Distribution patterns of subaerial corticolous microalgae in two European regions

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Background and aims – Subaerial phototrophic biofilms growing on tree bark represent one of the least-known micro-algal communities. Ecological distribution patterns of major micro-algal groups thriving in corticolous microhabitats on eleven host tree species in sub-Mediterranean and temperate European localities were investigated in the present study.

Methods – In total, 169 samples of corticolous biofilms were investigated by direct light microscopy. Microalgae were identified to the lowest putatively monophyletic groups that could be distinguished unambiguously. Among other abiotic factors, the pH of the bark surface was measured using a flathead electrode, and the proportion of open sky at individual sites was evaluated by image analysis of fisheye circular photographs.

Key results – The distribution of the putatively trebouxiophycean coccoid green algae was mostly related to microscale factors, such as sample orientation on trunks. Conversely, the distribution of Trentepohliales and Cyanobacteria was related mostly to bark pH values and to regional differences between temperate and sub-Mediterranean localities. The distribution of some streptophytan taxa, such as *Mesotaenium* or *Spirotaenia*, was closely related to particular host tree species.

Conclusions – Individual major groups of corticolous algae and Cyanobacteria in European ecosystems have distinctly different ecological strategies in relation to important abiotic factors.

Key words – Biodiversity, community ecology, corticolous biofilms, Cyanobacteria, micro-algal ecology, Streptophyta, subaerial algae, Trebouxiophyceae, Trentepohliales.

INTRODUCTION

Subaerial micro-algal biofilms growing on tree bark are omnipresent in a variety of ecosystems. However, terrestrial microhabitats have traditionally attracted far less attention from phycologists than marine and freshwater habitats, so their diversity and community structure is still very poorly known (Freystein & Reisser 2010, Rindi et al. 2010). Because of the lack of suitable discriminating characters and because species concepts are poor, species-level microscopic identification of most subaerial algae and Cyanobacteria is complicated (Ettl & Gärtner 1995). In comparison to that of planktic and benthic communities, the morphological diversity of terrestrial microalgae is strikingly low; most taxa have coccoid spherical to oval cells, or simple filamentous thalli (Hoffmann 1989, Ettl & Gärtner 1995). Consequently, most microscopic studies of subaerial assemblages have been based on either limited datasets acquired from approximate morphological identification of cultured strains (e.g. Nakano et al. 1991, Neustupa & Škaloud 2008), or direct observations of natural samples. Research on natural samples has

typically been concentrated on several conspicuous groups, such as Trentepohliales, *Klebsormidium*, and *Prasiola* (e.g. Rindi & Guiry 2004, Hedenås et al. 2007). Recent molecular studies of subaerial microalgae have mostly been focused on phylogenetics and taxonomy of new and little-known micro-algal and cyanobacterial lineages thriving in these microhabitats (Rindi et al. 2006, Zhang et al. 2008, Neustupa et al. 2013). They illustrated that the real phylogenetic diversity of subaerial microalgae was probably grossly underestimated by traditional morphological taxonomy. Molecular data revealed that the micro-algal communities of these habitats include numerous as yet unknown phylogenetic taxa. Some have been described as new micro-algal genera, such as *Spongiochrysis*, *Heveochlorella*, *Hylodesmus*, and *Leptochlorella* (Rindi et al. 2006, Zhang et al. 2008, Eliáš et al. 2010, Neustupa et al. 2013).

Despite methodological limitations, microscopy still provides the very core of current knowledge on ecology and distribution patterns of subaerial algae, including those in corticolous biofilms. Tropical corticolous biofilms are often

dominated by filamentous Trentepohliales, whereas bark microhabitats in temperate ecosystems are more typically dominated by coccoid green algae, mostly belonging to the Trebouxiophyceae (Printz 1939, Freystein & Reisser 2010, Rindi et al. 2010). However, corticolous trentepohliacean growths may also occur in temperate and boreal forests, and may even locally dominate the microbial phototrophic community (López-Bautista et al. 2002, Hedenås et al. 2007). Marini et al. (2011) showed that *Trentepohlia*-containing lichens are more abundant with increasing mean temperature accross Italy, and a similar pattern may occur in the free-living Trentepohliales. Conversely, the abundance of coccoid Trebouxiophyceae in lichens is related to the regional proportion of high forests, indicating their possible large-scale affinity to relatively shaded and humid conditions (Marini et al. 2011). In addition, Lüttge & Büdel (2010) illustrated that temperate trentepohliacean assemblages differed considerably from trebouxiophycean biofilms in their lower ability to recover from long-term desiccation. The abundance of corticolous coccoid green algal assemblages in Finnish boreal forests is positively correlated with atmospheric nitrogen deposition (Poikolainen et al. 1998). Likewise, coccoid green algae are more abundant on the needles of conifers in relatively more polluted areas of Sweden (Grandin 2011), and Freystein et al. (2008) identified potentially pollution-tolerant species of corticolous green algae, such as *Klebsormidium* and *Diplosphaera*, in urban areas of Leipzig, Germany.

Though large-scale variation in abundance has been studied, considerably fewer data are available on variation in community structure and abundance of corticolous microalgae on smaller scales, such as between different trees in a single locality, or on a single host tree. Neustupa & Škaloud (2008) illustrated pronounced differences in species composition between assemblages on bark samples from extremely shaded undergrowth in a tropical forest and those on bark from adjacent less-shaded synanthropic habitats. Likewise, Hedenås et al. (2007) found significantly more trentepohliacean algae on tree bark in a shaded, old-growth boreal forest than in more illuminated and less humid clear-cut areas. The other major components of corticolous micro-algal assemblages found in their study area, Nostocales and coccoid Trebouxiophyceae, were affected by microscale variation and were typically most abundant on the northern sides of trees. However, this effect was much more pronounced in clear-cut areas than in the old-growth forest, where the biomass of all the major groups was generally high (Hedenås et al. 2007). Small-scale variation in corticolous and epixylic biofilms was also illustrated by Neustupa & Škaloud (2010),who reported considerable differences in the species composition of micro-algal assemblages growing on living trees and decaying wood in the tropical forest of Singapore. Several trebouxiophycean species, such as *Dictyochloropsis* spp. and *Pseudomarvaniaaerophytica* (Neustupa & Sejnohová) Eliáš & Neustupa, strongly preferred bark substrate over the adjacent bare wood microhabitats. Likewise, small-scale microhabitat preferences were reported for the subaerial species of the genus *Prasiola* (Rindi & Guiry 2004). This genus is found in strongly eutrophic subaerial microhabitats of cold temperate and boreal ecosystems, such as at the bases

of urban walls or tree trunks (Knebel 1935, Rindi & Guiry 2004).

The pH of the bark surface has been considered one of the most important factors affecting the community structure of corticolous organisms. The relationship between pH and community structure is well known for epiphytic lichens and bryophytes (e.g. Marmor & Randlane 2007, Fritz et al. 2009) and myxomycetes (Scarborough et al. 2009). The host tree species is often taken as a proxy for the bark pH, but several studies have shown that the actual pH of bark samples may vary considerably between different individuals of the same tree species (Reisner & Ots 2002, Marmor & Randlane 2007). Spier et al. (2010) reported that the host tree species may be more closely correlated with the community structure of corticolous epiphytic lichens than the actual pH of the bark samples. To the best of our knowledge, the effects of pH on corticolous algae have not been tested for.

Therefore, in the present study, we evaluated the relationship between bark pH and abundance of major algal groups in corticolous biofilms from two European regions. To account for variation between individual host tree species, we did not rely on published records of characteristic bark pH of individual taxa but measured the actual pH of individual samples using a flathead electrode. Following the modified protocol of Hedenås et al. (2007), we carried out direct microscopy of individual biofilm samples rather than first cultivating them on agar plates. This approach allowed us to analyse a relatively high number of samples, but our taxonomic resolution was inevitably limited to major lineages and to several conspicuous and well-delimited genera. We chose two regions, the western parts of the Czech Republic (Bohemia) and the coastal areas surrounding the north-eastern Adriatic Sea (Italy, Slovenia, Croatia), to emphasize the differences between the temperate and the sub-Mediterranean climate that are reflected in significant differences in the mean temperature and precipitation pattern. In total, we investigated six autochthonous host tree species in Istria and five in Bohemia. To account for small-scale variation in the abundance of individual algal groups, the samples were taken at different heights from the ground from the northern and southern sides of trees. The effects of region on microalgae community structure were taken as a proxy for climatic factors, such as mean temperature and precipitation. Likewise, variation at the mesoscale level, represented by the individual host taxa within a region, accounted for the effects of abiotic factors varying primarily at the level of individual tree species, such as pH and bark roughness. Variation at the microscale level of individual trees primarily reflected local factors, such as the sample height and orientation. A random similarity structure of samples would indicate that purely neutral factors, such as small-scale dispersal, colonization or local extinctions, structured micro-algal assemblages, rather than environmental and spatial factors.

MATERIAL AND METHODS

Sampling

In total, 169 samples were collected in April to October 2012 from the bark of eleven tree species in two Euro-

pean regions, Central Bohemia (Czech Republic) and the north-eastern Adriatic coastal region of Istria (Italy, Slovenia, Croatia) (electronic appendix 1A), which belong to different phytogeographical and climatic regions. Central Bohemia has typically temperate climatic conditions (annual mean temperature: 7–9°C, precipitation: 450–650 mm). Conversely, Istria belongs to the sub-Mediterranean region with an annual mean temperature of 12–14°C and precipitation of 850–1100 mm. The Istrian samples were taken from *Arbutus unedo* L. (Ericaceae), *Cupressus sempervirens* L., *Juniperus oxycedrus* L. (Cupressaceae), *Pinus nigra* Arnold (Pinaceae), *Quercus ilex* L. and *Q. pubescens* Willd. (Fagaceae); the Central Bohemian samples from *Picea abies* (L.) H.Karst., *Pinus sylvestris* L. (Pinaceae), *Populus tremula* L. (Salicaceae), *Quercus robur* L. (Fagaceae) and *Tilia cordata* Mill. (Tiliaceae). The samples were taken randomly from the northern and/or southern ($\pm$ 10°) sides of trees. Each sample consisted of 3 cm^2 of bark that was placed in a sterile bag. Microhabitats covered by lichens were avoided, and remaining sporadic isolated lichen thalli were carefully removed prior to further analysis of samples. The open sky proportion (OSP), as a proxy for the illumination of samples, was quantified by image analysis of circular photographs taken at individual sampling points (Canon EOS 1100D camera; 8 mm fisheye lens). The images were analysed with Gap Light Analyzer, ver. 2.0 (Frazer et al. 1999).

The pH of the bark surface was measured in the laboratory by using the WTW pH-330 set with a flathead electrode (SenTix Sur). In total, 0.5 ml of a 0.1 M solution of KCl in water was dripped on individual pieces of epiphyte-free bark surface 60 seconds prior to measurement (Marmor & Randlane 2007, Rambo 2010). Bark roughness was visually estimated in three ordinal categories (1: smooth, 2: moderately coarse, 3: coarse). They mostly reflected the interspecific differences among the tree species, but, in some cases, considerable infraspecific variation in bark roughness among young and old specimens of the same tree species (such as *Tilia cordata*) resulted in their different estimated bark roughness values. The trunk diameter, the height of individual samples and their orientation were also recorded.

