Algal Research 51 (2020) 102062

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Symbiosis between river and dry lands: Phycobiont dynamics on river gravel bars

Lucie Vančurová[a,*], Veronika Kalníková[b], Ondřej Peksa[c], Zuzana Škvorová[a], Jiří Malíček[d], Patricia Moya[e], Kryštof Chytrý[b], Ivana Černajová[a], Pavel Škaloud[a]

[a] Charles University, Faculty of Science, Department of Botany, Benátská 2, 128 01 Prague 2, Czech Republic
[b] Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
[c] The West Bohemian Museum in Pilsen, Kopeckého sady 2, 301 00 Plzeň, Czech Republic
[d] The Czech Academy of Sciences, Institute of Botany, Zámek 1, 252 43 Průhonice, Czech Republic
[e] Dpto. Botánica, ICBIBE Fac. CC. Biológicas, Universitat de València, Dr. Moliner, 50, 46100 Burjassot, Valencia, Spain

ARTICLE INFO

Keywords:
Specificity
Lichen phycobiont
Succession
Community composition
Metabarcoding
Algal plurality

ABSTRACT

River gravel bars are dynamic and heterogeneous habitats straddling the transition between aquatic and terrestrial environments. Periodic flooding, low nutrient concentrations, frost, lack of stable sites, drought, and ground surface heat significantly influence the biota of these habitats. Mutualistic symbiosis may be a successful strategy for organisms to survive and proliferate under such harsh conditions. The lichen genus *Stereocaulon* was selected as a model symbiotic system from among the organisms living on river gravel bars. The goal of the current study was to determine the effect of this dynamic environment on phycobiont (*i.e.*, green eukaryotic photobiont) community structure. We analyzed 147 *Stereocaulon* specimens collected in the Swiss Alps using Sanger sequencing (fungal internal transcribed spacer (ITS) rDNA, algal ITS rDNA, and algal actin type I gene) and analyzed 8 selected thalli and 12 soil samples using Illumina metabarcoding (ITS2 rDNA). Phytosociological sampling was performed for all 13 study plots. Our analyses of communities of phycobionts, lichens, bryophytes, and vascular plants indicated a gradual change in the phycobiont community along a successional gradient. The particularly large phycobiont diversity associated with *Stereocaulon* mycobionts included algae, here reported as phycobionts for the first time. Each of the two *Stereocaulon* mycobiont operational taxonomic units had a distinct pool of predominant phycobionts. The thalli selected for Illumina metabarcoding contained a wide range of additional algae, *i.e.*, they showed algal plurality.

1. Introduction

River gravel bars are dynamic and heterogeneous habitats that occur in a range of ecosystems, from glacial floodplains and wide alpine river valleys to the piedmont [1–4]. Periodic flooding, together with variations in the speed and intensity of the water current, create richly braided rivers with a mosaic of channels, pools, bars, and islands [5–7]. The destruction and reformation of river gravel bars by floods result in wide structural changes and cyclic vegetation succession. The early successional vegetation type, together with subsequent vegetation types (mainly shrubs), form a mosaic of microhabitat conditions that are determined according to the disturbance levels [6,8–12]. Gravel bars in alpine zones significantly contribute to the regional diversity of the alpine environment [13]. The early- to mid-successional stands are often occupied by relatively diverse communities of vascular plants, which are characterized by high species richness and evenness, and relatively low vegetation cover. In later successional stages, the evenness and species richness decrease as organic matter and nutrients accumulate, and competition from established dominant species increases, as demonstrated in many studies [14–16].

Glacier-fed alpine rivers are highly influenced by daily flooding from the melting glaciers, which makes their conditions even more extreme (*e.g.*, [2,6,17]). Low nutrient concentrations in surficial substrates represent the most limiting environmental factor in glacial floodplains. Recently deglaciated terrain is characterized by bare soils, which do not contain any organic matter and initially lack a soil seed bank. Additionally, frost, lack of stable sites, drought, and ground surface heat significantly influence life in these habitats [4,18,19]. To help survive and persist in these conditions, common characteristics of gravel bar species include high diaspore dispersibility, fast growth,

* Corresponding author.
E-mail address: lucie.vancurova@natur.cuni.cz (L. Vančurová).

https://doi.org/10.1016/j.algal.2020.102062
Received 29 June 2020; Received in revised form 12 August 2020; Accepted 22 August 2020

tolerance of disturbance, clonal growth, and the ability to grow on nutrient-poor soils [20–25].

Under the harsh conditions of river gravel bars, mutualistic symbiosis may be a successful strategy for organisms to survive and proliferate [26,27]. Since lichens represent one of the oldest known and most recognizable examples of mutualistic symbiosis in stressful conditions [28], the lichen genus *Stereocaulon* was selected as a model symbiotic system from among the organisms living on river gravel bars. *Stereocaulon* is a widespread and ecologically successful pioneer lichen to be able to grow under harsh conditions on newly formed substrates [29,30]. Moreover, previous studies confirmed its ability to survive episodic submersion [31], even though it is not aquatic. Lichens are complex symbiotic systems, composed of various heterotrophic and autotrophic organisms. The presence of these various autotrophic and heterotrophic symbionts gives rise to a thallus with a typical phenotype [32]. The *Stereocaulon* mycobionts are associated with green algal symbionts (*i.e.*, phycobionts) and sometimes with additional cyanobionts located in specialized structures [33,34]. Recently, an exceptionally high diversity of phycobionts was discovered to be associated with *Stereocaulon*, including three ecologically diversified trebouxiophycean genera, *Asterochloris*, *Vulcanochloris*, and *Chloroidium* [35,36].

The ecological amplitude of the lichen mycobiont may be influenced by its specificity for the phycobionts [35,37]. Symbiotic interactions vary along environmental gradients [38] and could be affected by stressful environments [39,40]. Therefore, the goal of our study was to determine patterns in phycobiont diversity of *Stereocaulon* along a gradient of vegetation succession. Sanger sequencing of all 147 samples and Illumina metabarcoding of 8 selected thalli were applied to *Stereocaulon* specimens collected from 13 study plots to address the following questions: (1) is phycobiont diversity influenced by succession?; (2) how specific is *Stereocaulon* towards its phycobionts on river gravel bars?; and (3) does *Stereocaulon* growing on gravel bars exhibit algal plurality?

2. Material and methods

2.1. Study area and field sampling

The sampling was carried out in August 2017. Four localities, all situated on river gravel bars of glacial floodplains (1995–2070 m a.s.l.), were sampled across three glacial valleys: Morteratsch locality in the Morteratsch valley, Roseg I and Roseg II localities in the Roseg valley in the Bernina range, and Lonza locality in the Lötschental valley of the Lonza River in the Bernese Alps (a map is shown in Fig. S1). 13 vegetation plots (4 m × 4 m) were investigated. Study plots in each locality represented three successional stages [41]: (1) early stage (herbaceous early-successional scattered vegetation characterized by stands of alpine and scree-related herbs), (2) moderate (sparse scrub vegetation with willow species and *Myricaria germanica*), and (3) developed (stands with scattered trees of *Larix decidua* and scrubs of *Juniperus communis* subsp. *nana*), with the exception of the Roseg II locality where only the first and second stages were present (Tables 1, S1). Photographs of the study plots at different successional stages are given in Fig. S2.

Coordinates of each plot were recorded using a portable GPS (WGS-84 coordination system). The elevation of the gravel bar (as a distance from its highest point to the actual water level) and distance from the river were measured. One soil sample per plot was taken. All lichen, bryophyte and vascular plant taxa within the vegetation plots were recorded, with lichens and bryophytes collected from soil and stones. The cover of each species according to the extended Braun-Blanquet cover scale [42] and the total vegetation cover and the cover of each layer (tree, shrub, herb, moss, and lichen) were estimated in each plot. Vegetation plot data are listed in the Table S1. Ellenberg indicator values [43] and indicator values for bryophytes [44,45] were calculated. On each plot, a minimum of 10 *Stereocaulon* samples was collected; for each sample the type of substrate was noted. Only one morphospecies of *Stereocaulon* (*S. alpinum*) was found in the study area. Lichen morphospecies were identified in the field, as well as in the laboratory using standard microscopic and chemical methods, including spot tests and thin-layer chromatography (TLC). *Stereocaulon* vouchers were deposited in the Herbarium of Charles University in Prague (PRC) and vouchers of accompanying lichens in the personal herbarium of J. Malíček. Vascular plants and bryophytes unidentified in the field were collected for laboratory determination. All records for the vegetation plots were stored in the Gravel bar vegetation database – ID: EU-00-025 [46], which is included in the European Vegetation Archive [47]. Nomenclature follows Euro + Med PlantBase [48] for vascular plants, Hill et al. [49] for mosses, Grolle & Long [50] for liverworts, and Nimis et al. [51] for lichens.

