In addition to the variation in space, the other key part of community dynamics is its variation in time. Inclusion of temporal effects (continuous time and seasonal fluctuations) into our consideration of community development and persistence gives us a better insight and more accurate estimates of the present diversity. A vast majority of studies dealing with temporal effects on subaerial phototrophic communities have been focused on the early stages of development of biological soil crusts in arid environments. Here, phototrophic crusts have often been considered as the initial, pioneer phase of the succession processes that is eventually replaced by lichen and moss covers (Langhans *et al.* 2009; Zhang *et al.* 2009). Similar patterns of temporal succession have also been observed in studies investigating recolonization of soil surface after disturbance events such as fires, cleaning of man-made substrata (Johansen *et al.* 1984; Hallmann *et al.* 2013), or primary succession after glacial retreat (Kaštovská *et al.* 2005).

Little is known about the temporal variation of 'climax' subaerial phototrophic microcommunities such as corticolous biofilms on tree bark or mature soil surface crusts in arid ecosystems. Previous studies were too short-term to evaluate the periodical seasonal fluctuations (Johansen & Rushforth 1985; Bowker *et al.* 2002); nevertheless, they illustrated certain changes in algal abundance over time (Arnold *et al.* 2003).

The present study concerns small-scale spatio-temporal variation of microalgal biofilms thriving on the surface of a tree bark. These surfaces represent essentially an omnipresent and stable substrate for subaerial biofilms. In regions with overall lower humidity levels, these microalgal covers might persist tens of years without being overgrown by epiphytic lichens and mosses due to the extremely high levels of desiccation stress. Thus, tree bark represents a suitable stable habitat for the investigation of spatio-temporal microscale heterogeneity of microalgae thriving in subaerial biofilms. We described the within-trunk community structure (in terms of species composition and relative abundances). Then, we evaluated the relative effects of deterministic (niche-based) and dispersion-related (distance–decay) processes on the community turnover. Moreover, we also tested for the effect of temporal factors on the community structure, and we compared these effects with spatial heterogeneity and microhabitat-related factors.

Specifically, we investigated the effects of both spatial and temporal structures of corticolous algal communities thriving on two adjacent European beech (*Fagus sylvatica*) trees in a temperate region. We aimed to answer the following major questions: Do the studied phototrophic microcommunities on tree bark vary in time, at all? If yes, can this temporal variation be explained rather by continuous succession in time, or does it relate to periodic seasonal fluctuations of the climatic factors? Is the observed temporal variation characterized by some essential shifts in species composition, or is it rather typical by shifts in relative abundances of species? Furthermore, is bark microhabitat (rough or smooth patches of bark; height on the trunk; and sample orientation) important for the community diversity and structure? Lastly, what is the proportional importance of temporal, deterministic (niche-based and microhabitat-related), and stochastic (dispersion-related) factors on the observed community structure?

MATERIALS AND METHODS

Sampling

Two adjacent European beech (*F. sylvatica*, Fagaceae) trees growing in a closed forest stand were chosen for our investigation. The two beech trees had straight trunks, were about 5 m apart, and – at a height of 120 cm – had a trunk circumference of 250 and 280 cm, respectively. Both trunks branched at a height of about 4 m from the ground. The sampling locality was located in a temperate mixed forest on the northeastern slope of Beech Hill in Czech Central Mts., Czech Republic (50°32′50″N, 13°54′11″E, altitude of 620 m above sea level). Irradiation of the understory habitats in temperate deciduous forests varies widely in relation to season, cloud cover and diurnal rhythms; however, the particular values of individual spots usually have less than 20% of the irradiation reaching the canopy layer (Canham *et al.* 1990; McCarthy & Robinson 2003).

The area is characterized by an average annual precipitation of about 600 mm and mean temperature of 8.0°C. The average monthly temperatures range from –2°C (January) to 20°C (July). January to March is the driest period of the year (average monthly precipitation does not exceed 40 mm). On the contrary, the highest average monthly precipitation is typically from May to August. Our sampling strategy, which aimed at evaluating seasonal periodicity of microalgae on the trunks, reflected these fluctuations in precipitation patterns.

The samples were collected six times each autumn and spring in a time span of 3 years (17 October 2010, 22 March 2011, 23 October 2011, 10 April 2012, 10 November 2012, and 4 May 2013). This sampling design was chosen, because we wanted to discern and assess the effects of two components of temporal variation, continuous temporal distance, and periodic seasonal fluctuations.

A total of 72 bark samples were examined, amounting to 12 samples collected at one time (six samples randomly taken from each tree). The samples, consisting of pieces of bark (1 cm^2 each), were taken from randomly chosen spots on the trunks, they were peeled off the trunk with a sterilized knife and immediately placed in sterile paper bags. The height of the samples on the trunks and the directional aspect (azimuth) of the sample were recorded. The exact spots for sampling on the trunk were chosen randomly – but with regard to the need of even representation of studied variables. The roughness of the bark surface was visually inspected and assigned into two categories: smooth (i.e., without any visible splitting) or rough (bark with fissures), see Fig. 1.

Cultivation and identification

The cultivation-based studies of the microalgal communities often suffer from selectiveness of the growth media, which could facilitate or restrain growth of certain species and, thus, affects the observed diversity. In the present study, we wanted to maximize the number of processed samples, as well as to acquire comparable data for further ecological interpretations. Therefore, we used the standard Bold Basal Medium (Andersen 2005), which has originally been designed for culturing terrestrial green microalgae (Bischoff & Bold 1963). This medium has also been proven to be suitable for culturing multiple subaerial coccoid and filamentous microalgae (e.g., Ettl & Gärtner 1995; Flechtner 1999; Lewis & Flechtner 2002; Freystein *et al.* 2008; Neustupa & Škaloud 2008, 2010; Štifterová & Neustupa 2015).