Microscopy and identification

The biofilm at the surface of each sample was scrapped into the 1.5 ml Eppendorf tube and shaken at 1500 rev/min for 20 sec with 0.8 ml of liquid Bold Basal Medium and approximately 0.5 cm^3 of sterile glass beads (diameter: 0.75 mm). Then, 40 µl of the suspension of algal cells from the Eppendorf tube was observed at 1000× magnification under an Olympus BX 51 light microscope. In total, two microscope slides were prepared from each Eppendorf tube and the extraction procedure was repeated three times in three separate Eppendorf tubes for each sample. Consequently, for each sample, six slides were inspected. The abundance of each algal group observed on each slide was quantified as 1 (fewer than ten cells observed) or 2. Thus, the maximum possible abundance value for any group in a particular sample was 12, indicating a taxon that was present on all six slides and always observed as more than ten cells.

In general, the microalgae in samples were identified to the lowest putatively monophyletic groups that could be distinguished unambiguously by light microscopy. In some cases, individual traditional genera, such as *Mesotaenium* and *Spirotaenia* in Streptophyta, or *Scytonema* in Cyanobacteria, could be discerned, but the monophyly of the observed populations from different samples could not be unambiguously confirmed. Therefore, two parallel datasets were evaluated. The first included all these clear-cut morphological genera (electronic appendix 1B); the second included merged data for five major taxonomic groups: Cyanobacteria, Bacillariophyceae, Trentepohliales, Streptophyta and Trebouxiophyceae (electronic appendix 1C). The latter lineage mostly consisted of morphologically uniform coccoid green algae. Most of these corticolous taxa are known to belong to the Trebouxiophyceae (Ettl & Gärtner 1995, Rindi et al. 2010), but several morphologically very similar taxa from a sister lineage of Chlorophyceae have occasionally been reported from subaerial corticolous microhabitats (Němcová et al. 2011, Hodač et al. 2012). However, the vast majority of the observed specimens are highly likely to belong to the Trebouxiophyceae, so this group was tentatively called trebouxiophytes.

Data analysis

The effects of different abiotic factors on the measured pH values were evaluated by permutational multivariate analysis of variance (Per-MANOVA), implemented by the function *adonis* of the *vegan* package (Oksanen et al. 2011) in R, ver. 2.13.1 (R Development Core Team 2011). Variation in the dependent variable (pH of samples) was fitted to one or several independent variables, such as host species, orientation, and log transformed values, for height, trunk diameter, OSP and bark roughness. The Per-MANOVA tests were also used to evaluate the effect of individual spatial levels (region, host species, tree) on the community structure of the biofilms. Due to the nested structure of the data, the permutation tests evaluating effects of individual spatial levels were constrained such that randomizations occurred only within respective higher-order levels (i.e. samples were randomized within regions in tests evaluating effects of host species). Community structure was illustrated by principal component analysis in PAST, ver. 2.15 (Hammer et al. 2001).

Relationships between abiotic factors and the abundance of individual major lineages were illustrated by means of linear correlation analyses and partial linear correlation analyses. Finally, a set of multiple regression (MR) analyses was used to evaluate relationships between microalgae abundance data and abiotic factors in both regions. Optimal models for the MR analyses were chosen on the basis of Akaike's information criterion (AIC), using the *stepAIC* function of the MASS package in R, ver. 2.13.1 (Venables & Ripley 2002). The abiotic factors were standardized to zero mean and unit variance. A forward stepwise search for the optimal model, avoiding collinearity between closely related variables, was used (Burnham & Anderson 2004). MRs were conducted for each of the four major groups (Cyanobacteria, Streptophyta, Trentepohliales, Trebouxiophyceae). Diatoms were not included because they were only sporadically recorded in less

than 10% of the samples and Cyanobacteria were missing in the Bohemian samples. For each algal group and region, three MR models were constructed. Firstly, abiotic factors were evaluated with no consideration of spatial scale. Then, two MR models were constructed, in each of which an individual spatial level (host species, tree) was taken as a covariate that was partialled-out prior to the analysis.

RESULTS

The bark surface pH of samples taken from different host tree species differed significantly (F = 39.5, R^2 = 0.71, p < 0.001). The bark of most gymnosperm taxa, such as *Pinus nigra* and *Juniperus oxycedrus* among Istrian trees, or *Pinus sylvestris* and *Picea abies* among Central Bohemian taxa, was strongly acidic (fig. 1). Conversely, the angiosperm trees, such as *Quercus pubescens* and *Populus tremula*, had higher bark pH values. After accounting for the tree species, other factors, such as sample height, bark roughness, trunk diameter, OSP and sample orientation, had no significant effects on the pH values.

The microalgae detected in the samples of corticolous biofilms belonged to five major taxonomic groups (electronic appendix 1). Coccoid green algae, putatively belonging to Trebouxiophyceae, were dominant in almost all samples. Trentepohliales, streptophytan coccoid and filamentous green algae, Cyanobacteria and diatoms were also recorded. In three Istrian samples, Cyanobacteria were represented by the genus *Oscillatoria* (fig. 2A), but in most other cases, Nostocales were the typical cyanobacterial members of the corticolous assemblages (fig. 2B). Diatoms were only rarely detected. In total, five taxa were observed, *Pinnularia* cf. *borealis* Ehrenb., *Synedra capitata* Ehrenb.
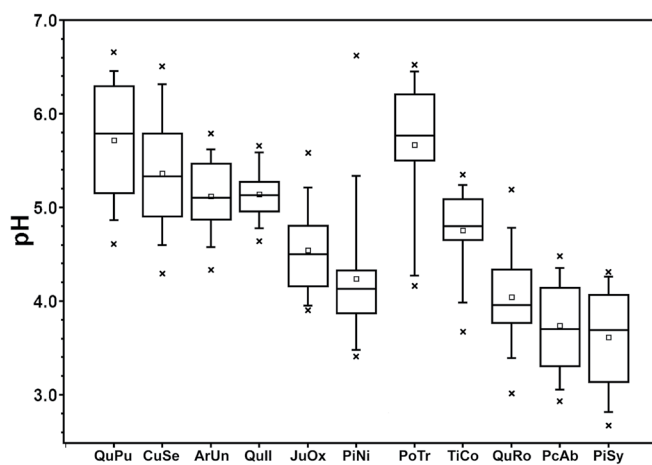
Figure 1 – Bark pH values of the trees sampled for corticolous algae in Istria: QuPu, *Quercus pubescens*; CuSe, *Cupressus semprevirens*; ArUn, *Arbutus unedo*; QuIl, *Quercus ilex*; JuOx, *Juniperus oxycedrus*; PiNi, *Pinus nigra*; and in Bohemia: PoTr, *Populus tremula*; TiCo, *Tilia cordata*; QuRo, *Quercus robur*; PcAb, *Picea abies*; PiSy, *Pinus sylvestris*. In individual box-whisker plots, the horizontal line indicates the median, the top and bottom of the box indicate the upper and lower quartiles, the whiskers indicate the 9th and the 91st percentile, and the crosses indicate the range. The small square indicates the mean pH for each tree.

(fig. 2C), *Hantzschia* sp., *Brachysira serians* (Bréb.) Round & D.G.Mann, and *Orthoseira* cf. *roeseana* (Rabenh.) Pfitzer (fig. 2D), and in all cases, among thousands of cells belonging to other algal groups, only one diatom cell *per* sample was observed. This indicated that diatoms probably did not make a significant contribution to the community structure of the corticolous biofilms we investigated. By contrast, trebouxiophycean algae typically dominated the samples. Non-lichenised cells, morphologically identifiable as *Trebouxia* or *Asterochloris* (fig. 2E–F), were frequently detected. However, the most abundant members of this group were usually *Apatococcus*-like populations represented by characteristic sarcinoid colonies (fig. 2G–H). Filamentous forms of *Prasiola crispa* (Lightf.) Kütz. (fig. 2I–J) were found in a few Central Bohemian samples. The rod-like cells and the short filaments of the trebouxiophycean genus *Stichococcus* (fig. 2K) also formed an important and occasionally dominant part of the investigated biofilms. The Trentepohliales were represented by filamentous populations, which were assigned to the morphological genus *Trentepohlia*. They were mostly formed by short-celled fragments with typical extraplastidial carotenoid globules of high cellular content (fig. 2L). The heterogenous lineage of streptophytan green algae was represented by several less common coccoid taxa, such as the genera *Mesotaenium* (fig. 2M) and *Spirotaenia* (fig. 2N). These morphologically well-defined micro-algal taxa were strongly related to individual host tree species, typically within a single region. Notably, *Mesotaenium* populations were only found in six out of fourteen samples from *Juniperus oxycedrus*. Likewise, the mucilaginous colonies and cells of *Spirotaenia* were only detected in six samples from *Arbutus unedo* and in a single sample from *Tilia cordata*. However, in most samples, the Streptophyta were represented by populations of the filamentous genus *Klebsormidium* (fig. 2O–P).

Variables at all the spatial scales of the sampling design significantly affected the community structure of the corticolous biofilms in both the full and reduced dataset (table 1). The effect of individual trees was weaker for the dataset that was reduced to five major lineages. This was also illustrated by the PCA of the community data (full dataset), which vaguely separated the Istrian and Bohemian samples, as well as the samples taken from individual host species (fig. 3). However, the first principal component (PC1), i.e. the main axis of the variation in the species composition of samples, apparently reflected the pH gradient. The samples taken from the host taxa characteristic by very low pH values were positioned in the negative extremes of PC1 and the trees with less acidic bark in the positive parts of the PC1 range.