2.2. DNA extraction, amplification, and Sanger sequencing

DNA was extracted from lichen thalli (total lichen DNA). Lichen thalli were examined under a dissecting microscope and washed with water before DNA extraction to remove possible surface contamination. Total genomic DNA was isolated from thallus fragments following the CTAB protocol [52]. Both algal and fungal nuclear internal transcribed spacers (ITS rDNA) and the algal actin type I gene (including one complete exon and two introns located at codon positions 206 and 248 [53]) were PCR amplified using primers listed in Table 2. PCRs were performed as described in Vančurová et al. [35]. All PCRs were performed in a volume of 20 µl using Red Taq Polymerase (Sigma) as described by Peksa and Škaloud [54] or with My Taq Polymerase. Negative controls, without DNA template, were included in every PCR run to eliminate false-positive results caused by contaminants in the reagents. The PCR products were sequenced using the same primers at Macrogen in Amsterdam, Netherlands. The newly obtained sequences were deposited in GenBank under accession numbers MT066249–MT066395, MT076321–MT076465, and MT093213–MT093219 (Table S2).

2.3. Sequence alignment and DNA analyses

Asterochloris datasets were analyzed both as a single locus for the ITS rDNA (data not shown) and as a concatenated dataset of ITS rDNA and actin type I loci. The *Asterochloris* ITS rDNA dataset consisted of 202 sequences (142 newly obtained and 60 previously published) from *Stereocaulon* and other lichens retrieved from GenBank. The actin type I dataset consisted of 67 sequences (7 newly obtained and 60 previously published). Actin type I locus was sequenced primarily in those samples where unique ITS rDNA barcodes were obtained, to increase the phylogenetic resolution. Since (1) ITS rDNA and actin type I topologies are highly congruent, and (2) the samples with identical ITS rDNA barcodes generally show identical actin type I locus sequences, actin type I sequences were not obtained for all studied strains. The alignment was automatically performed by MAFFT v.7 software [59] under the Q-INS-I strategy and manually edited according to the published secondary structures of ITS2 rDNA [58] using MEGA v.6 [60]. The actin type I sequences were aligned using MAFFT v.7 software [59] under the Q-INS-I strategy. After deleting identical sequences, the resulting concatenated alignment comprised 64 samples represented by unique ITS rDNA and actin type I sequences.

The ITS rDNA dataset of the *Stereocaulon* mycobiont comprised 171 sequences: 145 newly obtained sequences and 26 representative sequences selected to cover all the main clades 1–8 published by Högnabba [61]. The alignment was automatically performed by MAFFT v.7 software [59] under the Q-INS-I strategy. After removing identical sequences, the resulting alignment comprised 48 sequences. All DNA alignments are freely available on Mendeley Data: http://dx.doi.org/10.17632/jchg5h3t5k.1.

Phylogenetic relationships were inferred with the Bayesian

Table 1
Location of study plots.

Plot number	Successional stage	Locality	Altitude (m)	River distance (m)	Height above river (m)	GPS coordinates	
1	1	Morteratsch	2070	10.0	0.8	46.4308528	9.9357028
2	2	Morteratsch	2018	35.0	2.5	46.4305556	9.9350000
3	3	Morteratsch	2026	240.0	13.0	46.4332500	9.9332500
4	1	RosegI	2034	15.0	0.5	46.4253611	9.8604444
5	1	RosegI	2031	2.0	0.7	46.4250278	9.8602500
6	2	RosegI	2040	75.0	1.0	46.4238333	9.8590278
7	3	RosegI	2050	20.0	2.5	46.4215556	9.8586111
8	2	RosegII	2012	10.0	1.5	46.4341111	9.8649722
9	1	RosegII	1997	2.5	0.4	46.4376944	9.8702778
10	1	Lonza	1995	0.3	1.0	46.4459722	7.8999167
11	1	Lonza	2027	4.0	1.0	46.4473056	7.9040556
12	2	Lonza	2003	16.0	0.9	46.4463333	7.9000833
13	3	Lonza	2007	200.0	10.0	46.4465000	7.8997778

Inference (BI) carried out in MrBayes v.3.2.2 [62], maximum likelihood (ML) analysis implemented in GARLI v.2.0 [63], and maximum parsimony (MP) analysis using PAUP v.4.0b10 [64]. BI and ML analyses were carried out on a dataset partitioned into ITS1, 5.8 S and ITS2 rDNA, actin intron 206, actin intron 248, and actin exon regions. The best-fit substitution models (Table S3) were selected using the Bayesian information criterion (BIC) implemented in JModelTest2 [65,66]. ML analysis was carried out using default settings, five search replicates, with an automatic termination set at 5 million generations. The MP analysis was performed using heuristic searches with 1000 random sequence addition replicates and random addition of sequences (the number was limited to 10^4 per replicate). ML and MP bootstrap support values were obtained from 100 and 1000 bootstrap replicates, respectively. Only one search replicate was applied for ML bootstrapping.

2.4. Ecological community analyses

From a total of 147 samples with successfully sequenced phycobionts, two were excluded due to the absence of a mycobiont sequence. Since the mycobiont identity affects the phycobiont diversity [35], four samples belonging to the minority species-level lineage (OTU2) were also excluded. Thus, statistical analyses were carried out using 141 members of the prevailing mycobiont species-level lineage (OTU35) and their phycobionts. Since the number of samples per plot varied, it was impossible to perform analyses requiring an equal number of samples per plot with the original dataset. Therefore, the number of phycobiont species-level lineages was rarefied to the smallest sample size in the data set, *i.e.* five samples (Fig. S3). After excluding study plot no. 11 (with the sample size of 5), the smallest sample size in the data set increased to 10 samples. The rarefaction was performed using the *rarefy* function in *vegan* R package [67].

To visualize phycobiont diversity in the context of surrounding vegetation, an ordination model (non-metric multidimensional scaling; NMDS) was computed as available in vegan package of R [67]. The input dataset included vascular plants, bryophytes and lichens, whose cover values were converted to percentage and further log transformed. Afterwards, four variables (*i.e.*, successional stage, number of lichen species, number of phycobiont species rarefied to sample size 5 and proportion of locally common phycobionts) were passively fitted using the function *envfit* of vegan R package. *Asterochloris* StA5 and *A. phycobiontica* were considered as locally common phycobionts.

Thereafter, the relationship between species richness of *Stereocaulon alpinum* OTU35 phycobionts and overall lichen species richness was investigated in order to determine if there was a correlation between the number of phycobiont species-level lineages and number of lichen species. The linear regression was performed separately for the dataset including all plots and the dataset restricted to plots with sample size ≥ 10. Since the parametric regression analyses can be significantly biased in small sample sizes, the Bayesian linear regression was used and the number of phycobionts modeled as a function of lichen species richness.

The gradual change of phycobiont community composition was inspected as a correlation between the proportion of the two most abundant phycobiont species-level lineages ((number of *Asterochloris phycobiontica* samples + number of StA5 lineage samples)/number of all samples) and the successional stage (coded 1, 2 and 3). The proportion of the most abundant phycobionts was modeled as a function of the successional stage. The program JAGS v. 4.2.0 [68] through the *R2JAGS* package [69] in R was used to fit all regression models.

The vegetation plot data were stored in Turboveg for Windows v.2 database [70] and further managed with JUICE software [71] and in the R environment [72] with the help of the *vegan* R package [67].

Bipartite association networks were produced using *bipartite* R package [73].

2.5. Illumina metabarcoding of algal communities in selected lichen thalli and soil samples

In order to describe algal plurality in *Stereocaulon* thalli, Illumina metabarcoding was performed. Eight thalli (four assigned to mycobiont OTU35 and four to OTU2) were examined. The samples which showed difficulties with Sanger sequencing of predominant phycobiont, a probable/possible sign of the algal plurality, were selected [74]. Therefore, the evaluation of the frequency of this phenomenon is beyond the scope of this study. The samples were rehydrated with Milli-Q

Table 2
Primers used in this study.