In the laboratory, the microalgal biofilm was scraped off the samples with a sterile stylus and placed into 1.5 mL Eppendorf tubes containing 0.5 mL liquid Bold Basal Medium (Bischoff & Bold 1963). Then, sterile glass beads (ø 0.5 mm) were added to the tube, which was shaken for

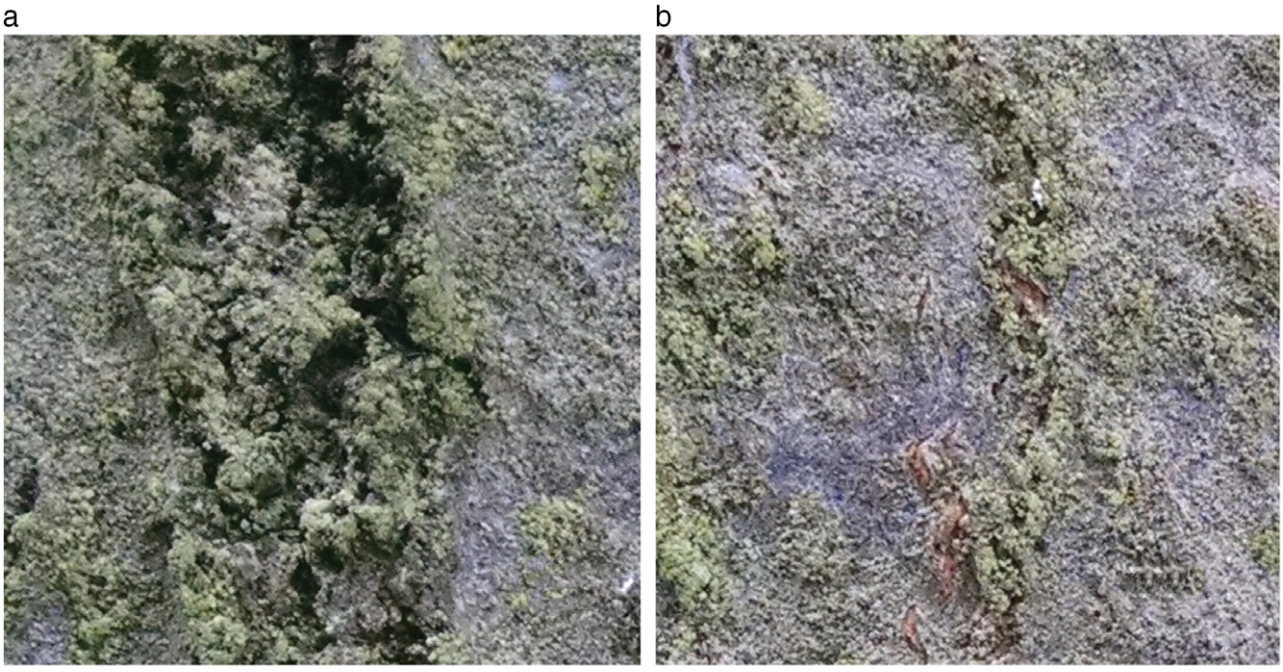

Fig. 1. Bark microhabitats. Rough (a) vs. smooth (b) portions of the bark surface.

15 s at 1200 rpm in a vortex mixer. The resulting suspension was inoculated onto two Petri dishes with agar-solidified BBM. After 3–5 weeks of cultivation (12-h L:D regime at 20°C and irradiance of ~60 μmol m^{-2} s), the plates were microscopically examined. Algal microcolonies were morphologically identified under an Olympus BX 31 microscope at a magnification of ×400–1000 at the lowest taxonomic level that could be unambiguously distinguished using relevant taxonomic references (Ettl & Gärtner 1995; Mikhailyuk *et al.* 2008; Darienko *et al.* 2010; Krienitz *et al.* 2011; Rindi *et al.* 2011; Guiry & Guiry 2016; Škaloud *et al.* 2016). In some cases, tentative identification at the species level was possible, but many taxa were only identified into genera or tentatively named morphotypes, which were used in subsequent analyses as operational taxonomic units (OTUs). The assessment of the semi-quantities of individual morphotypes in the samples was based on the assumption that each inspected microcolony originated from a single cell, colony, or a filament present in the original sample. In a given plate, after inspecting approximately 100 distinct colonies, the percentage proportions of each recognized morphotype were recorded. These values were then converted to a semi-quantitative, ordinal scale (0: absent; 1: 1–10%; 2: 11–40%; and 3: 41–100%).

Data analysis

Expected species accumulation curves for rarefied reference samples (i.e., sample-based rarefaction curves) with its unconditional confidence intervals were calculated in EstimateS 9 (Colwell *et al.* 2012; Colwell 2013). The 1st order jackknife total species richness estimator (suitable for binary data) was used for the approximation of the number of unseen species and was also calculated in EstimateS 9 (Colwell 2013).

We examined the patterns of sample α-diversity (i.e., sample species richness) and β-diversity (species turnover among samples, calculated as the Jaccard dissimilarity index) in relation to the studied factors—difference between two adjacent trees, i.e., beech 1 vs. beech 2; seasonal changes (spring vs. autumn sampling events); bark roughness (smooth vs. rough bark surface); directional aspect of sampling spots (radian data were transformed with cosine function to reflect north–south (cosine) aspect, Ager *et al.* 2003; Briggler & Prather 2003); and the height on trunks (within a range of 10–310 cm above the ground). Permutation Monte Carlo tests (a non-parametric equivalent to t statistics) were used for comparison of species richness among groups of samples. These tests were carried out in PAST, ver. 2.17c (Hammer *et al.* 2001). The matrix of Jaccard distances among the samples, computed by the function *vegdist* of the vegan package (Oksanen *et al.* 2011) in R, ver. 2.15.3 (R Development Core Team 2013), was used for the analysis of β-diversity values of communities. The β-diversity can be defined as the variability in species composition among sampling units for a given area. Significance of differences in variability of species composition (i.e., β-diversity based on Jaccard dissimilarities) among sampling units, e.g., among spring vs. autumn sampling events and smooth vs. rough bark, was tested by the permutation test for the homogeneity of multivariate dispersions (Anderson *et al.* 2006) in vegan package (Oksanen *et al.* 2011). The area-proportional Venn diagrams, which show proportions of unique or shared morphotypes, were drawn in venneuler R package (Wilkinson & Urbanek 2011).