Trentepohliales, Streptophyta and Cyanobacteria were more abundant in the sub-Mediterranean Istrian samples, and this pattern was also significant in the partial correlation analyses, i.e. after accounting for all the abiotic factors (table 2). Notably, the distribution of Cyanobacteria may have been strongly determined at the regional level, as they only occurred in the Istrian samples. The abundance patterns of the trebouxiophycean green algae were optimally explained by the MR models that invariably included sample orientation as the significant factor (abundance was increased on the northern side of trees). This effect was discernible in both regions and at different spatial levels. Interestingly, the effect
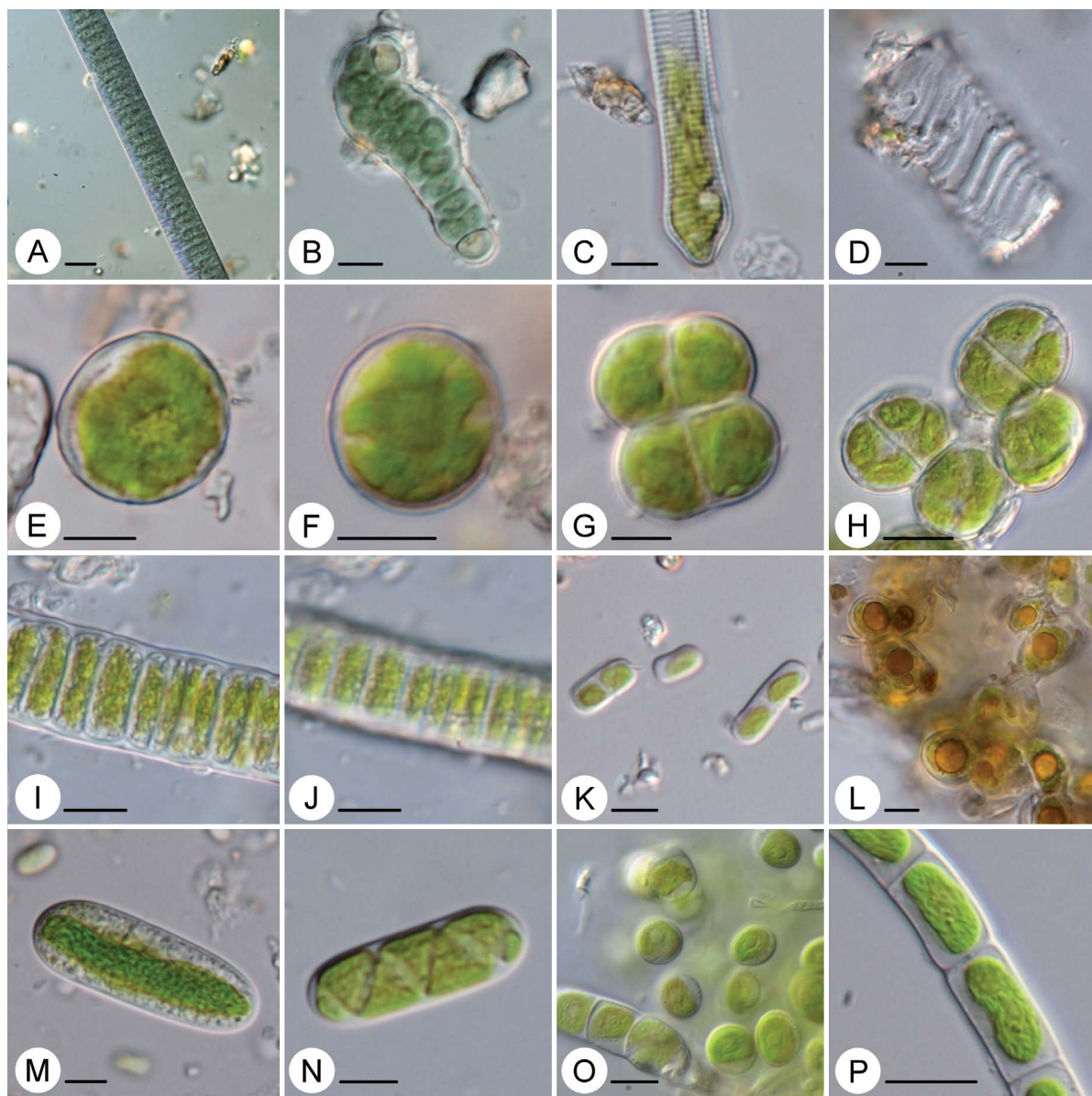
Figure 2 – Micrographs of selected characteristic microalgae occurring in the corticolous biofilms: A, *Oscillatoria* sp.; B, *Nostoc* sp.; C, *Synedra capitata*; D, *Orthoseira* cf. *roeseana*; E & F, *Trebouxia/Asterochloris* sp.; G & H, *Apatococcus*-like colonies; I, *Prasiola crispa*; J, *P. crispa*, longitudinal cell wall striation; K, *Stichococcus* sp.; L, *Trentepohlia* sp.; M, *Mesotaenium* sp.; N, *Spirotaenia* sp.; O, a mixture of *Klebsormidium* sp. filaments and unidentified coccoid trebouxiophycean algae; P, *Klebsormidium* sp. Scale bars represent 5 μm (A–D, I–O) or 10 μm (E–H, P).

Table 1 – Results of the permutational multivariate analyses of variance evaluating effects of individual spatial factors on community structure of corticolous microalgae.

Region: temperate Bohemia and sub-Mediterranean Istria; host species: eleven species of tree; tree: individual tree. The tests are sequential, which means that the terms are sequentially evaluated in the order as they appear in the formula. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p > 0.05$.

Full dataset						Reduced dataset					
Factor	Df	Sums of squares	Mean squares	F	*p*-value	Factor	Df	Sums of squares	Mean squares	F	*p*-value
Region	1	1.34	1.34	14.08	***	Region	1	1.01	16.89		***
Host species	9	3.97	0.44	4.62	***	Host species	9	0.22	3.73		***
Tree	73	11.81	0.16	1.70	***	Tree	73	0.09	1.49		*
Residuals	85	8.09	0.09			Residuals	85	0.06			

Table 2 – Results of the linear correlation analyses and partial correlation analyses to show relationships between individual abiotic factors and abundance of microalgal groups. Significant Pearson's correlation coefficients (r) / partial correlation coefficients (for analyses involving individual microalgal groups) are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; -, $p > 0.05$.

	Bark pH	Trunk diameter	Height	Open sky proportion	Bark roughness	Orientation (north)	Region (Istria)	Trebouxiophyceae	Streptophyta	Trentepohliales	Cyanobacteria
Bark pH	x	-0.20**	-	0.20**	-	-	0.35***	- / 0.21**	0.16* / -	0.31*** / 0.25**	0.34*** / 0.31***
Trunk diameter	-0.20**	x	-	-	0.47***	-	-0.42***	- / -	- / -	-0.28*** / -	- / -
Height	-	-	x	-	-	-	-	- / -	-0.15* / -	- / -	- / -
Open sky proportion	0.20**	-	-	x	-	-	0.25**	- / -	- / -	- / -0.22**	- / -
Bark roughness	-	0.47***	-	-	x	-	-0.23**	- / -	- / -	-0.28*** / -	- / 0.17*
Orientation (north)	-	-	-	-	-	x	-	0.36*** / 0.36***	0.22** / 0.23**	- / -	- / -
Region (Istria)	0.35***	-0.42***	-	0.25**	-0.23**	-	x	- / -0.16*	0.27*** / 0.24**	0.29*** / 0.18*	0.24** / 0.18*
Trebouxiophyceae	- / 0.21**	- / -	- / -	- / -	- / -	0.36*** / 0.36***	- / -0.16*	x	0.17*	-	-
Streptophyta	0.16* / -	- / -	-0.15* / -	- / -	- / -	0.22** / 0.23**	0.27*** / 0.24**	0.17*	x	-	-
Trentepohliales	0.31*** / 0.25**	-0.28*** / -	- / -	- / -0.22**	-0.28*** / -	- / -	0.29*** / 0.18*	-	-	x	0.17*
Cyanobacteria	0.34*** / 0.31***	- / -	- / -	- / -	- / 0.17*	- / -	0.24** / 0.18*	-	-	0.17*	x
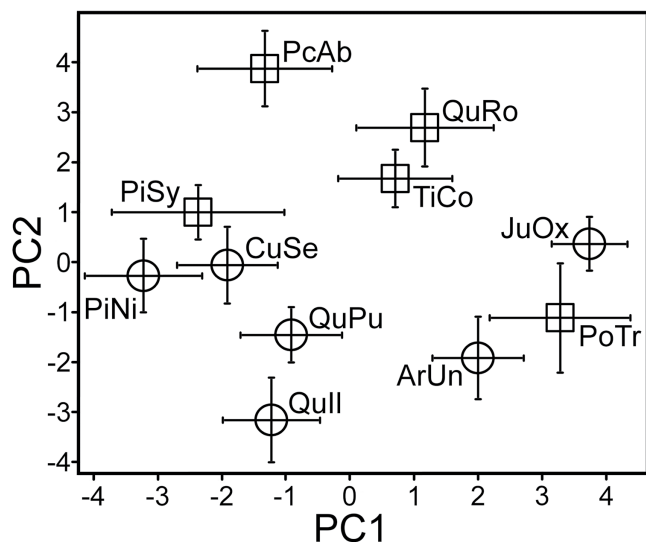
Figure 3 – The ordination plot of the first (25.3% of the variation) and second axes (18.1%) of the PCA of the community data of samples. The centroids and standard error bars for samples taken from individual host species are illustrated. Squares: Central Bohemia, circles: Istria. For abbreviations of tree species, see figure 1.

of pH on trebouxiophycean abundance was detectable only in the temperate samples, but it was insignificant in the models that partialled-out variation spanned by the host species and individual tree levels (table 3). Likewise, sample orientation was the only factor selected by the AIC procedure that significantly affected the abundance of streptophytesin the Istrian samples at all spatial levels (table 4). This factor was much less significant for the streptophyte algae in the Bohemian samples, but they were most strongly determined by the bark pH values typical for individual host tree species.

The distribution of Trentepohliales was affected by different factors in both regions. In the temperate samples it was most strongly affected by the pH of the bark (table 3). Their abundance increased with increasing bark pH, but this relationship was completely obscuredat the host species-level, i.e. the variation in trentepohliacean abundance between different tree species accounted for the relationship of these algae and bark pH. Several other factors were also marginally significant, such as bark roughness, which was negatively related to the abundance of Trentepohliales. This means that Trentepohliales in the Bohemian samples were slightly less abundant on the trees with coarser bark. Interestingly, this effect was also detectable within trees of the same species. The open sky proportion also slightly negatively influenced the abundance of this group in the temperate samples (table 3). Conversely, Trentepohliales in the Istrian samples were only weakly related to the evaluated abiotic factors. They were slightly more abundant in the shaded sub-mediterranean mi-

Table 3 – Results of the multiple regression analyses evaluating the effects of abiotic factors on the abundance of individual microalgal groups in samples taken at different spatial levels in the temperate Bohemian region.
Only the significant factors of individual models are depicted. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p > 0.05$.

Factor	Estimate	Standard error	t-statistic	p-value
Trebouxiophyceae, uncontrolled; F-ratio = 8.14*, R^2 = 0.33, adjusted R^2 = 0.29**				
pH	1.12	0.28	4.03	***
Orientation-N	0.82	0.28	2.96	**
Height	-0.56	0.28	-2.01	*
Trebouxiophyceae, controlled for host species; F-ratio = 6.65*, R^2 = 0.28, adjusted R^2 = 0.24**				
Orientation-N	1.87	0.51	3.65	***
Height	-0.01	0.004	-2.40	*
Trebouxiophyceae, controlled for tree; F-ratio = 9.90*, R^2 = 0.22, adjusted R^2 = 0.20**				
Orientation-N	1.56	0.42	3.77	***
Height	-0.01	0.003	-2.13	*
Streptophytes, uncontrolled; F-ratio = 7.96*, R^2: 0.19, adjusted R^2: 0.16**				
pH	0.59	0.17	3.46	***
Streptophytes, controlled for host species; F-ratio = 4.34*, R^2 = 0.06, adjusted R^2 = 0.04				
Orientation-N	0.69	0.33	2.08	*
Streptophytes, controlled for tree; F-ratio = 3.15n.s., R^2 = 0.04, adjusted R^2 = 0.03				
Trentepohliales, uncontrolled; F-ratio: 8.69*, R^2: 0.34, adjusted R^2: 0.30**				
pH	1.16	0.28	4.17	***
Bark roughness	-0.74	0.27	-2.73	**
Open sky proportion	-0.68	0.28	-2.45	*
Trentepohliales, controlled for host species; F-ratio: 5.78*, R^2: 0.26, adjusted R^2: 0.21**				
Bark roughness	-0.99	0.46	-2.14	*
Trentepohliales, controlled for tree; F-ratio: 2.21n.s., R^2: 0.03, adjusted R^2: 0.02				

Table 4 – Results of the multiple regression analyses evaluating the effects of abiotic factors on the abundance of individual microalgal groups in samples taken at different spatial levels in the sub-mediterranean Istrian region.
Only the significant factors of individual models are depicted. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p > 0.05$.