Name	Sequence		Reference
nr-SSU-1780-5′	5′-CTG CGG AAG GAT CAT TGA TTC-3′	Algal ITS region, algal-specific	[55]
ITS1-F-5′	5′-CTT GGT CAT TTA GAG GAA GTA A-3′	Fungal ITS region, fungal-specific	[56]
ITS4-3′	5′-TCC TCC GCT TAT TGA TAT GC-3′	Algal and fungal ITS region, universal	[57]
ActinF2 Astero-5′	5′-AGC GCG GGT ACA GCT TCA C-3′	Actin type I locus, algal specific	[58]
ActinR2 Astero-3′	5′-CAG CAC TTC AGG GCA GCG GAA-3′	Actin type I locus, algal specific	[58]
1378-Chlorophyta	5′-TTG CCT TGT CAG GTT GAT TCC GG-3′	Illumina sequencing of ITS2	This study
5.8F-Chlorophyta	5′-GAA TTC CGT GAA CCA TCG AAT CTT T-3′	Illumina sequencing of ITS2	This study

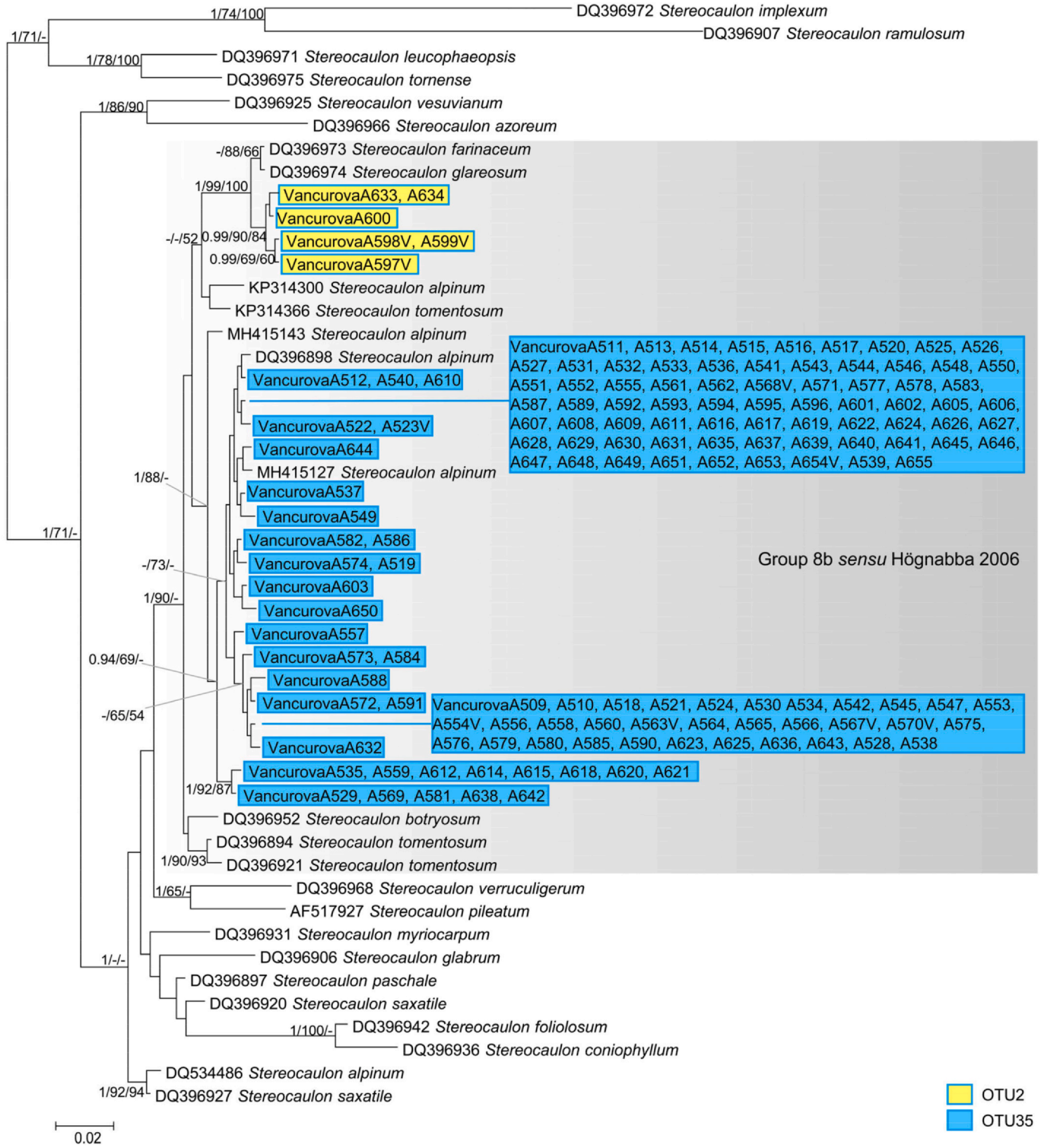


Fig. 1. Unrooted phylogenetic hypothesis of *Stereocaulon* resulting from the Bayesian analysis of ITS rDNA. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are in boxes. All new sequences belong to Group 8b *sensu* Högnabba [61], as marked.

sterile water one day before being processed and stored in a growth chamber at 20 °C under a 12 h/12 h light/dark cycle (15 µmol/m^2/s). Thalli were cleaned under a stereomicroscope to remove soil particles and then superficially sterilized following Arnold et al. [75]. Fragments from different parts of each thallus were randomly excised and pooled together (0.1 mg). Total genomic DNA was isolated and purified using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

Soil samples, one from each study plot, were sieved to remove contamination. Total genomic DNA was isolated and purified using the Soil DNA Isolation Plus Kit® (Norgen Biotek Corp.), following the manufacturer's instructions. Since the soil sample from plot number 7 was not processed successfully, only 12 soil samples were analyzed in the next steps.

Chlorophyta algal communities associated with the eight thalli and

12 soil samples were assayed using Illumina high-throughput sequencing of ITS2 of the rRNA operon, proposed as a universal barcode across eukaryotic kingdoms [76]. High-coverage PCR primers at conserved sites were designed using a customized database for the algal phylum Chlorophyta (Table 2).

Amplicons for Illumina MiSeq sequencing were generated from nested PCR: in the first PCR the forward 1378-Chlorophyta (newly designed; Table 2) and the reverse ITS4 primers [57] were used and 27 amplification cycles were run, in the second PCR three replicates were amplified using the primers 5.8F-Chlorophyta (newly designed; Table 2) and ITS4 modified with Illumina overhang adaptors (forward overhang: 5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG-3′; reverse overhang: 5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3′) and 22 amplification cycles were run. These three replicates were then pooled together. PCR reactions were performed as described in Moya et al. [77]: All PCRs (25 μl) contained 2.5 μl of 10× buffer, 0.4 μM primers, 0.2 mM dNTPs, and 0.6 u/μl of ExTaq (Takara, Shiga, Japan), and sterile Milli-Q water was used to bring to correct volume. The PCR conditions were 1 cycle of 95 °C for 2 min; 27 or 22 number of cycles (as described above) of 94 °C for 30 s, 56 °C for 45 s, and 72 °C for 1 min; and a final extension of 72 °C for 5 min.

PCR products were purified using AMPure XP beads (Beckman Coulter). Indexing PCR and addition of Nextera sequence adapters were performed using Nextera XT Index kit (Illumina Inc., San Diego, CA, USA) following the protocol for Illumina L library preparation. Finally, a second purification round was carried out using AMPure XP beads. Libraries were then quantified and pooled together. The libraries were sequenced on Illumina MiSeq platform using the MiSeq Reagent Kit v3 (paired end 2 × 300 bp), at STAB Vida, Lisbon, Portugal and Genomics Core Facility at the University of Valencia, Spain.

2.6. Bioinformatics analyses

Quality control analysis of the Illumina MiSeq paired-end reads was performed using the FastQC *v.0.11.8*. Raw reads were processed using Quantitative Insights Into Microbial Ecology 2 (QIIME2 v.2018.11 [78]). Demultiplexed paired-end sequence reads were pre-processed using DADA2 [79], a package integrated into Qiime2 that accounts for quality filtering, denoising, joining paired ends, and removal of chimeric sequences. The first 20 bp were trimmed from forward and reverse reads before merging to remove adaptors. In order to remove lower quality bases, amplicon sequence variants (ASVs) were truncated at position 210 based on the FastQC reports during this step.

Subsequent analyses were based on the ASV table, which contained the count for each unique sequence in each sample. Only ASVs with frequency ≥100 were further analyzed. BLAST searches were used to confirm the sequence identity. Exclusively algal sequences were further analyzed. A phylogenetic tree (Fig. S4) was inferred with Bayesian Inference (BI) using MrBayes v.3.2.2 [62] as described above. Euler diagrams were produced using *eulerr* R package [80].