Total variance, attributed to individual factors that influenced biofilm community composition, was partitioned using the redundancy analysis (RDA) on standardized semi-quantity dataset (i.e., on a dataset scaled to zero mean and unit variance by the function *decostand* of R). The variance partition was carried out by the function *varpart* of the vegan package (Oksanen *et al.* 2011) in R. The percentages of the variance explained by individual factors, including the bark roughness, season, time, and spatial distance, could thus be determined as the adjusted R^2 values acquired by the function *varpart*. Subsequent ANOVA-like permutation tests (function *cca.anova* in vegan package) were used to assess the significance of the RDA constraints. Following RDA, permutational analysis of variance (Per–MANOVA), implemented by the function *adonis* of the vegan package, was also used to further evaluate individual main factors as aspect, bark roughness, height and tree. Each factor was evaluated in a separate Per-MANOVA run after partialling-out the sums of squares of the remaining effects. Community structure was illustrated by non-metric MDS ordination analysis, implemented in PAST, ver. 2.17c (Hammer *et al.* 2001). The distance–decay relationships in the community compositional dissimilarity (measured by Jaccard indices among samples) were evaluated by Mantel tests (function *mantel* in vegan package). The indicator species analysis, implemented by function *multipatt* in indicspecies package (De Cáceres *et al.* 2010) of R, ver. 2.15.3, was used for the analysis of the seasonal and microhabitat (bark roughness) effects on the corticolous microalgal community composition. This analysis assessed the strength and statistical significance of the relationship between species abundances and groups of sites (Dufrêne & Legendre 1997; De Cáceres 2013).

RESULTS

Patterns of species diversity

The investigated beech trees harbored a diverse community of subaerial corticolous microalgae. In total, we successfully distinguished 55 unique microalgal morphotypes (see Appendix S1 in the Supporting Information for the list of species). Moreover, the unsaturated relationship between the number of processed samples (n = 72, i.e., 72 biofilm samples covering a total of 72 cm^2 of the bark surface) and the total species richness indicates that more OTUs would be uncovered with additional sampling efforts (Fig. 2). Similarly, the estimation of 70 present morphotypes (as provided by the first order Jackknife total species richness estimator) also suggests that the true diversity is even higher.

The majority of taxa (26) belonged to the green algal class Trebouxiophyceae, which is known to comprise most of the coccoid and filamentous algae capable to survive and grow in subaerial conditions, as well as a vast majority of lichen photobionts (Rindi *et al.* 2009). In addition, members of Chlorophyceae, Streptophyta, Xanthophyceae, Ulvophyceae, and Eustigmatophyceae were also recovered (Fig. S1 in the Supporting Information).

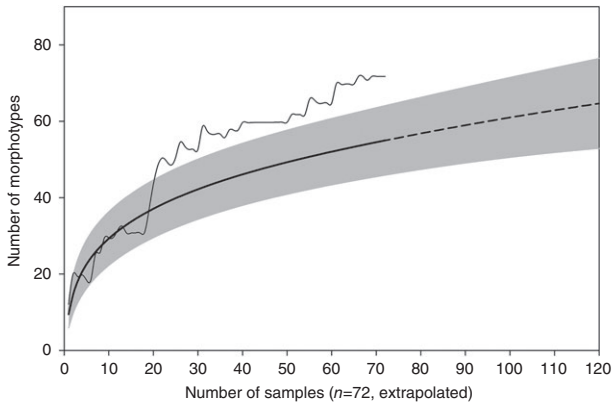

Fig. 2. Unsaturated relationship between the number of processed samples and the total species richness shown by extrapolated sample-based rarefaction curve and the first order jackknife total species richness estimator. The grey area represents 95% unconditional confidence interval of the rarefaction curve.

The local species richness, i.e., a number of OTUs on the bark area of 1 cm^2, ranged from two to 19 with a mean value of 9.5 morphotypes per sample. No differences were observed between both trees with respect to mean number of OTUs found in a single sample and species turnover among samples (Fig. 3a–c).

The season of sampling (spring or autumn) did not affect the number of found morphotypes. However, a significant effect of sampling season on the species turnover among samples (β-diversity) was detected (permutation test for homogeneity of multivariate dispersion $P = 0.002$). Species turnover among the spring samples was significantly higher than among the autumn samples, i.e., the autumn samples were significantly more similar to each other (Fig. 3d–f). Conversely, bark roughness considerably affected the α-diversity; the rough bark with distinct fissures harbored significantly richer communities than the smoother bark (permutation t-test $P = 0.0002$), but bark roughness did not affect the community β-diversity (Fig. 3g,h). Bark roughness also had a very pronounced effect on the number of unique morphotypes found on smooth vs. rough bark. On both types of bark surface, 35 morphotypes were found, but the smooth bark samples harbored only three unique morphotypes, whereas the rough bark samples included no less than 17 unique OTUs (Fig. 3i). Moreover, the height and azimuth of the sampling spots had no significant effect on their species richness (Fig. 4) and composition (see Figs. S2, S3 in the Supporting Information).

Community structure

The RDA and subsequent ANOVA-like permutation tests demonstrated that the effects of bark roughness, spatial