Factor	Estimate	Standard error	*t*-statistic	*p*-value
Trebouxiophyceae, uncontrolled; F-ratio = 18.60***, R^2 = 0.16, adjusted R^2 = 0.15				
Orientation-N	1.07	0.25	4.31	***
Trebouxiophyceae, controlled for host species; F-ratio = 19.93***, R^2 = 0.17, adjusted R^2 = 0.16				
Orientation-N	2.16	0.48	4.46	***
Trebouxiophyceae, controlled for tree; F-ratio = 11.22**, R^2 = 0.11, adjusted R^2 = 0.10				
Orientation-N	1.03	0.31	3.35	**
Streptophytes, uncontrolled; F-ratio: 5.94***, R^2: 0.16, adjusted R^2: 0.13				
Orientation-N	0.77	0.34	2.28	*
Height	-0.79	0.33	-2.36	*
Open sky proportion	-0.78	0.33	-2.34	*
Streptophytes, controlled for host species; F-ratio = 7.60**, R^2 = 0.07, adjusted R^2 = 0.06				
Orientation-N	1.81	0.66	2.76	**
Streptophytes, controlled for tree; F-ratio = 13.34***, R^2 = 0.12, adjusted R^2 = 0.11				
Orientation-N	1.50	0.41	3.65	***
Trentepohliales, uncontrolled; F-ratio: 4.36*, R^2: 0.08, adjusted R^2: 0.07				
Open sky proportion	-0.79	0.34	-2.34	*
Trentepohliales, controlled for host species; F-ratio: 4.16*, R^2: 0.04, adjusted R^2: 0.03				
Orientation-N	1.31	0.64	2.04	*
Trentepohliales, controlled for tree; F-ratio: 9.21**, R^2: 0.09, adjusted R^2: 0.08				
Orientation-N	1.27	0.42	3.03	**
Cyanobacteria, uncontrolled; F-ratio: 13.90***, R^2: 0.23, adjusted R^2: 0.21				
pH	1.13	0.23	4.82	***
Bark roughness	0.52	0.23	2.23	*
Cyanobacteria, controlled for host species; F-ratio: 4.23*, R^2: 0.04, adjusted R^2: 0.03				
pH	0.68	0.33	2.06	*
Cyanobacteria, controlled for tree; F-ratio: 2.43n.s., R^2: 0.02, adjusted R^2: 0.01				

crohabitats and on the northern side of trees in the MR models describing trentepohliacean abundance with the effects of the microscale level partialled-out prior to the analysis (table 4). Abundance of Cyanobacteria was also strongly related to increasing bark pH. Interestingly, this effect remained weakly significant even in the MR model that partialled-out the host species differences prior to the analysis. Cyanobacteria were also positively influenced by increasing bark roughness, although this was explained by differences between the host tree species (table 4).

DISCUSSION

Most of the samples were dominated by the green algal *Apatococcus*-like sarcinoid microalgae. Previously, these morphologically relatively uniform green algae were classified into several traditional trebouxiophycean genera, such as *Apatococcus*, *Desmococcus*, and *Prasiococcus* (Ettl & Gärtner 1995, Rindi 2007, Freystein & Reisser 2010). However, neither the monophyly, nor the infrageneric diversity of these taxa has ever been tested by molecular methods. Therefore, in this study, the *Apatococcus*-like populations are treated as part of the broadly defined group of trebouxiophycean corticolous microalgae. Despite the obvious omnipresence

of the sarcinoid *Apatococcus*-like microalgae in temperate corticolous biofilms, they have often been under-represented in cultivation studies (Freystein et al. 2008, Khaybullina et al. 2010). Gustavs et al. (2010) suggested that the difficult culturing and comparatively slow growth of *Apatococcus lobatus* (Chodat) J.B.Petersen may be related to the mixotrophy of this alga that, on the other hand, enhances its ecological success in the natural conditions (Hallmann et al. 2013).

Three major taxonomic groups, Cyanobacteria, Trentepohliales and streptophytes, were significantly more abundant in the sub-Mediterranean localities, and samples from these sites were typically more diversified than the temperate biofilms. This pattern strongly contributed to the significance of the regional effects on the community structure of corticolous algae. However, whether these differences may be ascribed to the higher annual precipitation or to the higher mean temperature of the Istrian region cannot be discerned. *Prasiola crispa* was only detected in several temperate samples, which concurs with the presumed affinity of the genus *Prasiola* with colder climatic conditions (Rindi & Guiry 2004, Rindi et al. 2007). Populations corresponding to the genus *Stichococcus*, which is phylogenetically closely related to *Prasiola* (Leliaert et al. 2012), were not limited to the

colder region but were frequently found in the sub-Mediterranean samples.

The significant effects of bark pH on the abundance of Trebouxiophyceae, Cyanobacteria and Trentepohliales were mostly explained by the differences in the host species. Consequently, the host species proved to be a better predictor of the abundance of these micro-algal groups than the actual pH value of the sample. The fact that several micro-algal taxa have a close affinity to a particular host tree species, such as the genus *Spirotaenia* on *Arbutus unedo*, or the genus *Mesotaenium* on *Juniperus oxycedrus*, also indicates that species-specific characteristics of tree bark may significantly influence the community structure of biofilms. We can conclude that corticolous micro-algal communities are significantly influenced by factors acting at the host species-level. Therefore, their local distribution on different trees likely cannot be explained solely by neutral factors, such as dispersal, immigration or local extinctions (Hubbell 2001). Likewise, significant effects of abiotic factors acting at the meso- and macroscale levels were reported for corticolous microalgae in a boreal forest (Hedenås et al. 2007), as well as for epilithic subaerial microalgae (Rindi et al. 1999, Rindi & Guiry 2004).

Individual major groups of corticolous microalgae proved to be primarily influenced by different abiotic factors. Whereas the abundance of Trentepohliales and Cyanobacteria was positively related to warmer and/or more humid climatic conditions and, in parallel, to host tree species with higher bark pH, the abundance of trebouxiophytes and streptophytes was primarily determined by microscale factors, such as the sample orientation. Hedenås et al. (2007) reported that, as well as having an effect on the coccoid trebouxiophytes, the north-south orientation of samples on trunks also significantly influenced the abundance of Cyanobacteria. Such a pattern was not detected in our study, perhaps because we used a different sampling design. Whereas Hedenås et al. (2007) concentrated on patterns of microalgae distribution on *Populus tremula*, a species characterised by high bark pH values, we compared biofilms from a variety of host taxa growing in two climatically different regions and took lower numbers of samples from each tree species. Therefore, patterns of microscale distribution in groups limited to a few host tree species with higher bark pH, such as Cyanobacteria, may have been obscured because relatively low numbers of samples included this microbial group. Interestingly, the differences in the bark pH values, based on the host species diversity, more strongly influenced the abundances of individual micro-algal lineages in the temperate samples. The pH values were invariably recovered as the prime abiotic factor for the streptophytes, Trebouxiophyceae and Trentepohlialesin the samples from the Bohemian region. Conversely, pH seemed to be considerably less important for micro-algal groups in the sub-mediterranean region, where only Cyanobacteria were significantly related to this factor. Whether this difference in micro-algal strategies in both climatically different regions may represent a more general phenomenon remains to be tested in future studies.

The streptophytes and trebouxiophytes we found in the biofilms were composed of multiple generic lineages. Therefore, macro- and mesoscale factors, such as climate or the host species, may also influence the distribution of lower taxonomic levels of these groups. However, many genus- and species-level lineages of the subaerial coccoid green algae are morphologically almost indistinguishable (Darienko et al. 2010, Hodač et al. 2012, Neustupa et al. 2013). This may be especially true for the omnipresent *Apatococcus*-like taxa. They are often difficult to cultivate and their real phylogenetic diversity still remains unexplored (Gustavs et al. 2010, Hallmann et al. 2013). We believe that the next generation of sequencing methods, or methods allowing the detection of individual lineages using specific ribosomal RNA oligonucleotide probes, may help to resolve this. Then, it may also be possible to assess whether the ecological strategies of the higher-level lineages of corticolous subaerial microalgae, which were illustrated in this study, are shared by individual species and genera.

SUPPLEMENTARY DATA

Supplementary data are available in pdf format at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingentaconnect.com/content/botbel/plecevo/supp-data), and consist of the three tables with the abiotic and algae composition data of samples.

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Electronic appendix to:
Jiří Neustupa & Anna Štifterová (2013)
Distribution patterns of subaerial corticolous microalgae in two European regions

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Appendix – The tables with the abiotic and algae composition data of samples. A, abiotic factors; B,

community composition of microalgae based on identification of individual groups and genera (full

dataset); C, community composition of microalgae based on abundances of the major taxonomic groups

(merged dataset).

Electronic appendix, part A

	region	host species	tree	trunk (cm)	height (cm)	BR	OSP (%)	pH	orientation	coordinates
B1	B	TiCo	TiCo1	45	130	3	25.9	5.13	N	50°09′25.79″N 13°54′09.70″E
B2	B	TiCo	TiCo1	45	130	3	21.0	4.48	S	50°09′25.79″N 13°54′09.70″E
B3	B	TiCo	TiCo1	50	20	3	27.1	5.35	N	50°09′25.79″N 13°54′09.70″E
B4	B	PcAb	PcAb1	40	110	2	32.9	4.14	N	50°09′33.61″N 13°54′09.86″E
B5	B	PcAb	PcAb1	40	100	2	45.7	4.27	S	50°09′33.61″N 13°54′09.86″E
B6	B	TiCo	TiCo2	40	130	2	7.6	4.74	N	50°14′18.70″N 14°05′30.93″E
B7	B	TiCo	TiCo2	50	10	2	19.6	4.84	N	50°14′18.70″N 14°05′30.93″E
B8	B	PiSy	PiSy1	25	150	3	21.5	3.91	N	50°14′17.65″N 14°05′27.12″E
B9	B	PiSy	PiSy1	25	150	3	15.6	3.95	S	50°14′17.65″N 14°05′27.12″E
B10	B	PcAb	PcAb2	30	150	2	17.5	4.02	N	50°14′17.11″N 14°05′25.98″E
B11	B	PcAb	PcAb2	40	15	2	17.1	4.19	N	50°14′17.11″N 14°05′25.98″E
B12	B	PcAb	PcAb2	40	15	2	8.1	3.96	S	50°14′17.11″N 14°05′25.98″E
B13	B	TiCo	TiCo3	50	120	3	15.0	4.71	S	50°14′15.98″N 14°05′21.40″E
B14	B	QuRo	QuRo1	30	160	3	13.2	4.53	N	50°14′16.78″N 14°05′20.58″E
B15	B	QuRo	QuRo1	30	10	3	18.2	5.19	N	50°14′16.78″N 14°05′20.58″E
B16	B	QuRo	QuRo2	20	150	3	20.1	4.56	S	50°14′16.22″N 14°05′21.83″E
B17	B	PcAb	PcAb3	25	140	2	21.8	4.48	N	50°14′15.25″N 14°05′17.32″E
B18	B	TiCo	TiCo4	20	120	2	12.6	4.96	S	50°14′15.23″N 14°05′15.73″E
B19	B	PiSy	PiSy2	25	130	3	25.4	4.23	N	50°14′13.96″N 14°05′11.27″E