3. Results

3.1. Species composition in the study plots

In total, 88 vascular plant taxa, 19 bryophyte taxa, and 45 lichen taxa were recorded within the 13 study plots. Table S4 contains a summary of the species richness for individual plots.

3.2. Diversity of phycobionts and mycobionts

To address the overall diversity of the *Stereocaulon* mycobionts and their phycobionts in the study area, a phylogenetic analysis of the internal transcribed spacer (ITS) rDNA loci of both partners was performed. A phylogram resulting from the Bayesian analysis of the ITS rDNA sequences of the *Stereocaulon* mycobionts is shown in Fig. 1. The majority of the recovered mycobiont sequences formed a well-supported lineage delimited as operational taxonomic unit 35 (OTU35) by Vančurová et al. [35]. Five sequences matched the distantly related OTU2 (sister to DQ396973 and DQ396974), despite the morphological similarity of all the studied samples. Both OTU35 and OTU2 fall into Group 8b *sensu* Högnabba [61].

The predominant phycobiont (*i.e.*, the most abundant alga within a particular thallus [74]) detected in 97% of the *Stereocaulon* samples belonged to the genus *Asterochloris*, while only five specimens represented other trebouxiophycean algae. In the case of these five specimens (coded A574, A574.1, A633, A634, and A634V) the identity of the phycobionts was confirmed by a Blast search against the GenBank database. Significant matches from 99% to 87% were obtained for A574 as *Coccomyxa viridis* HG973000, A574.1 as *Elliptochloris reniformis* LT560354, and A633, A634, and A634V as uncultured Trebouxiophyceae FJ554399. These latter three sequences formed a well-supported clade with the more distantly related sequence KF907701 (86% sequence similarity; Fig. S4), which was previously assigned to clade URa28 [81]. These sequences are referred to by this nomenclature hereafter.

The phylogenetic hypothesis resulting from the Bayesian analysis of the ITS rDNA and actin type I sequences of *Asterochloris* (Fig. 2) was congruent with that of previous studies [35,54,82–84]. The species boundaries delimited by Vančurová et al. [35] and the nomenclature used [35] were maintained. A total of 14 lineages, including one novel lineage, here referred to as StA9, were recorded. Eleven of these lineages were previously determined as phycobionts of *Stereocaulon* ([35] and references therein); namely, *A. glomerata*, *A. irregularis*, *A. italiana*, *A. lobophora*, *A. phycobiontica*, *A.* aff. *italiana*, *Asterochloris* clade 8, *Asterochloris* clade 12, *Asterochloris* StA3, *Asterochloris* StA4, and *Asterochloris* StA5. Two of these 14 lineages (*A. echinata* and *A. leprarii*) were found in association with a *Stereocaulon* mycobiont for the first time in this study. The most frequently occurring phycobionts were linked with the lineages *Asterochloris* StA5 and *A. phycobiontica*.

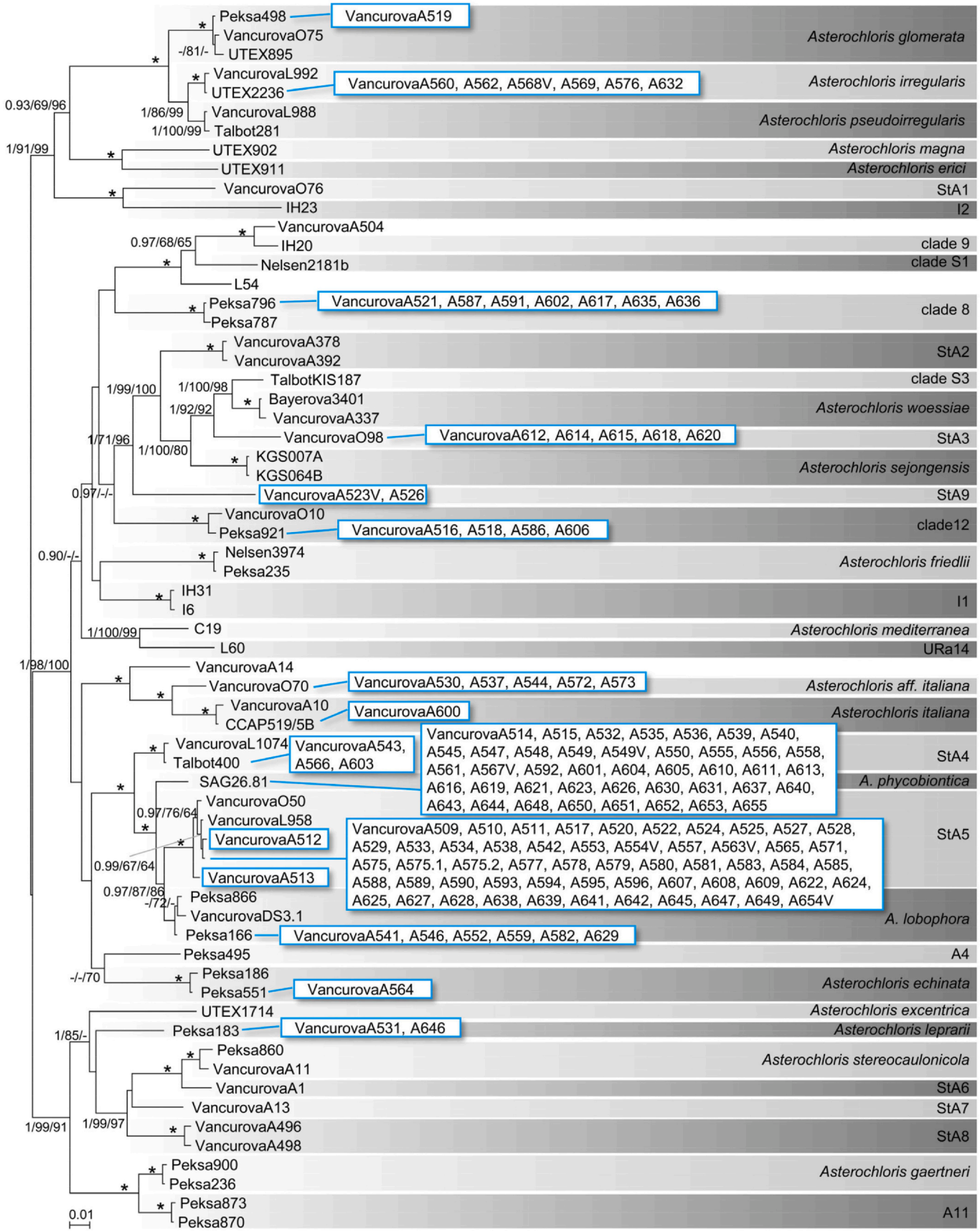
3.3. Phycobiont community structure and its changes along a successional gradient

Stereocaulon alpinum OTU2 (n = 4) was only sampled in two study plots (nos. 8 and 11). One sample was assigned to *A. italiana* and three were assigned to the trebouxiophycean lineage URa28. None was shared with the dominant *Stereocaulon* mycobiont (OTU35) in the study area (Fig. 3).

Stereocaulon alpinum OTU35 (n = 141) was associated with 15 distinct species-level lineages of the phycobionts in the study area: 13 lineages of *Asterochloris*, 1 *Coccomyxa*, and 1 *Elliptochloris*. Phycobionts from two to six species-level lineages were recorded in each of the 13 study plots (Fig. S5). When the sample size was reduced to five, 2.0–3.7 phycobiont species per plot were expected. For a sample size of 10 (excluding plot 11, which only had five samples), 2.8–5.3 phycobiont species per plot were expected (Fig. S3; Table 1).

To visualize the phycobiont diversity in the context of the surrounding vegetation (vascular plants, bryophytes, and lichens), an ordination model was computed. The non-metric multidimensional scaling (NMDS) ordination (stress value 0.132) mostly reflected the successional gradient (Fig. 4). The fitted variable of succession (fit in the ordination: $r^2 = 0.3689$) was positively correlated with number of lichens ($r^2 = 0.7273$) and the number of phycobiont species ($r^2 = 0.1872$); in contrast it was strongly negatively correlated with the proportion of locally common phycobionts ($r^2 = 0.1988$).