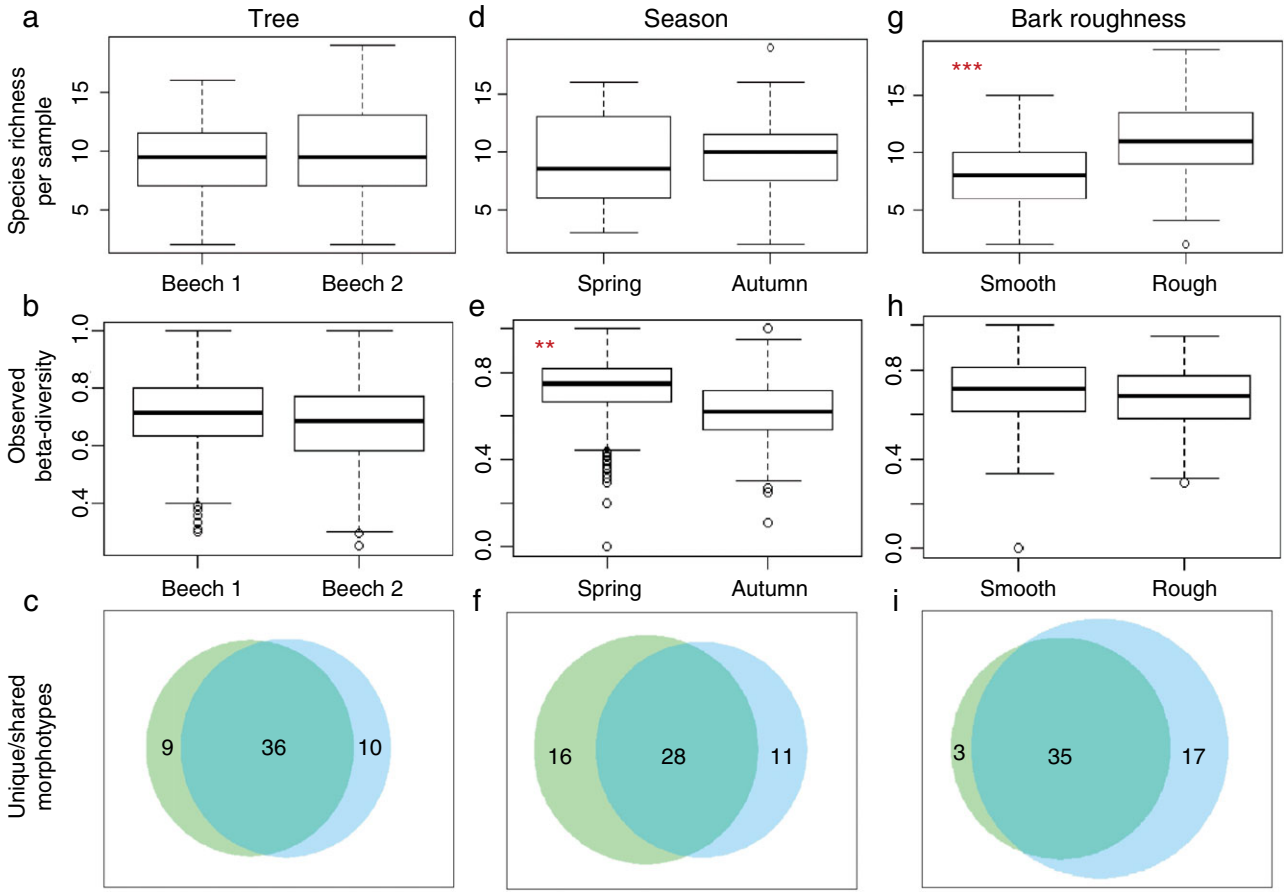

Fig. 3. The effects of tree (a–c), seasonality (d–f), and bark roughness (g–i) on the species diversity patterns.

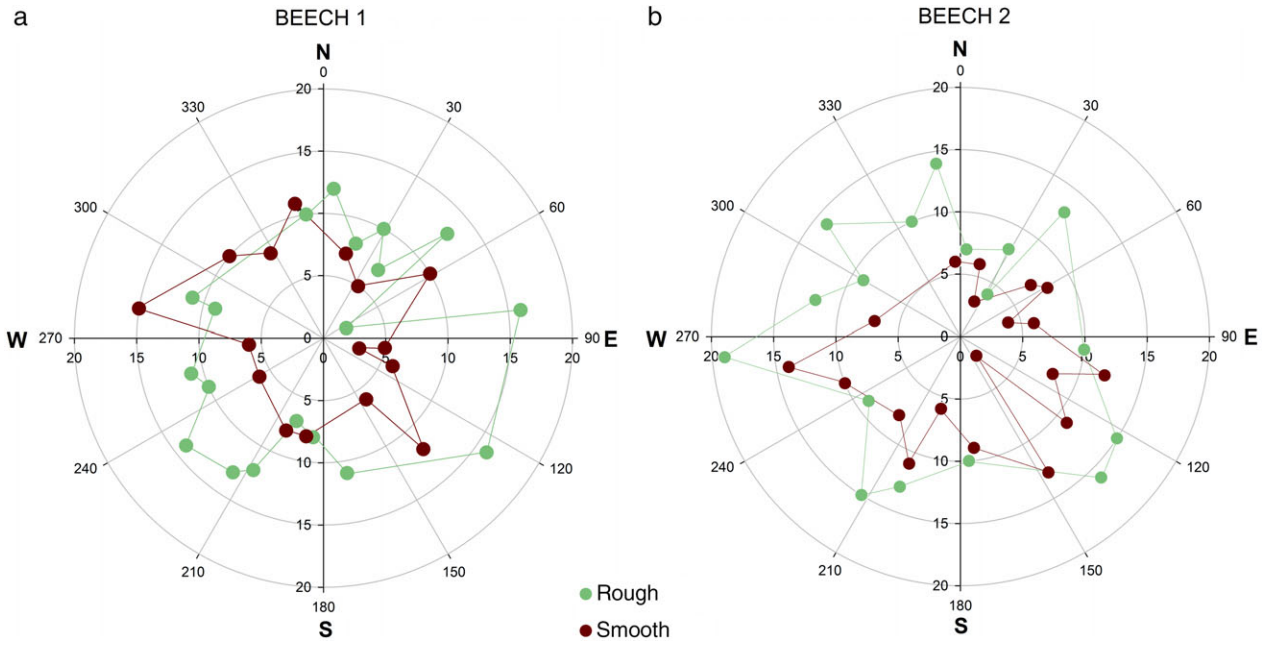

Fig. 4. The sample species richness in relation to directional aspect of sampling spots around the trunks (azimuth) and bark roughness for each studied tree (a,b). Note that the effect of bark roughness clearly exceeds that of directional aspect.

distance, season, and time significantly structured the observed microalgal species composition (Fig. 5). Among all samples, the effects of microhabitat (bark roughness) and season were more substantial than the effects of time and spatial distance. Bark roughness explained 2.5% (beech 1) and 2.2% (beech 2) of the variability in community species composition (Fig. 5). The effect of seasonality was even more pronounced as it explained greater amount of variability in community species composition—8.1% (beech 1) and 3% (beech 2), respectively. Permutational multivariate analyses of variance (Per–MANOVA) further demonstrated that the effect of bark roughness (controlled for the other effects of height, tree and aspect) is consistently important (Table 1). The non-metric MDS ordination diagrams also illustrated the studied effects (Fig. 6a–d). The important effects of the bark roughness and the season on the microalgal community structure can be seen although the group clusters were not separated—they overlapped only partially (Fig. 6b–c).