	region	host species	tree	trunk (cm)	height (cm)	BR	OSP (%)	pH	orientation	coordinates
B20	B	PiSy	PiSy2	25	10	3	22.5	4.29	N	50°14′13.96″N 14°05′11.27″E
B21	B	QuRo	QuRo3	45	150	3	5.3	3.83	S	50°16′21.05″N 13°54′35.61″E
B22	B	QuRo	QuRo3	50	30	3	5.1	4.38	S	50°16′21.05″N 13°54′35.61″E
B23	B	PiSy	PiSy3	45	140	3	7.6	3.20	N	50°16′21.21″N 13°54′35.06″E
B24	B	PiSy	PiSy3	50	20	3	7.9	3.08	N	50°16′21.21″N 13°54′35.06″E
B25	B	PiSy	PiSy3	50	20	3	6.2	3.61	S	50°16′21.21″N 13°54′35.06″E
B26	B	QuRo	QuRo4	65	170	3	14.7	4.21	N	50°16′19.94″N 13°54′27.82″E
B27	B	QuRo	QuRo4	65	170	3	7.1	3.62	S	50°16′19.94″N 13°54′27.82″E
B28	B	PcAb	PcAb4	45	150	2	7.5	3.53	N	50°16′20.01″N 13°54′28.66″E
B29	B	PiSy	PiSy4	40	160	3	7.6	2.67	N	50°16′20.21″N 13°54′24.96″E
B30	B	PoTr	PoTr1	50	160	2	15.4	5.50	N	50°16′19.24″N 13°54′22.12″E
B31	B	PoTr	PoTr1	50	160	2	8.5	5.53	S	50°16′19.24″N 13°54′22.12″E
B32	B	PcAb	PcAb5	40	150	2	15.4	3.68	N	50°16′16.10″N 13°54′05.06″E
B33	B	PcAb	PcAb5	40	150	2	7.8	3.70	S	50°16′16.10″N 13°54′05.06″E
B34	B	TiCo	TiCo5	20	150	1	12.6	4.77	S	50°16′15.25″N 13°53′58.79″E
B35	B	TiCo	TiCo5	20	150	1	7.8	4.83	N	50°16′15.25″N 13°53′58.79″E
B36	B	TiCo	TiCo6	30	150	2	8.2	5.14	S	50°16′11.44″N 13°53′31.51″E
B37	B	TiCo	TiCo6	30	155	1	10.9	5.18	N	50°16′11.44″N 13°53′31.51″E
B38	B	PoTr	PoTr2	25	130	2	13.4	6.42	S	50°15′58.39″N 13°52′34.62″E
B39	B	PoTr	PoTr2	25	130	2	19.7	5.96	N	50°15′58.39″N 13°52′34.62″E
B40	B	PoTr	PoTr2	25	15	2	22.8	5.53	N	50°15′58.39″N 13°52′34.62″E
B41	B	PoTr	PoTr2	25	15	2	11.5	6.29	S	50°15′58.39″N 13°52′34.62″E
B42	B	QuRo	QuRo5	55	140	3	6.3	4.02	S	50°33′15.69″N 14°01′49.07″E
B43	B	QuRo	QuRo5	55	130	3	7.4	3.86	N	50°33′15.69″N 14°01′49.07″E
B44	B	QuRo	QuRo6	60	130	3	8.4	3.75	S	50°33′13.76″N 14°01′53.44″E
B45	B	QuRo	QuRo6	70	15	3	10.9	4.16	S	50°33′13.76″N 14°01′53.44″E
B46	B	QuRo	QuRo6	60	135	3	12.2	3.60	N	50°33′13.76″N 14°01′53.44″E
B47	B	QuRo	QuRo6	70	15	3	13.4	3.89	N	50°33′13.76″N 14°01′53.44″E
B48	B	PiSy	PiSy5	40	150	3	15.6	3.69	S	50°33′12.85″N 14°02′05.55″E
B49	B	PiSy	PiSy5	40	150	3	20.7	4.18	N	50°33′12.85″N 14°02′05.55″E
B50	B	QuRo	QuRo6	30	135	3	17.4	3.67	S	50°33′04.19″N 14°02′11.11″E

	region	host species	tree	trunk (cm)	height (cm)	BR	OSP (%)	pH	orientation	coordinates
B51	B	QuRo	QuRo7	25	145	3	19.1	4.45	N	50°32′43.47″N 14°02′15.93″E
B52	B	QuRo	QuRo7	40	10	3	17.5	4.16	N	50°32′43.47″N 14°02′15.93″E
B53	B	PoTr	PoTr3	80	150	3	15.9	6.36	N	50°13′34.46″N 14°06′08.71″E
B54	B	PoTr	PoTr3	80	150	3	18.4	5.96	S	50°13′34.46″N 14°06′08.71″E
B55	B	TiCo	TiCo7	45	160	3	29.9	4.15	N	50°13′53.90″N 14°05′39.67″E
B56	B	TiCo	TiCo7	45	10	3	23.4	4.64	N	50°13′53.90″N 14°05′39.67″E
B57	B	TiCo	TiCo7	45	160	3	28.8	4.16	S	50°13′53.90″N 14°05′39.67″E
B58	B	TiCo	TiCo7	45	20	3	29.3	4.40	S	50°13′53.90″N 14°05′39.67″E
B59	B	TiCo	TiCo8	7	160	1	7.6	4.82	S	50°10′18.42″N 13°53′20.99″E
B60	B	TiCo	TiCo8	7	160	1	10.3	4.81	N	50°10′18.42″N 13°53′20.99″E
B61	B	PcAb	PcAb6	45	160	2	27.1	3.28	S	50°10′23.65″N 13°53′18.99″E
B62	B	PcAb	PcAb6	45	160	2	41.9	3.31	N	50°10′23.65″N 13°53′18.99″E
B63	B	PoTr	PoTr4	10	150	2	16.5	5.77	S	50°10′32.36″N 13°53′13.40″E
B64	B	PoTr	PoTr4	10	15	2	14.6	5.76	S	50°10′32.36″N 13°53′13.40″E
B65	B	PoTr	PoTr4	10	130	2	16.8	5.30	N	50°10′32.36″N 13°53′13.40″E
B66	B	PoTr	PoTr4	10	10	2	19.7	4.33	N	50°10′32.36″N 13°53′13.40″E
B67	B	PiSy	PiSy6	40	140	2	17.4	2.96	S	50°10′33.31″N 13°53′11.78″E
B68	B	PiSy	PiSy6	40	10	2	16.5	3.14	S	50°10′33.31″N 13°53′11.78″E
B69	B	PiSy	PiSy6	40	130	2	18.1	2.93	N	50°10′33.31″N 13°53′11.78″E
B70	B	PiSy	PiSy6	40	10	2	17.6	3.01	N	50°10′33.31″N 13°53′11.78″E
B71	B	PoTr	PoTr5	60	160	3	50.7	6.51	N	50°09′46.46″N 13°54′09.18″E
B72	B	PoTr	PoTr5	60	10	3	65.0	6.62	N	50°09′46.46″N 13°54′09.18″E
I1	I	PiNi	PiNi1	30	130	3	68.8	3.63	N	45°46′44.80″N 13°37′20.22″E
I2	I	QuPu	QuPu1	25	175	3	37.5	5.87	S	45°46′51.87″N 13°37′21.40″E
I3	I	QuPu	QuPu1	25	140	3	22.6	6.08	N	45°46′51.87″N 13°37′21.40″E
I4	I	QuIl	QuIl1	25	130	3	18.5	4.64	N	45°46′56.23″N 13°37′16.05″E
I5	I	QuIl	QuIl1	25	170	3	22.8	5.19	S	45°46′56.23″N 13°37′16.05″E
I6	I	PiNi	PiNi2	20	160	2	37.4	3.41	S	45°47′04.29″N 13°36′19.77″E
I7	I	QuIl	QuIl2	25	100	2	18.4	5.08	N	45°46′36.70″N 13°35′41.23″E
I8	I	QuIl	QuIl2	35	20	2	16.6	5.57	N	45°46′36.70″N 13°35′41.23″E
I9	I	QuIl	QuIl2	25	125	2	12.5	4.93	S	45°46′36.70″N 13°35′41.23″E

	region	host species	tree	trunk (cm)	height (cm)	BR	OSP (%)	pH	orientation	coordinates
I10	I	QuIl	QuIl3	20	140	2	18.7	4.96	S	45°46′36.52″N 13°35′34.95″E
I11	I	QuIl	QuIl4	15	100	3	15.7	5.21	N	45°46′44.44″N 13°35′25.30″E
I12	I	CuSe	CuSe1	40	150	2	20.3	4.85	N	45°47′18.16″N 13°35′24.08″E
I13	I	CuSe	CuSe1	50	25	2	16.2	5.08	N	45°47′18.16″N 13°35′24.08″E
I14	I	PiNi	PiNi3	40	140	3	19.3	4.10	N	45°46′39.70″N 13°35′44.08″E
I15	I	PiNi	PiNi3	40	150	3	18.8	4.27	S	45°46′39.70″N 13°35′44.08″E
I16	I	PiNi	PiNi3	50	35	3	16.9	3.84	N	45°46′39.70″N 13°35′44.08″E
I17	I	QuIl	QuIl5	20	130	2	13.7	4.92	N	45°46′39.49″N 13°35′43.90″E
I18	I	QuIl	QuIl5	20	130	2	15.1	5.06	S	45°46′39.49″N 13°35′43.90″E
I19	I	QuIl	QuIl5	30	40	2	15.3	5.38	S	45°46′39.49″N 13°35′43.90″E
I20	I	QuIl	QuIl6	20	140	2	18.5	5.11	N	45°46′39.52″N 13°35′41.86″E
I21	I	QuIl	QuIl6	20	140	2	14.6	5.13	S	45°46′39.52″N 13°35′41.86″E
I22	I	CuSe	CuSe2	40	160	1	13.7	5.35	N	45°46′38.43″N 13°35′39.94″E
I23	I	CuSe	CuSe2	40	150	1	19.9	5.57	S	45°46′38.43″N 13°35′39.94″E
I24	I	QuIl	QuIl7	5	130	1	20.0	5.20	N	45°46′36.69″N 13°35′38.56″E
I25	I	QuIl	QuIl7	5	130	1	15.4	5.27	S	45°46′36.69″N 13°35′38.56″E
I26	I	QuIl	QuIl8	15	120	2	16.5	5.28	S	45°08′13.84″N 13°38′13.17″E
I27	I	QuIl	QuIl8	15	120	2	13.4	5.66	N	45°08′13.84″N 13°38′13.17″E
I28	I	JuOx	JuOx1	10	60	2	13.7	4.09	S	45°08′13.61″N 13°38′13.57″E
I29	I	JuOx	JuOx1	10	60	2	14.0	4.12	N	45°08′13.61″N 13°38′13.57″E
I30	I	QuIl	QuIl9	10	120	1	16.0	4.91	S	45°08′13.90″N 13°38′13.37″E
I31	I	QuIl	QuIl9	10	120	1	15.3	4.62	N	45°08′13.90″N 13°38′13.37″E
I32	I	ArUn	ArUn1	20	130	2	15.7	5.47	S	45°08′16.48″N 13°38′11.67″E
I33	I	ArUn	ArUn1	20	130	2	16.0	5.43	N	45°08′16.48″N 13°38′11.67″E
I34	I	ArUn	ArUn1	30	20	2	18.4	5.48	N	45°08′16.48″N 13°38′11.67″E
I35	I	ArUn	ArUn2	10	130	2	18.4	5.17	N	45°08′16.21″N 13°38′12.27″E
I36	I	ArUn	ArUn3	20	130	2	10.7	5.59	S	45°08′13.15″N 13°38′42.13″E
I37	I	ArUn	ArUn3	20	130	2	15.9	4.75	N	45°08′13.15″N 13°38′42.13″E
I38	I	JuOx	JuOx2	20	50	2	17.5	4.59	S	45°08′13.11″N 13°38′39.42″E
I39	I	JuOx	JuOx2	20	50	2	13.8	3.92	N	45°08′13.11″N 13°38′39.42″E
I40	I	CuSe	CuSe3	50	110	2	58.1	5.63	S	45°22′56.82″N 13°43′25.00″E