These results were supported by the Bayesian linear regression: the species richness of the *S. alpinum* OTU35 phycobionts significantly increased with the species richness of all the lichens recorded at a study plot (Fig. 5), while the proportion of the two most abundant phycobiont species-level lineages (*A. phycobiontica* and *Asterochloris* StA5) significantly decreased with increasing successional stage in favor of other



(caption on next page)

Fig. 2. Unrooted phylogenetic hypothesis of *Asterochloris* resulting from the Bayesian analysis of combined ITS rDNA and actin type I sequences. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are in boxes. Clade affiliations: clade 8, clade 9 *sensu* Škaloud and Peksa [58], A4, A11 *sensu* Peksa and Škaloud [54], URa14 *sensu* Ruprecht et al. [81], I1, I2 *sensu* Řídká et al. [85], S1, S3 *sensu* Nelsen and Gargas [86], *A. aff. italiana* and StA1 – StA8 *sensu* Vančurová et al. [35]. StA9 lineage was identified as new in present study. Table S5 contains accession numbers of reference sequences retrieved from GenBank.

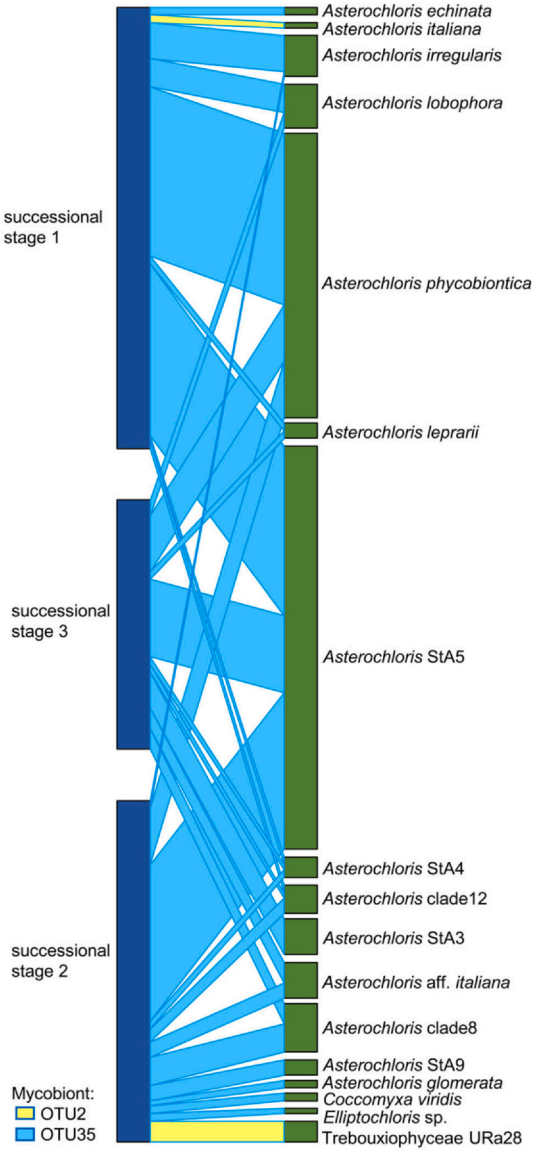


Fig. 3. Bipartite association network between successional stages and phycobiont species-level lineages. The width of the links is proportional to the number of specimens forming the association.

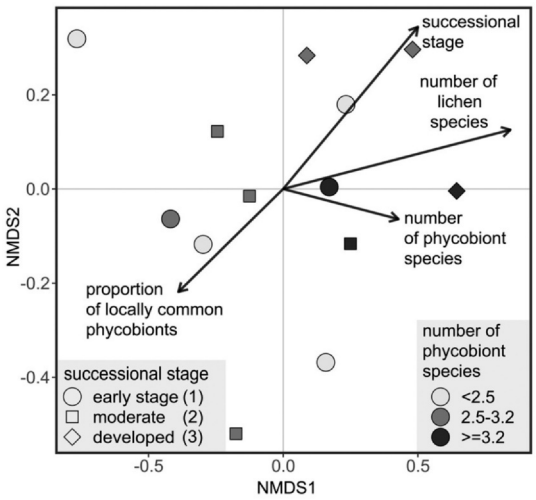


Fig. 4. Ordination diagram of non-metric multidimensional scaling (NMDS). The stress of the model is 0.132. The shape of the symbols represents the successional stage and their color the three levels of number of phycobiont species. Four variables (successional stage, number of lichen species, number of phycobiont species and proportion of locally common phycobionts) were passively superimposed onto the ordination plot.

species recovered at a lower frequency (Fig. 6). The phycobiont communities of the early-successional stages were relatively species-poor and mostly consisted of species that were generally abundant in the study area. With ongoing succession, the number of locally rare phycobiont species increased, together with the total number of phycobiont and lichen species.

3.4. Algal plurality detected using microalgal metabarcoding

Phycobiont diversity within particular lichen thalli (n = 8) was inspected using Illumina metabarcoding. A total of 1,945,186 raw readings were generated, of which 1,240,063 passed the demultiplexing step and quality filter. This represented an average of 155,007 algal reads (minimum = 38,329, maximum = 246,121, median = 179,415) per sample. The filtered metabarcoding dataset consisted of 116 hits (4 to 44 per sample).

The abundances of the recovered algal clades by sample are shown in Fig. S6, with the predominant phycobiont comprising 52.4–98.8% of the readings. Thalli A523M, A554M, A563M, and A570M (assigned to the mycobiont OTU35) contained various *Asterochloris* species as the predominant phycobiont, which were assigned to lineage StA9 (sample A523M), *A. irregularis* (A563M), and *A. phycobiontica*/StA4/StA5 (A554M and A570M). The species-level lineages StA4, StA5, and *A. phycobiontica* were indistinguishable using the ITS2 rDNA marker. The relative frequency of amplicon sequence variants (ASVs) linked to *Asterochloris* spp. by sample is shown in Fig. S7. Thalli A597M, A598M, A633M, and A634M (assigned to mycobiont OTU2) predominantly contained phycobionts from the trebouxiophycean lineage URa28.

3.5. Soil as a potential reservoir for phycobionts

The phycobiont diversity in 12 soil samples was analyzed using Illumina metabarcoding. A total of 1,524,198 raw reads were generated, 876,596 of which passed the demultiplexing step and quality filter. This represented an average of 73,049 algal reads (minimum = 18,255, maximum = 164,643, and median = 63,827) per sample. The filtered metabarcoding dataset consisted of 427 hits (4 to 89 per sample). The phylogenetic hypothesis resulting from the Bayesian analysis of the ITS2 rDNA sequences obtained by the metabarcoding of soil samples, selected lichen samples, and reference sequences from GenBank is shown in Fig. S4. Sequences were recovered in 44 well-supported clades, 27 of which exclusively contained soil algae, 2 exclusively contained phycobionts, and 15 were shared by these two groups. The occurrence of particular clades of soil algae in each study plot is depicted in Fig. S8.

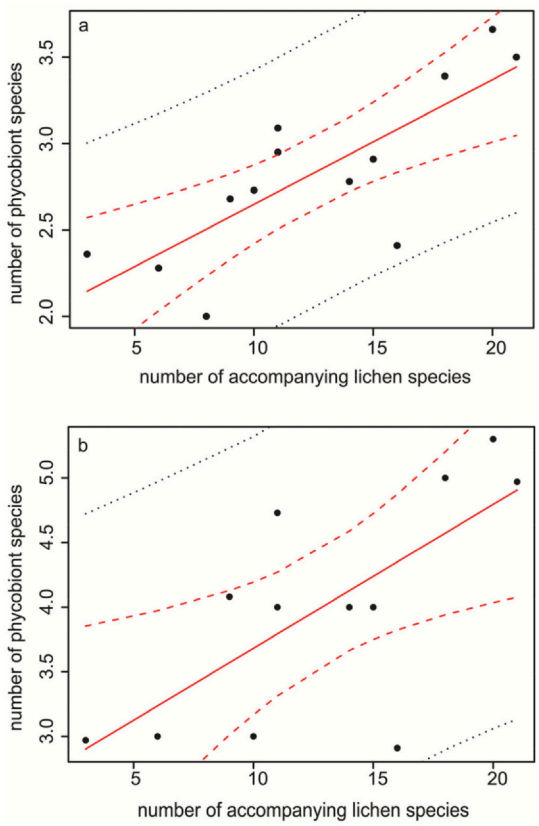


Fig. 5. Bayesian linear regression of number of accompanying lichen species as a predictor of the number of phycobionts associated with mycobiont OTU35 rarefied to **a** sample size of 5, **b** sample size of 10 per plot. Dashed lines show the 95% CRI around the regression line.