The distance–decay analyses, evaluating the effect of spatial distance among the sampling spots on similarity in their species composition, did not recover any clear patterns and demonstrated relative homogeneity of microalgal community composition within the studied spatial range of tens of centimeters to meters within a single trunk (Fig. 7). However, the trees differed in their spatial autocorrelation patterns as illustrated by the Mantel tests. While there was a significant relationship between the spatial distance of samples and their community dissimilarity on tree no. 2, species composition of the samples collected from tree no. 1 did not depend on their spatial distance. This pattern was congruent with the results of the variance partition based on the RDA (Fig. 5).

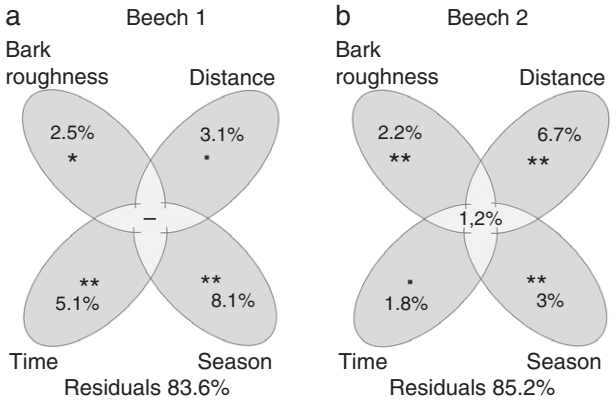

Fig. 5. Variance partition based on RDA. Proportions of variance resolved by bark roughness, distance, season, time, and their combinations for each tree (a, b).

Response of individual microalgal morphotypes

The multilevel indicator species analysis showed that *Interfilum terricolum*, *Diplosphaera chodatii*, and *Klebsormidium* sp. were significantly favored by the spring conditions (Fig. 8a), whereas higher abundance of *Symbiochloris irregularis*, *Apatococcus lobatus*, *Trebouxia* sp., and *Pseudococcomyxa simplex* was rather associated with the autumn season (Fig. 8b). The indicator species analysis also identified species preferring different microhabitats—smooth vs. rough bark surface (Fig. 9). *I. terricolum*, *P. simplex*, and *Stichococcus bacillaris* were significantly associated with the rough bark samples (Fig. 9). Finally, none of the microalgal morphotypes was significantly favored by the smooth bark surface microhabitat.

Table 1. Results of permutational multivariate analyses of variance (Per-MANOVA) evaluating the effects of individual factors on the community structure

Factor	Df	Sums of squares	Mean squares	F	R^2	*P*-value
Semi-quantities						
Aspect	1	148.0	148.01	1.46	0.01924	0.163
Bark roughness	1	379.4	379.43	3.77	0.04932	0.001***
Height	1	244.1	244.08	2.42	0.03173	0.017*
Tree	1	232.6	232.61	2.31	0.03024	0.021*
Presence/absence						
Aspect	1	0.3544	0.354 43	1.4585	0.02012	0.108
Bark roughness	1	0.4864	0.486 43	2.0017	0.02762	0.022*
Height	1	0.3618	0.361 78	1.4888	0.02054	0.108
Tree	1	0.3339	0.333 86	1.3739	0.01896	0.151

The Manhattan distance index (semi-quantities) and the Jaccard similarity index (presence/absence) were used. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