	region	host species	tree	trunk (cm)	height (cm)	BR	OSP (%)	pH	orientation	coordinates
I41	I	CuSe	CuSe3	50	110	2	48.7	5.93	N	45°22′56.82″N 13°43′25.00″E
I42	I	CuSe	CuSe4	50	20	2	62.2	5.31	N	45°31′22.81″N 13°34′54.40″E
I43	I	CuSe	CuSe5	50	130	2	23.7	4.65	S	45°31′26.17″N 13°34′50.23″E
I44	I	CuSe	CuSe5	50	10	2	32.4	6.51	S	45°31′26.17″N 13°34′50.23″E
I45	I	PiNi	PiNi4	50	150	3	19.4	3.50	N	45°31′16.77″N 13°34′52.69″E
I46	I	PiNi	PiNi5	25	140	3	22.2	4.29	N	45°31′16.31″N 13°34′52.49″E
I47	I	QuPu	QuPu2	40	100	2	27.6	5.35	S	45°31′16.81″N 13°34′48.69″E
I48	I	QuPu	QuPu3	50	170	2	24.9	5.03	N	45°31′16.37″N 13°34′50.89″E
I49	I	PiNi	PiNi6	40	10	3	21.3	4.30	N	45°34′41.71″N 13°44′36.30″E
I50	I	PiNi	PiNi6	40	10	3	37.8	3.89	S	45°34′41.71″N 13°44′36.30″E
I51	I	QuPu	QuPu4	25	160	3	18.8	5.70	N	45°34′43.17″N 13°44′39.70″E
I52	I	QuPu	QuPu4	25	10	3	22.8	5.82	N	45°34′43.17″N 13°44′39.70″E
I53	I	QuPu	QuPu4	25	150	3	22.4	5.45	S	45°34′43.17″N 13°44′39.70″E
I54	I	QuPu	QuPu5	30	100	3	36.2	4.93	N	45°34′47.05″N 13°45′00.95″E
I55	I	QuPu	QuPu5	30	100	3	34.7	4.91	S	45°34′47.05″N 13°45′00.95″E
I56	I	QuPu	QuPu6	20	80	3	22.8	5.09	N	45°34′45.25″N 13°44′52.89″E
I57	I	QuPu	QuPu6	20	80	3	45.6	4.61	S	45°34′45.25″N 13°44′52.89″E
I58	I	PiNi	PiNi7	30	10	3	44.1	4.91	S	45°34′35.83″N 13°44′14.40″E
I59	I	PiNi	PiNi7	30	150	3	31.2	4.41	S	45°34′35.83″N 13°44′14.40″E
I60	I	CuSe	CuSe6	30	10	2	52.1	6.28	N	45°34′35.77″N 13°44′13.45″E
I61	I	CuSe	CuSe6	30	130	2	42.5	5.28	N	45°34′35.77″N 13°44′13.45″E
I62	I	CuSe	CuSe7	40	120	2	27.1	5.40	N	45°46′09.69″N 13°22′14.93″E
I63	I	CuSe	CuSe7	40	120	2	69.4	5.84	S	45°46′09.69″N 13°22′14.93″E
I64	I	CuSe	CuSe8	50	10	2	36.8	5.09	S	45°46′16.76″N 13°22′15.06″E
I65	I	CuSe	CuSe8	50	10	2	23.2	4.83	N	45°46′16.76″N 13°22′15.06″E
I66	I	CuSe	CuSe8	50	150	2	34.6	4.29	S	45°46′16.76″N 13°22′15.06″E
I67	I	CuSe	CuSe8	50	150	2	14.4	4.79	N	45°46′16.76″N 13°22′15.06″E
I68	I	CuSe	CuSe9	30	20	2	36.9	5.90	N	45°46′27.24″N 13°22′10.67″E
I69	I	ArUn	ArUn4	20	60	2	18.4	5.24	N	45°08′15.33″N 13°37′41.73″E
I70	I	ArUn	ArUn4	20	70	2	20.6	4.93	S	45°08′15.33″N 13°37′41.73″E
I71	I	PiNi	PiNi8	40	100	3	37.1	4.03	S	45°08′17.51″N 13°37′35.84″E

	region	host species	tree	trunk (cm)	height (cm)	BR	OSP (%)	pH	orientation	coordinates
I72	I	PiNi	PiNi8	40	130	3	24.7	4.04	N	45°08′17.51″N 13°37′35.84″E
I73	I	ArUn	ArUn5	10	50	2	18.8	4.86	N	45°08′16.03″N 13°38′04.58″E
I74	I	ArUn	ArUn5	10	50	2	14.7	4.99	S	45°08′16.03″N 13°38′04.58″E
I75	I	ArUn	ArUn6	7	90	2	16.3	5.04	N	45°08′17.83″N 13°38′06.10″E
I76	I	QuPu	QuPu7	15	120	2	11.3	6.33	N	45°08′24.95″N 13°38′19.79″E
I77	I	QuPu	QuPu8	20	140	3	23.1	6.42	N	45°27′35.29″N 13°39′44.56″E
I78	I	QuPu	QuPu8	20	140	3	18.2	6.35	S	45°27′35.29″N 13°39′44.56″E
I79	I	QuPu	QuPu9	15	150	3	17.6	5.76	N	45°27′35.45″N 13°39′44.57″E
I80	I	QuPu	QuPu9	15	150	3	14.1	6.25	S	45°27′35.45″N 13°39′44.57″E
I81	I	QuPu	QuPu10	25	150	3	22.9	6.66	N	45°27′36.01″N 13°39′44.39″E
I82	I	QuPu	QuPu10	35	7	3	11.2	6.31	N	45°27′36.01″N 13°39′44.39″E
I83	I	JuOx	JuOx3	9	50	2	14.7	4.35	S	45°08′10.76″N 13°41′45.64″E
I84	I	JuOx	JuOx3	9	40	2	16.6	4.80	N	45°08′10.76″N 13°41′45.64″E
I85	I	JuOx	JuOx4	10	100	2	19.7	3.96	N	45°08′13.16″N 13°41′53.45″E
I86	I	JuOx	JuOx4	10	100	2	19.9	4.18	S	45°08′13.16″N 13°41′53.45″E
I87	I	JuOx	JuOx5	12	160	2	36.3	5.58	N	45°08′15.00″N 13°41′47.18″E
I88	I	JuOx	JuOx6	10	100	2	19.4	5.09	S	45°08′15.00″N 13°41′47.18″E
I89	I	JuOx	JuOx7	8	120	1	23.4	4.79	S	45°08′09.03″N 13°41′14.90″E
I90	I	ArUn	ArUn7	15	100	1	17.2	5.46	N	45°08′15.66″N 13°37′38.20″E
I91	I	ArUn	ArUn7	15	17	1	13.8	5.79	N	45°08′15.66″N 13°37′38.20″E
I92	I	JuOx	JuOx8	7	100	1	19.7	4.49	N	45°08′15.57″N 13°37′36.19″E
I93	I	JuOx	JuOx8	7	10	1	18.4	4.51	N	45°08′15.57″N 13°37′36.19″E
I94	I	JuOx	JuOx8	7	75	1	18.8	4.97	S	45°08′15.57″N 13°37′36.19″E
I95	I	ArUn	ArUn8	12	80	1	13.8	4.67	N	45°08′13.86″N 13°37′31.59″E
I96	I	ArUn	ArUn8	12	90	1	13.5	4.33	S	45°08′13.86″N 13°37′31.59″E
I97	I	ArUn	ArUn8	12	10	1	13.4	5.47	S	45°08′13.86″N 13°37′31.59″E

ArUn – *Arbutus unedo*, CuSe – *Cupressus sempervirens*, JuOx – *Juniperus oxycedrus*, PcAb – *Picea abies*, PiNi – *Pinus nigra*, PiSy – *Pinus sylvestris*, PoTr – *Populus tremula*, QuIl – *Quercus ilex*, QuPu – *Quercus pubescens*, QuRo – *Quercus robur*, TiCo – *Tilia cordata*

BR – bark roughness, OSE – open sky proportion, N – north, S – south, B – Bohemia, I – Istria