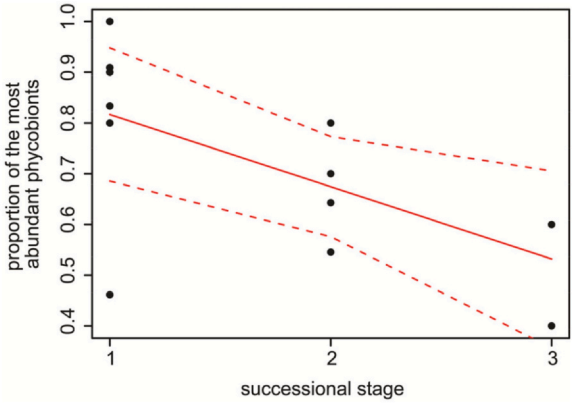


Fig. 6. Bayesian linear regression of number of a successional stage as a predictor of the proportion of the most abundant phycobiont species-level lineages ((number of *Asterochloris phycobiontica* samples + number of StA5 lineage samples) / number of all samples). Dashed lines show the 95% CRI around the regression line.

To determine the shared pool of algae between the lichen phycobionts and free-living soil algae, the occurrence of particular algal ITS2 haplotypes was analyzed, including the whole dataset obtained by Sanger sequencing (probably predominant phycobionts; Fig. 7a). Nine of the haplotypes obtained by Sanger sequencing were also found by Illumina sequencing of the soil and lichens. The vast majority of haplotypes were unique for the soil (n = 256) or Illumina lichen (n = 79) datasets. However, 27 haplotypes were shared by the soil and lichens but were not detected by Sanger sequencing using DNA extractions

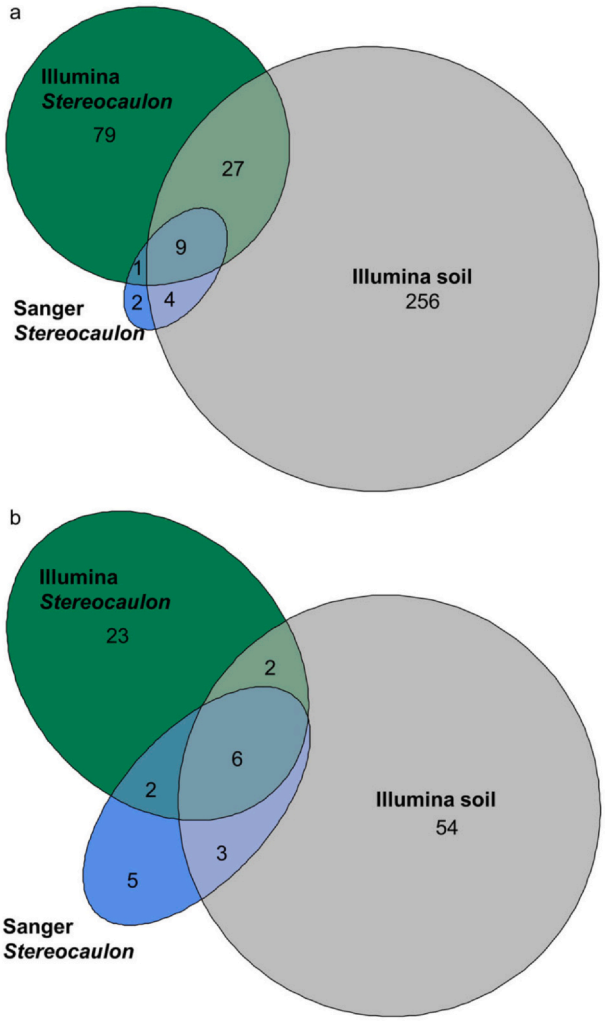


Fig. 7. Euler diagrams depicting sets of algal ITS2 rDNA haplotypes recovered from selected *Stereocaulon* thalli (n = 8) using Illumina metabarcoding, from all *Stereocaulon* samples (n = 147) using Sanger sequencing and from soil samples (n = 12) using Illumina metabarcoding. In case of Illumina metabarcoding sets, only haplotypes with frequency **a** ≥100 and **b** ≥1000 were included.

from *Stereocaulon* in the study area. The same analysis that was restricted to haplotypes with a frequency ≥ 1000 (to eliminate possible bias produced by errors from the polymerase chain reaction and sequencing [87]) showed a similar pattern (Fig. 7b).

4. Discussion

4.1. Change of community structure along a successional gradient

Succession on river gravel bars is an important driver of both species composition and diversity. It is well documented in the case of vascular plants (e. g., [8,16]), but also applies to microorganisms, such as soil bacteria or mycorrhizal fungi [88,89]. However, different groups of taxa respond differently to this gradient; for example, vascular plants are known to follow a nested-community pattern, where the highest species diversity is associated with early- to mid-successional stages, and community diversity declines with ongoing succession (*e.g.*, [14,90,91]). This pattern of vascular plant species richness was observed in the current study.

The pattern observed for the phycobiont communities of *Stereocaulon* lichens differed. The phycobiont communities of the early-vegetation stages were composed of relatively few species-level

lineages, such as *A. phycobiontica* and *Asterochloris* StA5 (Fig. 3), which are alpine and psychrophilic [35,54]. As locally adapted lineages, they are probably common in populations surrounding the study plots. Therefore, newly emerged river gravel bars are easily colonized by the *A. phycobiontica* and *Asterochloris* StA5 lineages. In subsequent stages, the observed species richness of the phycobiont algae mostly increased and these two species-level lineages were gradually substituted by other lineages (Fig. 6). Some of these lineages could be specialized to slightly different microhabitat conditions within particular plots; for example, clades 8, 12, and StA3 tolerate a higher pH [35,55,92,93]. On the river gravel bars of glacial floodplains, organisms with various substrate optima could coexist due to the heterogeneity of the substrate transported by a river or glacier from distant localities and various substrate layers. In the study area, acidophilic vascular plants and bryophytes dominated, but the occurrence of basophilic species, such as *Didymodon fallax*, *Lophozia excisa*, *Syntrichia ruralis*, and *Veronica fruticans*, as found in our data, was considered an indication of the basic fractions of the substrate.

On the river gravel bars, the species richness of the phycobionts was positively correlated with that of lichens (Fig. 5). This correlation possibly indicates the phycobionts and mycobionts use similar dispersal strategies while colonizing newly exposed gravel bar stands. However, it could also be connected with other variables, such as changing microhabitat heterogeneity. The species richness of terricolous lichens on glacier forelands in the Alps was positively correlated with the time since deglaciation [94], analogous to lichen species richness on deglaciated plots in maritime Antarctica [95]. In both cases, most species, once established, persisted until the oldest successional stages. Beck et al. [96] found two haplotypes of *Stichococcus antarcticus*, a phycobiont of the *Placopsis* lichen in maritime Antarctica, exclusively occurred in areas that had been deglaciated for a long time and had a more developed soil and lichen community. The succession of vegetation causes numerous physical and chemical changes in the soil, as the abundance of organic matter in the soil increases with increasing plant cover. Therefore, the correlation between the species richness of various organisms and successional stages is frequently connected with changing soil characteristics, such as nutrient concentrations [14,41,88,89].

There are two possible sources of phycobionts for the lichens on river gravel bars: algae that continually colonize the gravel bars from surrounding areas or soil algae *in situ* [97–99]. Several algal clades were found in both the soil and the lichens (Fig. S4), but the phycobiont pool appeared to be independent of the soil algae; for example, the most frequent ITS2 rDNA haplotype among Sanger sequences (*A. phycobiontica*/StA4/StA5, which were recovered in 69% of all samples) was present in only two soil samples at very low abundances (212 (2.4%) and 135 (3.1%) of algal readings). In addition, other haplotypes were abundant in the lichens and rare in the soil or *vice versa*. Approximately, only ten ITS2 haplotypes belonging to the genera *Asterochloris*, *Elliptochloris*, and clade URa28 were abundant in both soil and lichen thalli. In Fig. 8, a comparison of the relative abundances of the algal clades in soil samples and lichen thalli is presented. Only plots 4, 5, 6, and 8, with soil samples generating > 5000 algal reads and with *Stereocaulon* samples analyzed using Illumina metabarcoding, are displayed. The discrepancy between the communities of soil and lichen algae supports our hypothesis that phycobionts originating from the surrounding area (probably from other lichen populations) colonize the recently emerged plots, without a substantial contribution from the "soil seed bank." However, these results should be perceived as the basis for future research. The number of soil samples was rather limited, and some taxa could have remained undetected [100]. Nevertheless, the taxonomic composition of the algae occurring on the river gravel bars was comparable to the pool of soil algae detected in the foreland of the Damma glacier in the Swiss Alps [101]. Notably, a significantly different algal community was found in the early-successional stage, which in that case was represented by bare soil near a receding glacier. The lichen phycobionts, including *Asterochloris*, were reported from the soil in the transitional and developed stages. One of *Asterochloris* sequences recovered by Frey et al. [101] corresponds with the lineage StA9, which was first reported as a lichen phycobiont in the present study.