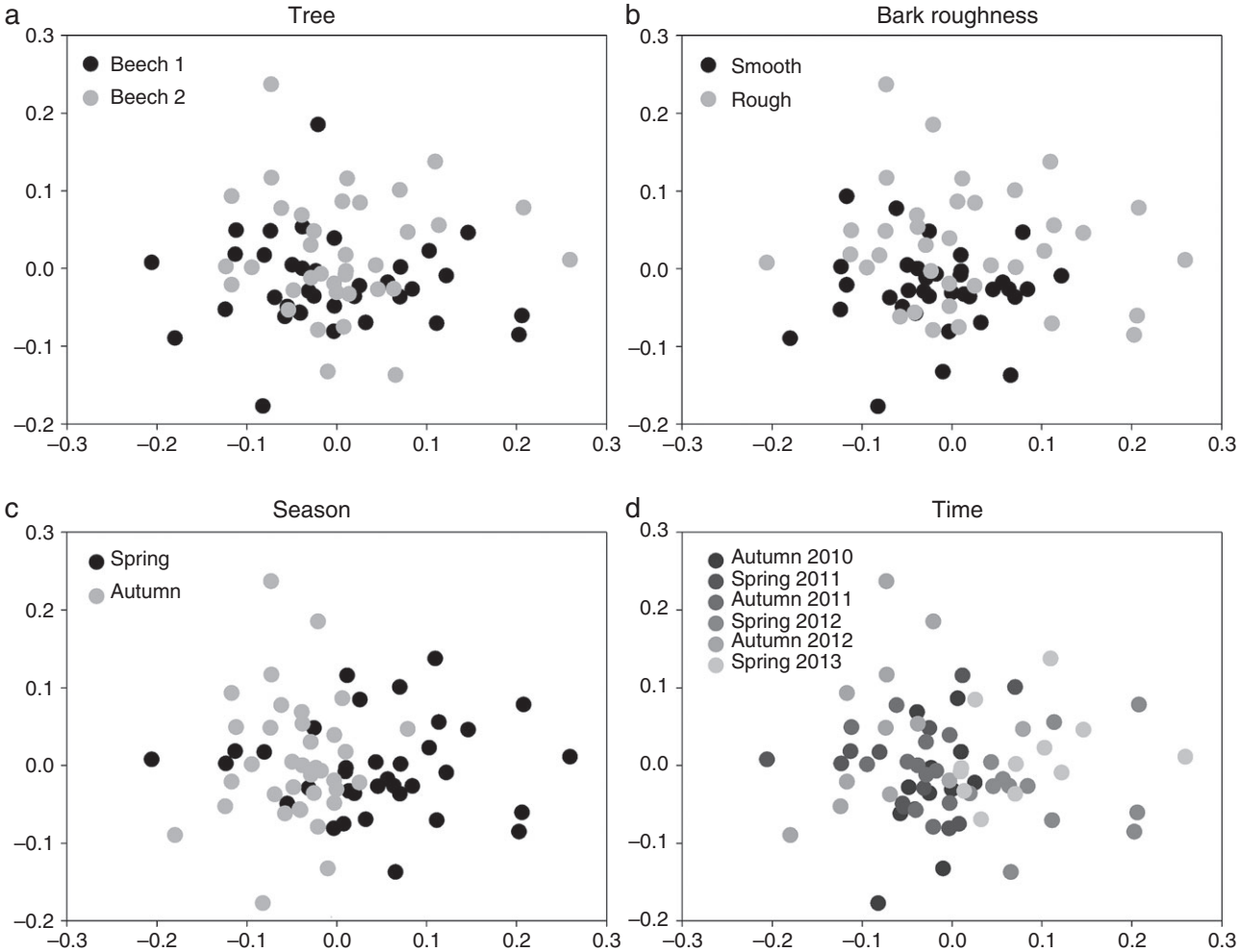

Fig. 6. Non-metric MDS ordination diagrams showing the differences in species composition of the samples with regard to individual factors (a – tree, b – bark roughness, c – season, d – time). Semi-quantity data matrix based on the Manhattan distances was used. 2D stress = 0.2915, 3D stress = 0.2477.

DISCUSSION

Species richness

Both beech trees, investigated in the present study, hosted rich microalgal corticolous communities. In one sample, i.e., on a piece of bark with an area of 1 cm^2, there were on average 9.5 microalgal morphotypes. In total, we recognized 55 unique morphotypes—we found 45 and 46 morphotypes on the studied European beech trees. Interestingly, these numbers were considerably higher than comparative data on species richness of corticolous microalgae in various forest ecosystems published by Nakano *et al.* (1991) and

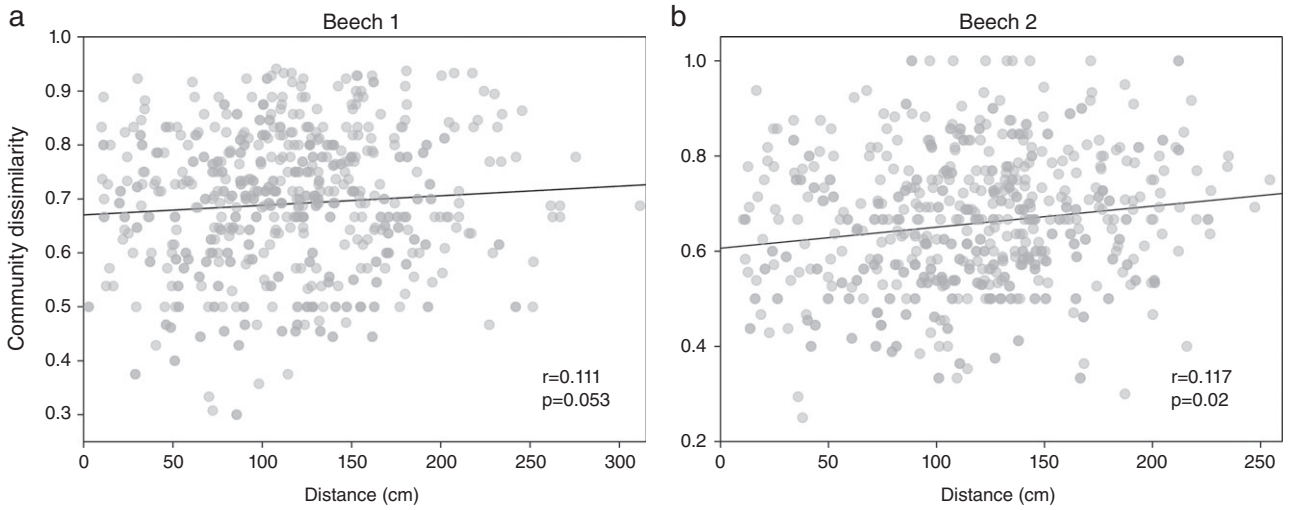

Fig. 7. Distance–decay in the community compositional dissimilarity (measured as Jaccard dissimilarity). Incidence data for beech 1 (a) and beech 2 (b), respectively. The Mantel test was used to test the strength and significance of the correlations.