Electronic appendix, part B

	tree	APT	COC	PRS	STC	TRB	KLB	SPR	MST	TRN	DIA	NST	SCT	SNC	OSC
B1	TiCo1	12	8	8	4	2	0	0	0	0	1	0	0	0	0
B2	TiCo1	12	2	0	2	0	0	0	0	0	0	0	0	0	0
B3	TiCo1	12	2	11	3	0	0	0	0	0	0	0	0	0	0
B4	PcAb1	12	4	0	5	7	0	0	0	0	0	0	0	0	0
B5	PcAb1	5	1	0	0	7	0	0	0	0	0	0	0	0	0
B6	TiCo2	12	2	0	8	2	0	0	0	0	0	0	0	0	0
B7	TiCo2	10	0	0	0	0	0	3	0	0	0	0	0	0	0
B8	PiSy1	10	2	0	2	8	0	0	0	0	0	0	0	0	0
B9	PiSy1	7	0	0	0	2	0	0	0	0	0	0	0	0	0
B10	PcAb2	9	0	0	8	8	0	0	0	0	0	0	0	0	0
B11	PcAb2	12	8	0	7	0	0	0	0	2	0	0	0	0	0
B12	PcAb2	6	0	0	0	0	0	0	0	0	0	0	0	0	0
B13	TiCo3	11	0	0	3	4	0	0	0	0	0	0	0	0	0
B14	QuRo1	8	2	0	0	0	0	0	0	8	0	0	0	0	0
B15	QuRo1	11	2	0	5	0	0	0	0	4	0	0	0	0	0
B16	QuRo2	10	0	0	0	7	0	0	0	0	0	0	0	0	0
B17	PcAb3	9	0	0	7	6	0	0	0	0	0	0	0	0	0
B18	TiCo4	6	0	0	0	0	0	0	0	0	0	0	0	0	0
B19	PiSy2	9	8	0	5	0	0	0	0	0	0	0	0	0	0
B20	PiSy2	12	10	0	8	0	0	0	0	0	0	0	0	0	0
B21	QuRo3	2	0	0	11	3	0	0	0	4	0	0	0	0	0
B22	QuRo3	9	8	0	10	8	0	0	0	5	0	0	0	0	0
B23	PiSy3	0	0	0	2	0	0	0	0	0	0	0	0	0	0
B24	PiSy3	2	2	0	9	0	0	0	0	0	0	0	0	0	0
B25	PiSy3	2	0	0	9	0	0	0	0	0	0	0	0	0	0
B26	QuRo4	10	8	0	10	5	0	0	0	3	0	0	0	0	0
B27	QuRo4	5	3	0	10	0	0	0	0	0	0	0	0	0	0
B28	PcAb4	7	0	0	9	0	0	0	0	0	0	0	0	0	0
B29	PiSy4	0	0	0	1	0	0	0	0	0	0	0	0	0	0
B30	PoTr1	9	6	0	3	0	9	0	0	7	0	0	0	0	0
B31	PoTr1	10	9	0	7	0	0	0	0	4	0	0	0	0	0
B32	PcAb5	0	0	0	12	0	0	0	0	0	0	0	0	0	0
B33	PcAb5	5	5	0	8	2	0	0	0	0	0	0	0	0	0
B34	TiCo5	8	8	0	5	0	0	0	0	5	0	0	0	0	0
B35	TiCo5	10	10	0	5	4	0	0	0	5	0	0	0	0	0
B36	TiCo6	10	7	0	3	4	0	0	0	0	0	0	0	0	0
B37	TiCo6	10	9	0	6	0	0	0	0	8	0	0	0	0	0
B38	PoTr2	11	7	0	0	4	0	0	0	0	0	0	0	0	0
B39	PoTr2	12	10	0	6	3	8	0	0	6	0	0	0	0	0
B40	PoTr2	11	12	0	3	0	0	0	0	6	0	0	0	0	0
B41	PoTr2	11	6	0	0	0	0	0	0	10	0	0	0	0	0
B42	QuRo5	9	0	0	5	0	0	0	0	0	0	0	0	0	0
B43	QuRo5	12	0	0	6	0	0	0	0	0	0	0	0	0	0
B44	QuRo6	11	0	0	7	6	0	0	0	0	0	0	0	0	0
B45	QuRo6	12	3	0	7	0	0	0	0	0	0	0	0	0	0
B46	QuRo6	12	0	0	6	2	0	0	0	0	0	0	0	0	0
B47	QuRo6	12	9	0	7	3	0	0	0	3	0	0	0	0	0
B48	PiSy5	5	0	0	0	0	0	0	0	0	1	0	0	0	0
B49	PiSy5	10	11	0	2	0	0	0	0	0	0	0	0	0	0
B50	QuRo6	2	0	0	0	0	0	0	0	0	0	0	0	0	0
B51	QuRo7	12	10	0	0	6	0	0	0	0	0	0	0	0	0
B52	QuRo7	12	8	0	10	3	0	0	0	0	1	0	0	0	0
B53	PoTr3	11	0	0	4	7	0	0	0	0	0	0	0	0	0
B54	PoTr3	5	0	0	0	0	0	0	0	0	0	0	0	0	0
B55	TiCo7	12	0	0	7	2	0	0	0	0	0	0	0	0	0
B56	TiCo7	12	3	0	6	0	0	0	0	0	0	0	0	0	0
B57	TiCo7	6	0	0	5	0	0	0	0	0	0	0	0	0	0
B58	TiCo7	9	0	0	7	0	0	0	0	0	0	0	0	0	0
B59	TiCo8	7	0	0	6	3	0	0	0	6	0	0	0	0	0
B60	TiCo8	7	5	PRS	3	4	KLB	SPR	MST	TRN	DIA	NST	SCT	SNC	OSC
B61	PcAb6	6	0	0	3	0	0	0	0	0	1	0	0	0	0
B62	PcAb6	3	2	0	11	4	0	0	0	0	0	0	0	0	0

	tree	APT	COC	PRS	STC	TRB	KLB	SPR	MST	TRN	DIA	NST	SCT	SNC	OSC
B63	PoTr4	8	10	0	0	0	0	0	0	0	0	0	0	0	0
B64	PoTr4	6	10	0	3	0	0	0	0	8	0	0	0	0	0
B65	PoTr4	6	9	0	6	0	0	0	0	3	0	0	0	0	0
B66	PoTr4	9	9	0	0	0	0	0	0	7	0	0	0	0	0
B67	PiSy6	3	0	0	0	0	0	0	0	0	0	0	0	0	0
B68	PiSy6	5	0	0	3	0	0	0	0	0	0	0	0	0	0
B69	PiSy6	4	0	0	0	0	0	0	0	0	0	0	0	0	0
B70	PiSy6	6	9	0	3	0	0	0	0	0	0	0	0	0	0
B71	PoTr5	10	0	0	6	0	5	0	0	3	0	0	0	0	0
B72	PoTr5	12	0	6	8	7	3	0	0	0	1	0	0	0	0
I1	PiNi1	8	0	0	0	4	0	0	0	0	0	0	0	0	0
I2	QuPu1	6	0	0	0	4	0	0	0	2	0	11	1	0	0
I3	QuPu1	6	0	0	0	0	7	0	0	6	0	5	0	4	0
I4	QuIl1	8	0	0	0	0	8	0	0	4	0	0	0	0	0
I5	QuIl1	8	2	0	4	4	4	0	0	0	0	0	0	0	0
I6	PiNi2	5	0	0	0	7	0	0	0	0	0	0	0	0	0
I7	QuIl2	10	0	0	0	0	0	0	0	10	0	0	0	0	0
I8	QuIl2	6	0	0	0	0	0	0	0	9	0	0	0	0	0
I9	QuIl2	4	0	0	0	0	0	0	0	0	0	0	0	0	0
I10	QuIl3	8	0	0	0	0	0	0	0	0	0	0	0	0	0
I11	QuIl4	10	0	0	0	0	0	0	0	6	0	0	0	0	0
I12	CuSe1	12	0	0	2	0	0	0	0	0	0	0	0	0	0
I13	CuSe1	12	0	0	0	0	0	0	0	6	0	0	0	0	0
I14	PiNi3	12	0	0	0	0	10	0	0	0	0	0	0	0	0
I15	PiNi3	12	0	0	0	0	0	0	0	0	0	0	0	0	0
I16	PiNi3	11	2	0	6	0	11	0	0	0	0	0	0	0	0
I17	QuIl5	8	4	0	7	2	10	0	0	9	0	0	0	0	0
I18	QuIl5	6	0	0	0	0	0	0	0	10	0	0	0	0	0
I19	QuIl5	6	0	0	4	0	0	0	0	12	0	0	0	0	0
I20	QuIl6	6	0	0	0	0	0	0	0	7	0	0	0	0	0
I21	QuIl6	6	0	0	0	6	0	0	0	3	0	0	0	0	0
I22	CuSe2	12	0	0	3	0	0	0	0	3	0	0	0	0	0
I23	CuSe2	9	0	0	3	9	0	0	0	0	0	0	0	0	0
I24	QuIl7	6	4	0	0	4	0	0	0	3	0	0	0	0	0
I25	QuIl7	8	0	0	0	5	0	0	0	2	1	0	0	0	0
I26	QuIl8	9	4	0	5	8	0	0	0	6	0	0	0	0	0
I27	QuIl8	10	0	0	0	0	3	0	0	10	0	0	10	0	0
I28	JuOx1	10	5	0	2	5	0	0	0	4	0	0	0	0	0
I29	JuOx1	11	6	0	0	2	0	0	5	0	0	0	0	0	0
I30	QuIl9	12	5	0	0	8	0	0	0	0	0	0	0	0	0
I31	QuIl9	12	8	0	0	0	0	0	0	8	0	0	0	0	0
I32	ArUn1	6	8	0	3	0	4	8	0	8	0	0	2	0	0
I33	ArUn1	8	12	0	1	0	0	8	4	2	0	0	10	0	0
I34	ArUn1	10	10	0	0	0	0	10	0	6	0	0	9	0	0
I35	ArUn2	8	0	0	3	2	0	0	0	6	1	0	0	0	0
I36	ArUn3	8	6	0	0	0	0	5	0	3	0	0	0	0	0
I37	ArUn3	9	8	0	4	0	0	0	0	0	0	0	0	0	0
I38	JuOx2	12	5	0	8	2	0	0	5	5	0	0	0	0	0
I39	JuOx2	12	9	0	4	4	0	0	3	2	0	0	0	0	0
I40	CuSe3	6	3	0	0	7	0	0	0	0	1	0	0	4	0
I41	CuSe3	12	3	0	5	9	0	0	0	0	0	0	0	4	0
I42	CuSe4	12	0	0	0	0	0	0	0	6	0	0	0	0	0
I43	CuSe5	3	0	0	0	0	0	0	0	7	0	0	0	0	0
I44	CuSe5	4	0	0	0	0	0	0	0	0	0	0	0	0	0
I45	PiNi4	12	0	0	0	9	0	0	0	0	0	0	0	0	0
I46	PiNi5	9	4	0	2	0	0	0	0	9	0	0	0	0	0
I47	QuPu2	4	4	0	2	3	0	0	0	5	0	0	0	0	6
I48	QuPu3	12	9	0	9	0	0	0	0	6	0	0	0	0	4
I49	PiNi6	9	0	0	6	7	0	0	0	8	0	0	0	0	0
I50	PiNi6	3	COC	PRS	STC	TRB	KLB	SPR	MST	TRN	DIA	NST	SCT	SNC	OSC
I51	QuPu4	10	3	0	0	2	0	0	0	3	0	0	0	0	0
I52	QuPu4	11	0	0	6	8	4	0	0	7	0	0	0	0	4
I53	QuPu4	10	0	0	5	0	0	0	0	7	1	0	0	0	0
I54	QuPu5	8	2	0	6	3	0	0	0	8	0	0	0	0	0