A similar pattern was demonstrated for corals, with little overlap between the pool of photosynthetic symbionts in the sediment and the host [102]; however, Ali et al. [103] demonstrated a significant influence of the sediment on coral symbiosis establishment.

4.2. Low specificity as an adaptive strategy

The specificity (*i.e.*, the taxonomic range of acceptable partners [104–106]) of both mycobionts and phycobionts has been considered a crucial characteristic of lichen interactions. A reduced specificity of symbiotic partners was frequently reported as an advantageous strategy in harsh environments [39,40].

Both species-level lineages of *Stereocaulon* recorded in the study area (Fig. 1) were morphologically identical and indistinguishable in the field. The overwhelming majority of samples belonged to OTU35, which was reported to have low specificity towards its phycobionts [35]. On river gravel bars, the mycobionts belonging to this lineage frequently associated with algae that are generally known as the phycobionts of *Lepraria* lichens (*A. phycobiontica*, *A. echinata*, and *A. leprarii* [84]). Such low specificity (OTU35 was associated with 13 species-level lineages of *Asterochloris* and, in two cases, with representatives of other trebouxiophycean phycobionts) could facilitate the colonization of heterogeneous and harsh habitats, including river gravel bars of glacial floodplains.

On the other hand, *S. alpinum* OTU2 was associated with different phycobionts, despite growing in the same environment as *S. alpinum* OTU35. The OTU2 mycobiont is mostly associated with the trebouxiophycean alga URa28. This alga was previously detected in a soil sample from Canada, either as a soil alga or possibly as a phycobiont of *Stereocaulon* sp., which was recorded in the same location [107].

One of the alternative hypotheses concerning the specificity towards the phycobionts is that lichens tightly attached to the substrate were considered less specific than lichens with a fruticose growth form [108,109]. However, *S. alpinum* has a well-developed fruticose thallus, and our samples were no exception to this, despite harsh environmental conditions. Within the genus *Stereocaulon*, other mycobiont species were reported with low specificity towards their phycobionts and were able to establish symbiosis with more than one algal genus. The existence of more algal genera associated with *Stereocaulon* lichens, in addition to *Asterochloris*, *Chloroidium* [110], *Vulcanochloris* [35,36], *Elliptochloris*, *Coccomyxa*, and the clade URa28, could be expected.

4.3. Algal plurality

The occurrence of more than one phycobiont species in a single lichen thallus (*i.e.*, algal plurality) was an overlooked phenomenon but revealed to be quite common in lichens (*e.g.*, [77,92,111–113]), and has been documented in *Stereocaulon* [35]. However, various lichen species differ in the prevalence of algal plurality. Dal Grande et al. [114] revealed the occurrence of more than one phycobiont species in 49.2% of *Lasallia hispanica* thalli but in only 1.7% of *L. pustulata* thalli. Leavitt et al. [109] proposed the hypothesis that those lichens, which are unspecific towards their phycobionts, more frequently exhibit algal plurality.

Using Illumina metabarcoding, more than one phycobiont species was found in all selected samples from both the mycobiont species-level lineages (Fig. S6). However, these samples were selected because of difficulties with Sanger sequencing, which in itself could indicate algal plurality [74]. Illumina metabarcoding as well as Sanger sequencing uncovered URa28 as the predominant phycobiont of *S. alpinum* OTU2. In most cases, the phycobiont determined by Sanger sequencing corresponded with the predominant phycobiont according to high-

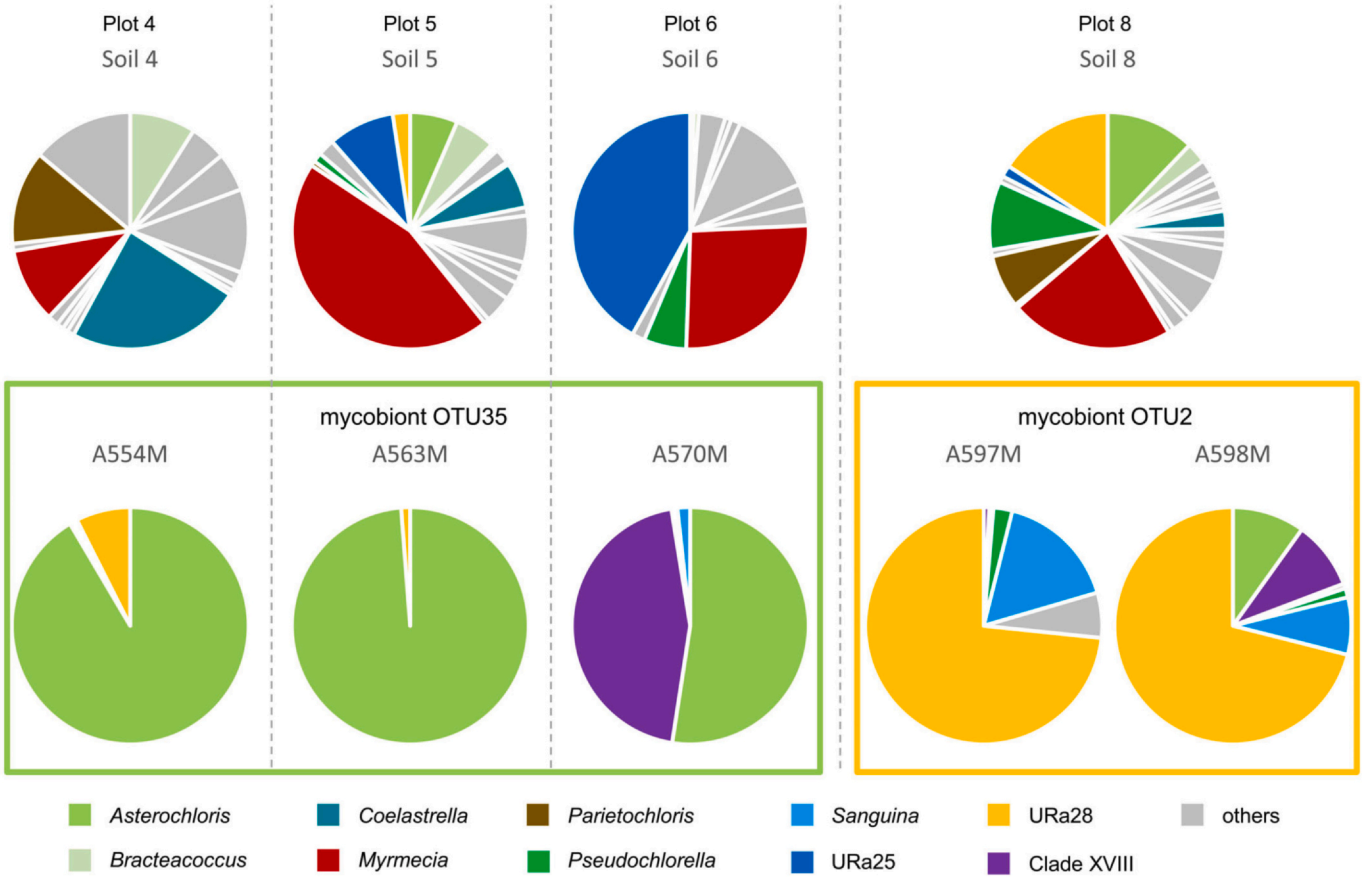


Fig. 8. Relative abundances of algal clades within soil (first line) and *Stereocaulon* (second line) samples. Solely plots 4, 5, 6, and 8 with soil samples generating > 5000 algal reads and with *Stereocaulon* samples analyzed using Illumina metabarcoding were displayed. Clade affiliations: URa25, URa28 *sensu* Ruprecht et al. [81]. Clade XVIII was identified as new in present study. Amplicon sequence variants (ASVs) were sorted into these clades based on phylogenetic hypothesis presented in Fig. S4.

throughput sequencing [74,115].