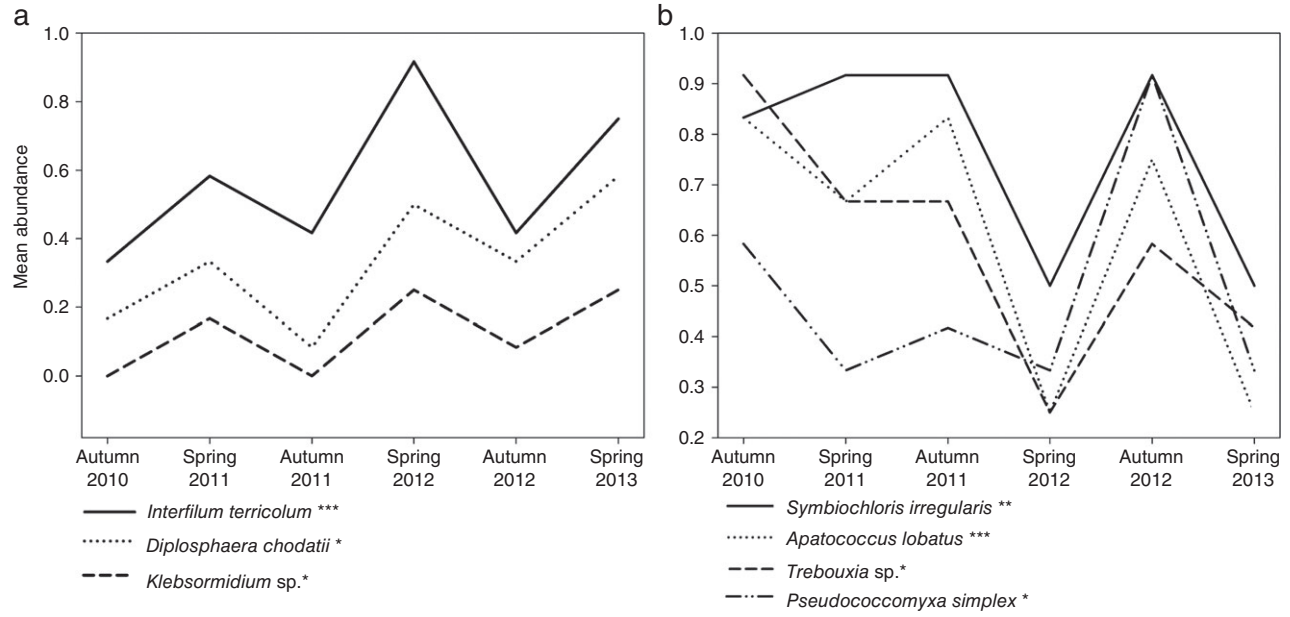

Fig. 8. Seasonal fluctuations in relative abundances of the microalgal morphotypes. OTUs significantly associated with spring (a) and autumn (b) seasons, respectively, are displayed.

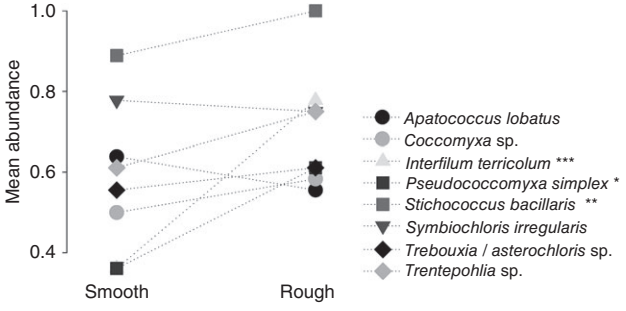

Fig. 9. Preference of microhabitat. The most abundant morphotypes (abundance in at least one group >0.5) are displayed.

Neustupa and Škaloud (2008, 2010). These researchers, using similar methods of cultivation and identification of strains, recovered on average 4.8–8.9 morphotypes on a single tree. However, this difference may have primarily been caused by different designs of the present study. In contrast to the above-mentioned previous studies, we sampled the corticolous microcommunity on a smaller scale with relatively high number of individual samples and in different seasons. All this probably resulted in relatively higher detail of our data than those of the previous studies.

Microhabitat—bark roughness, height, and aspect

The microhabitat type, evaluated as bark roughness, proved to be an important factor that affected both the species richness

of the morphotypes in the samples, as well as the community structure. Species richness was significantly higher in spots with a distinctly cracked, rough bark surface than in the samples collected from spots with a rather smooth bark. In addition, we found 17 morphospecies that occurred exclusively in the samples collected from spots with a rough bark, but only two limited to samples from smooth bark spots. Bark roughness determines variation in humidity of different bark microhabitats. In general, bark fissures have higher retention of the stemflow, and they have higher water-holding capacity than smooth surfaces do (Valová & Bieleszová 2008; Büdel 2011; Ellis 2012). Water availability and substrate humidity have been considered as key factors for successful colonization, growth, and photosynthetic activity of the subaerial microhabitats by algae (Hoffmann 1989; Karsten *et al.* 2014; Lamit *et al.* 2015). Therefore, the higher diversity of the rough bark samples, observed in the present study, may probably also be explained by higher mean humidity of these microhabitats.

In addition to higher water availability, bark fissures probably also differ from the smooth surface in their physicochemical conditions, related to different tree tissues exposed to the microalgal growth (Whitmore 1962). Fritz *et al.* (2009) showed, that in the case of beech trees, pH values of outer and inner bark portions does not differ (in contrast to conifers, where the fissures have significantly higher pH values than smooth portions of bark, Legrand *et al.* 1996; Satake *et al.* 1996; Kuang *et al.* 2006). Still, the rough microhabitats possess unique characteristics – bark fissures are considerably shadier, retains snow and dust and are more sheltered against wind (Barkman 1958).

Previous studies investigating the within-trunk structure of the corticolous communities of bryophytes, lichens, and algae have repeatedly illustrated the significant effects of two main gradients, i.e., height of the sample on the trunk and its orientation, on the community structure McCune *et al.* 2000; Ellis 2012; Neustupa & Štifterová 2013). Despite the relatively short height gradient of our samples (10–310 cm above the ground), we could demonstrate its significant effect on species structure of the observed communities. However, the effect of height was limited to the dataset based on the semi-quantitative estimates of the species abundances; it was not significant in the dataset with the presence/absence data (Table 1). This means that the height of the samples on the trunks did not primarily restrict the occurrence of individual species but rather their ability to establish abundant populations at a particular microsite on a trunk. This pattern was probably caused by diverse autecological preferences of the species. Nutrients are usually more available in lower portions of the trunks (Ellis 2012) and, thus, this may have limited or facilitated the occurrence of particular species in relation to this factor. However, several studies have also pointed out that the bark-dwelling invertebrates, including the non-selective algae-grazers such as gastropods, oribatid mites, and springtails, are extremely sensitive to height, and their herbivory affects vertical distribution of the corticolous epiphytes (André 1985; Asplund *et al.* 2010).

Conversely, the environmental gradient related to aspect has not significantly influenced the studied communities. Despite frequent observations of conspicuous quantitative patterns such as the more extensive biofilm cover on the more humid and shadowed northern sides of the trunks (Barkman 1958), we did not find any relation of this factor with species richness or community structure. However, it should be noted that both studied trees were positioned in a relatively homogeneous forest stand with generally humid microclimate, which could significantly limit the effect of the sample orientation on the trunks. Our previous study (Neustupa & Štifterová 2013), also focused on this effect, was conducted in considerably more diverse environmental conditions and on a larger continental macroscale. That study illustrated that the aspect, correlated with higher irradiation and lower humidity of south facing microhabitats on the trunks, affected the distribution of coccoid trebouxiophycean microalgae, especially on solitary trees with high potential irradiance of the trunks.