	tree	APT	COC	PRS	STC	TRB	KLB	SPR	MST	TRN	DIA	NST	SCT	SNC	OSC
I55	QuPu5	10	2	0	0	4	0	0	0	0	0	0	0	0	0
I56	QuPu6	8	6	0	0	0	0	0	0	0	0	0	0	0	0
I57	QuPu6	6	2	0	0	0	0	0	0	0	0	0	0	0	0
I58	PiNi7	4	0	0	0	0	0	0	0	0	0	0	0	0	0
I59	PiNi7	8	0	0	0	6	0	0	0	0	0	0	0	0	0
I60	CuSe6	12	0	0	0	0	0	0	0	0	0	0	0	0	0
I61	CuSe6	8	0	0	5	7	0	0	0	0	0	0	0	0	0
I62	CuSe7	12	0	0	6	0	0	0	0	7	0	0	0	0	0
I63	CuSe7	6	0	0	0	7	0	0	0	4	0	0	0	0	0
I64	CuSe8	5	0	0	0	0	0	0	0	0	0	0	0	0	0
I65	CuSe8	12	3	0	3	3	10	0	0	8	0	0	0	0	0
I66	CuSe8	1	0	0	0	0	0	0	0	0	0	0	0	0	0
I67	CuSe8	12	2	0	5	0	8	0	0	0	0	0	0	0	0
I68	CuSe9	12	0	0	6	0	10	0	0	3	0	0	0	0	0
I69	ArUn4	8	0	0	4	0	7	0	0	0	0	0	0	0	0
I70	ArUn4	9	2	0	7	3	0	0	0	4	0	0	0	0	0
I71	PiNi8	7	0	0	0	0	0	0	0	0	0	0	0	0	0
I72	PiNi8	0	0	0	0	0	0	0	0	5	0	0	0	0	0
I73	ArUn5	8	3	0	7	0	0	0	0	2	0	0	0	0	0
I74	ArUn5	7	3	0	5	0	0	0	0	0	0	0	0	0	0
I75	ArUn6	10	3	0	0	0	9	0	0	0	0	0	0	0	0
I76	QuPu7	9	2	0	0	0	0	0	0	5	0	0	0	0	0
I77	QuPu8	10	5	0	0	0	0	0	0	7	0	9	0	0	0
I78	QuPu8	8	0	0	0	0	0	0	0	3	0	9	0	0	0
I79	QuPu9	8	4	0	3	0	0	0	0	0	0	0	0	0	0
I80	QuPu9	6	1	0	3	0	0	0	0	0	0	0	0	0	0
I81	QuPu10	9	3	0	5	0	0	0	0	0	0	4	0	2	0
I82	QuPu10	6	0	0	0	0	0	0	0	3	0	5	0	0	0
I83	JuOx3	7	5	0	2	2	8	0	0	0	0	0	0	0	0
I84	JuOx3	10	7	0	8	0	11	0	0	0	0	0	0	0	0
I85	JuOx4	9	3	0	7	0	0	0	0	9	0	0	0	0	0
I86	JuOx4	10	5	0	7	0	0	0	0	3	0	0	0	0	0
I87	JuOx5	11	6	0	8	0	3	0	0	0	1	0	0	0	0
I88	JuOx6	8	6	0	3	2	0	0	0	3	0	0	0	0	0
I89	JuOx7	10	5	0	7	0	0	0	0	7	0	0	0	0	0
I90	ArUn7	9	6	0	0	2	5	0	0	8	0	0	0	0	0
I91	ArUn7	6	4	0	4	0	0	0	0	0	0	0	0	0	0
I92	JuOx8	8	7	0	8	0	0	3	3	4	1	0	0	0	0
I93	JuOx8	9	7	0	8	0	6	7	7	3	0	0	0	0	0
I94	JuOx8	7	5	0	0	5	0	0	0	6	0	0	0	0	0
I95	ArUn8	6	5	0	3	0	0	0	0	8	0	0	0	0	0
I96	ArUn8	7	6	0	4	0	0	0	0	4	0	0	0	0	0
I97	ArUn8	8	8	0	3	0	10	0	0	6	0	0	0	0	0

APT – *Apatococcus*-like, COC – *Coccomyxa*, PRS – *Prasiola*, STC – *Stichococcus*, TRB – *Trebouxia*, KLB – *Klebsormidium*, SPR – *Spirotaenia*, MST – *Mesotaenium*, TRN – *Trentepohlia*, DIA – diatoms, NST – *Nostoc*, SCT – *Scytonema*, SNC – *Synechococcus*-like, OSC – *Oscillatoria*

Electronic appendix, part C

	tree	trebouxiophytes	streptophytes	Trentepohlia	Diatoms	Cyanobacteria
B1	TiCo1	12	0	0	1	0
B2	TiCo1	12	0	0	0	0
B3	TiCo1	12	0	0	0	0
B4	PcAb1	12	0	0	0	0
B5	PcAb1	7	0	0	0	0
B6	TiCo2	12	0	0	0	0
B7	TiCo2	10	3	0	0	0
B8	PiSy1	10	0	0	0	0
B9	PiSy1	7	0	0	0	0
B10	PcAb2	9	0	0	0	0
B11	PcAb2	12	0	2	0	0
B12	PcAb2	6	0	0	0	0
B13	TiCo3	11	0	0	0	0
B14	QuRo1	8	0	8	0	0
B15	QuRo1	11	0	4	0	0
B16	QuRo2	10	0	0	0	0
B17	PcAb3	9	0	0	0	0
B18	TiCo4	6	0	0	0	0
B19	PiSy2	9	0	0	0	0
B20	PiSy2	12	0	0	0	0
B21	QuRo3	11	0	4	0	0
B22	QuRo3	10	0	5	0	0
B23	PiSy3	2	0	0	0	0
B24	PiSy3	9	0	0	0	0
B25	PiSy3	9	0	0	0	0
B26	QuRo4	10	0	3	0	0
B27	QuRo4	10	0	0	0	0
B28	PcAb4	9	0	0	0	0
B29	PiSy4	1	0	0	0	0
B30	PoTr1	9	9	7	0	0
B31	PoTr1	10	0	4	0	0
B32	PcAb5	12	0	0	0	0
B33	PcAb5	8	0	0	0	0
B34	TiCo5	8	0	5	0	0
B35	TiCo5	10	0	5	0	0
B36	TiCo6	10	0	0	0	0
B37	TiCo6	10	0	8	0	0
B38	PoTr2	11	0	0	0	0
B39	PoTr2	12	8	6	0	0
B40	PoTr2	12	0	6	0	0
B41	PoTr2	11	0	10	0	0
B42	QuRo5	9	0	0	0	0
B43	QuRo5	12	0	0	0	0
B44	QuRo6	11	0	0	0	0
B45	QuRo6	12	0	0	0	0
B46	QuRo6	12	0	0	0	0
B47	QuRo6	12	0	3	0	0
B48	PiSy5	5	0	0	1	0
B49	PiSy5	11	0	0	0	0
B50	QuRo6	2	0	0	0	0
B51	QuRo7	12	0	0	0	0
B52	QuRo7	12	0	0	1	0
B53	PoTr3	11	0	0	0	0
B54	PoTr3	5	0	0	0	0
B55	TiCo7	12	0	0	0	0
B56	TiCo7	12	0	0	0	0
B57	TiCo7	6	0	0	0	0
B58	TiCo7	9	0	0	0	0
B59	TiCo8	7	0	6	0	0
B60	TiCo8	7	0	0	0	0
B61	PcAb6	6	0	0	1	0
B62	PcAb6	11	0	0	0	0

	tree	trebouxiophytes	streptophytes	Trentepohlia	Diatoms	Cyanobacteria
B63	PoTr4	10	0	0	0	0
B64	PoTr4	10	0	8	0	0
B65	PoTr4	9	0	3	0	0
B66	PoTr4	9	0	7	0	0
B67	PiSy6	3	0	0	0	0
B68	PiSy6	5	0	0	0	0
B69	PiSy6	4	0	0	0	0
B70	PiSy6	9	0	0	0	0
B71	PoTr5	10	5	3	0	0
B72	PoTr5	12	3	0	1	0
I1	PiNi1	8	0	0	0	0
I2	QuPu1	6	0	2	0	11
I3	QuPu1	6	7	6	0	5
I4	QuIl1	8	8	4	0	0
I5	QuIl1	8	4	0	0	0
I6	PiNi2	7	0	0	0	0
I7	QuIl2	10	0	10	0	0
I8	QuIl2	6	0	9	0	0
I9	QuIl2	4	0	0	0	0
I10	QuIl3	8	0	0	0	0
I11	QuIl4	10	0	6	0	0
I12	CuSe1	12	0	0	0	0
I13	CuSe1	12	0	6	0	0
I14	PiNi3	12	10	0	0	0
I15	PiNi3	12	0	0	0	0
I16	PiNi3	11	11	0	0	0
I17	QuIl5	8	10	9	0	0
I18	QuIl5	6	0	10	0	0
I19	QuIl5	6	0	12	0	0
I20	QuIl6	6	0	7	0	0
I21	QuIl6	6	0	3	0	0
I22	CuSe2	12	0	3	0	0
I23	CuSe2	9	0	0	0	0
I24	QuIl7	6	0	3	0	0
I25	QuIl7	8	0	2	1	0
I26	QuIl8	9	0	6	0	0
I27	QuIl8	10	3	10	0	10
I28	JuOx1	10	0	4	0	0
I29	JuOx1	11	5	0	0	0
I30	QuIl9	12	0	0	0	0
I31	QuIl9	12	0	8	0	0
I32	ArUn1	8	8	8	0	2
I33	ArUn1	12	8	2	0	10
I34	ArUn1	10	10	6	0	9
I35	ArUn2	8	0	6	1	0
I36	ArUn3	8	5	3	0	0
I37	ArUn3	9	0	0	0	0
I38	JuOx2	12	5	5	0	0
I39	JuOx2	12	3	2	0	0
I40	CuSe3	7	0	0	1	4
I41	CuSe3	12	0	0	0	4
I42	CuSe4	12	0	6	0	0
I43	CuSe5	3	0	7	0	0
I44	CuSe5	4	0	0	0	0
I45	PiNi4	12	0	0	0	0
I46	PiNi5	9	0	9	0	0
I47	QuPu2	4	0	5	0	6
I48	QuPu3	12	0	6	0	4
I49	PiNi6	9	0	8	0	0
I50	PiNi6	3	0	0	0	0
I51	QuPu4	10	0	3	0	0
I52	QuPu4	11	4	7	0	4
I53	QuPu4	10	0	7	1	0
I54	QuPu5	8	0	8	0	0

	tree	trebouxiophytes	streptophytes	Trentepohlia	Diatoms	Cyanobacteria
I55	QuPu5	10	0	0	0	0
I56	QuPu6	8	0	0	0	0
I57	QuPu6	6	0	0	0	0
I58	PiNi7	4	0	0	0	0
I59	PiNi7	8	0	0	0	0
I60	CuSe6	12	0	0	0	0
I61	CuSe6	8	0	0	0	0
I62	CuSe7	12	0	7	0	0
I63	CuSe7	7	0	4	0	0
I64	CuSe8	5	0	0	0	0
I65	CuSe8	12	10	8	0	0
I66	CuSe8	1	0	0	0	0
I67	CuSe8	12	8	0	0	0
I68	CuSe9	12	10	3	0	0
I69	ArUn4	8	7	0	0	0
I70	ArUn4	9	0	4	0	0
I71	PiNi8	7	0	0	0	0
I72	PiNi8	0	0	5	0	0
I73	ArUn5	8	0	2	0	0
I74	ArUn5	7	0	0	0	0
I75	ArUn6	10	9	0	0	0
I76	QuPu7	9	0	5	0	0
I77	QuPu8	10	0	7	0	9
I78	QuPu8	8	0	3	0	9
I79	QuPu9	8	0	0	0	0
I80	QuPu9	6	0	0	0	0
I81	QuPu10	9	0	0	0	4
I82	QuPu10	6	0	3	0	5
I83	JuOx3	7	8	0	0	0
I84	JuOx3	10	11	0	0	0
I85	JuOx4	9	0	9	0	0
I86	JuOx4	10	0	3	0	0
I87	JuOx5	11	3	0	1	0
I88	JuOx6	8	0	3	0	0
I89	JuOx7	10	0	7	0	0
I90	ArUn7	9	5	8	0	0
I91	ArUn7	6	0	0	0	0
I92	JuOx8	8	3	4	1	0
I93	JuOx8	9	7	3	0	0
I94	JuOx8	7	0	6	0	0
I95	ArUn8	6	0	8	0	0
I96	ArUn8	7	0	4	0	0
I97	ArUn8	8	10	6	0	0