Effect of seasonality

The sampling season proved to be an important factor that affected the biofilms. For instance, the spring samples were generally more variable, i.e., their species turnover was significantly higher. Interestingly, this variation could not be explained by increased spatial autocorrelation (the Mantel tests did not yield any significant autocorrelation patterns, see Appendix S2 in the Supporting Information). Thus, it was rather a random variability of species composition among the samples with no regard to their spatial distance on the trunks. We propose that this increase in β-diversity of spring samples may be explained by preceding winter disturbance. Environmental stress, such as freezing periods, coupled with desiccation of the bark surface may have caused local extinctions of individual taxa on the trunks. Consequently, higher variability of the spring samples could then be related to rapid differential recolonization of individual spots on the trunks facilitated by the onset of the vegetative season. Similar patterns of increased β-diversity of spring samples in localities with pronounced seasonal climate have also been observed in freshwater microphytobenthos (Neustupa *et al.* 2012). In the course of the vegetative season, the β-diversity declined possibly as a result of homogenizing the biofilm composition due to the increased activity of bark-dwelling invertebrates (André 1985; Majer *et al.* 2003; Prinzing 2005; Erdmann *et al.* 2007). Thus, as a result of the transfer of algal propagules (cells) attached to their bodies, the species turnover among samples was reduced.

Seasonal variation also proved to be one of the key factor explaining patterns of the community structure of the biofilms. The abundance of some important species forming the biofilm communities apparently fluctuated in relation to the season. Several taxa, such as *I. terricolum*, *D. chodatii*, and *Klebsormidium* sp., were significantly more abundant in spring samples. Conversely, *S. irregularis*, *A. lobatus*, *Trebouxia* sp., and *P. simplex* typically dominated the autumn samples. Thus, it seems that autecological features of different common microalgae, which occur in corticolous communities, may be contrasting in relation to seasonal fluctuations. This may explain relatively high local diversity of these assemblages. Individual taxa prefer different meteorological conditions, which mean that they are able to form abundant populations in their favorable seasonal time span. Owing to high population numbers, they are then able to survive the adverse periods, which are, however, favorable to other members of the biofilm community (Chesson *et al.* 2004). These

findings generally concur with the recent findings by Beck *et al.* (2014), who described seasonal fluctuations of microfungal corticolous communities. In total, it seems that epiphytic microcommunities on perennial substrates, such as tree bark, have rather substantial seasonal variability. Despite their typical physiognomic uniformity and omnipresence, their variability and species structure are sensitive to seasonal fluctuations in abiotic conditions, which are in a way similar to their benthic and planktonic counterparts.

Temporal and spatial distances

The analyses of spatial and temporal effects on the community structure did not yield unequivocal results. Both effects significantly explained the variation in species composition of microalgae on one tree, but it was marginally insignificant in the other one. Despite the weakness of the results, they indicate the possible importance of community forming processes interconnected to temporal (i.e., temporal succession independent of the effects of seasonality) and spatial distances.

Spatial autocorrelation is an intrinsic property of many geographic and ecological systems on different scales (Legendre 1993; Shurin *et al.* 2009). In general, poor dispersers are expected to show more clumped distributions or greater spatial autocorrelation in community composition than good dispersers (Shurin *et al.* 2009). Body size, relative motility, and local abundance have been considered as key factors affecting the dispersal ability of individual taxa (Hillebrand *et al.* 2001; Finlay 2002). Subaerial microalgae apparently form relatively more abundant local populations than other eukaryotes. In addition, their bodies, i.e., the cells, filaments, or cellular clumps, are relatively very small, and they commonly occur in aerial plankton. Aerial transport of aerosolized microalgal cells, colonies, or filaments is thought to be the most effective means of their dispersal (Sharma *et al.* 2007). Extensive dispersion of the propagules should lead to incessant homogenization of the species pool of potential new colonisers (Sharma & Singh 2010). Differences in actual species composition of individual spots are then determined by species-sorting processes related to niche variability of the taxa in relation to substrate characteristics, such as water retention. The results of our distance–decay analyses, as well as weak spatial autocorrelation, showed that on a scale of tens of centimeters, the biofilms were not spatially clustered. In general, this concurred with the paradigm of species-sorting responding to microstructural heterogeneity as a major pattern structuring the corticolous microcommunities.

It has to be mentioned that our analysis of community structure (RDA, Fig. 5) identified significant drivers of the community structure, but the residual variabilities were relatively high. This unexplained variability could have been caused by other, not-measured biotic and abiotic factors (such as local differences of irradiance of individual sampled spots, microscale fluctuations in nutrient availability, or different levels of microalgal herbivory by invertebrates). However, a considerable part of this variability might have also been the result of neutral, random variability of species composition of the samples. This neutrality could have been accentuated by the methodology used in the study, which has been based on cultivation of the biofilms. For example, on Petri dishes we might have recorded also the taxa, which were only present as inactive propagules (cells) in the natural habitats. These inactive cells arrived to the sampling spots by aerial dispersion, but they did not form a part of the actively growing populations within a particular community.

CONCLUSIONS

The results of our study do not contradict the application of subaerial phototrophic biofilms in biomonitoring of anthropogenic pollution (Poikolainen *et al.* 1998; Freystein *et al.* 2008; Marmor & Degtjarenko 2014; Nováková & Neustupa 2015). However, our findings suggest that such surveys should also take into account the microscale variation caused by microstructural heterogeneity of the substrate, as well as spatio-temporal dynamics of the communities, reflecting the seasonal changes and non-periodic development related to probably relatively high rates of dispersal and subsequent colonization of the substrate by newly arriving taxa.

Our results showed that microalgae in corticolous subaerial biofilms growing on the trunk of a single beech tree form structurally diverse and variable communities. Despite relative homogeneity and small spatial extent of the substrate, we detected significant differences reflecting the microstructural differences in bark surface, probably related to variation in humidity of individual spots. In addition, we observed that the communities changed in time, both in relation to seasonal changes in temperature and humidity, as well as a result of non-periodic dynamics.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. List of identified species.

Appendix S2. Results of Mantel tests.

Fig. S1. Number of OTUs from individual major microalgal groups.

Fig. S2. Effect of aspect on sample species richness and β-diversity.

Fig. S3. Effect of height on sample species richness and β-diversity.