Even though the two mycobiont species-level lineages, OTU2 and OTU35, differed in their predominant phycobiont pools, they shared the pool of other intrathalline algae, unlike two *Circinaria* spp. collected at the same location; these shared the predominant phycobiont pool but showed a completely different pool of other intrathalline algae [115]. The *Stereocaulon* OTUs significantly differed in the frequency of intrathalline algae (Fig. S6). Above all, most of the OTU35 thalli (with *Asterochloris* as the predominant phycobiont) contained a small amount of URa28 algae, and most of the OTU2 thalli (with URa28 as the predominant phycobiont) contained a small amount of *Asterochloris*. Several algal clades interacted exclusively with one mycobiont species-level lineage, but their frequency was generally low. A comparable phycobiont pair, *Trebouxia jamesii/Trebouxia* sp. TR9, found in the *Ramalina farinacea* lichen, is assumed to physiologically benefit that symbiotic system [116,117]. Gasulla et al. [118] found two strains of *Coccomyxa* phycobionts in the thalli of the basidiolichen *Lichenomphalia*; one of the strains was restricted to lower altitudes, one to higher altitudes, and both were present in the thalli growing at intermediate altitudes. Alternatively, the minor phycobionts could occur in thalli without affecting the lichen and may be used as a source of algal symbionts for other lichens in the locality. This hypothesis is in concordance with our results, which point to a lack of symbiotic algae in the soil.

5. Conclusions

The main goal of this study was to determine the connections between phycobiont diversity and the successional gradient. The diversity of phycobionts on river gravel bars shifted along the successional vegetation gradient, from early-successional herbaceous stages through to the scrub and then young tree stages. The phycobiont communities of the early-successional stages were composed of relatively few species lineages. In subsequent stages, the observed species richness of the phycobionts mostly increased, while the species-level lineages typical for early-successional stages were gradually substituted by others that were probably adapted to the heterogenous microhabitat conditions of the river gravel bars. Moreover, a positive correlation was revealed between the species richness of the phycobionts and that of the accompanying lichens in the locality.

The second question addressed was related to the specificity of *Stereocaulon* lichens towards their phycobionts on river gravel bars. The substantial phycobiont diversity (including 14 *Asterochloris* species-level lineages and three additional trebouxiophycean algae) that was recovered from the river gravel bars suggested low specificity of *Stereocaulon* mycobionts. This range of phycobionts may help them to cope with the heterogeneous and dynamic conditions of the river gravel bars in glacial floodplains.

Finally, algal plurality was examined; more than one phycobiont species was found in samples belonging to both mycobiont OTUs (OTU2 and OTU35). *Asterochloris* phycobionts were recovered as the predominant phycobionts of OTU35, while the trebouxiophycean lineage URa28 was the predominant phycobiont of OTU2. However, the broad community of other intrathalline algae was shared by both mycobionts.

Besides novel insights into the community structure of symbiotic microorganisms under the harsh and dynamic conditions of river gravel

bars, this study presents challenging questions concerning cryptic lichen species and specificity towards the phycobionts, the dispersal of microscopic symbionts, the ecological function of additional intrathalline algae, and an observed discrepancy between the communities of soil and lichen algae.

CRediT authorship contribution statement

Lucie Vančurová: Conceptualization, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Funding acquisition. **Veronika Kalníková:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Ondřej Peksa:** Conceptualization, Methodology, Writing - review & editing. **Zuzana Škvorová:** Investigation, Writing - review & editing. **Jiří Malíček:** Investigation, Writing - review & editing, Funding acquisition. **Patricia Moya:** Investigation, Writing - original draft. **Kryštof Chytrý:** Formal analysis, Writing - original draft, Visualization. **Ivana Černajová:** Methodology, Writing - review & editing. **Pavel Škaloud:** Conceptualization, Formal analysis, Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to Helmut Mayrhofer and Jan Vondrák for identifying of a few critical lichens, Svatava Kubešová for help with bryophyte identification, Vít Grulich and Jiří Danihelka for identifying some vascular plants, and the anonymous reviewers for their valuable comments on the original version of the manuscript. This work was supported by the Charles University Science Foundation project GAUK 946417, the Primus Research Programme of Charles University no. SCI/13 and by the long-term research development project RVO 67985939.

No conflicts, informed consent, or human or animal rights are applicable to this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2020.102062.

References

[1] S. Hohensinner, C. Hauer, S. Muhar, River morphology, channelization, and habitat restoration, in: S. Schmutz, J. Sendzimir (Eds.), Riverine Ecosystem Management, Springer International Publishing, Cham, 2018, pp. 41–65.
[2] F. Malard, U. Uehlinger, R. Zah, K. Tockner, Flood-pulse and riverscape dynamics in a braided glacial river, Ecology 87 (2006) 704–716, https://doi.org/10.1890/04-0889.
[3] D.R. Montgomery, J.M. Buffington, Chanel processes, classification, and response, in: R. Naiman, R. Bilby (Eds.), River Ecology and Management, Springer-Verlag, New York, 1998, pp. 13–42.
[4] K. Tockner, A. Paetzold, U. Karaus, et al., Ecology of braided rivers, in: G.H.S. Smith, J.L. Best, C.S. Bristow, G.E. Petts (Eds.), Braided Rivers, Blackwell Publishing, Oxford, UK, 2006, pp. 339–359.
[5] W.J. Junk, P.B. Bayley, R.E. Sparks, The flood pulse concept in river-floodplain systems, Can. J. Fish. Aquat. Sci. 106 (1989) 110–127.
[6] K. Tockner, F. Malard, J.V. Ward, An extension of the flood pulse concept, Hydrol. Process. 14 (2000) 2861–2883.
[7] J.V. Ward, K. Tockner, D.B. Arscott, C. Claret, Riverine landscape diversity, Freshw. Biol. 47 (2002) 517–539, https://doi.org/10.1046/j.1365-2427.2002.00893.x.
[8] D. Gilvear, R. Francis, N. Willby, A.M.A. Gurnell, Gravel bars: a key habitat of gravel-bed rivers for vegetation, in: H. Habersack, H. Piégay, M. Rinaldi (Eds.), Gravel-bed Rivers VI: From Process Understanding to River Restauration, Developments in Earth Surface Processes, Elsevier, Amsterdam, 2008, pp. 677–700.
[9] N. Müller, River dynamics and floodplain vegetation and their alterations due to human impact, Large Rivers 9 (1996) 477–512, https://doi.org/10.1127/lr/9/1996/477.
[10] N.E. Pettit, R.H. Froend, Variability in flood disturbance and the impact on riparian tree recruitment in two contrasting river systems, Wetl. Ecol. Manag. 9 (2001) 13–25, https://doi.org/10.1023/A:1008471100136.
[11] K. Prach, L. Tichý, K. Lencová, et al., Does succession run towards potential natural vegetation? An analysis across seres, J. Veg. Sci. 27 (2016) 515–523, https://doi.org/10.1111/jvs.12383.
[12] C. Wellstein, U. Uehlinger, R. Zah, Terrestrial floodplain vegetation, in: J.V. Ward, U. Uehlinger (Eds.), Ecology of a Glacial Flood Plain, Springer Netherlands, Dordrecht, 2003, pp. 109–121.
[13] K. Tockner, F. Malard, Channel typology, in: J.V. Ward, U. Uehlinger (Eds.), Ecology of a Glacial Flood Plain, Springer Netherlands, Dordrecht, 2003, pp. 57–73.
[14] D. Corenblit, J. Steiger, A.M. Gurnell, et al., Control of sediment dynamics by vegetation as a key function driving biogeomorphic succession within fluvial corridors, Earth Surf Process Landforms 34 (2009) 1790–1810, https://doi.org/10.1002/esp.1876.
[15] V. Kalníková, K. Chytrý, M. Chytrý, Early vegetation succession on gravel bars of Czech Carpathian streams, Folia Geobot 53 (2018) 317–332, https://doi.org/10.1007/s12224-018-9323-6.
[16] K. Prach, P. Petřík, Z. Brož, J.-S. Song, Vegetation succession on river sediments along the Nakdong River, South Korea, Folia Geobot 49 (2014) 507–519, https://doi.org/10.1007/s12224-014-9195-3.
[17] A.M. Milner, G.E. Petts, Glacial rivers: physical habitat and ecology, Freshw. Biol. 32 (1994) 295–307, https://doi.org/10.1111/j.1365-2427.1994.tb01127.x.
[18] S. Marcante, B. Erschbamer, O. Buchner, G. Neuner, Heat tolerance of early developmental stages of glacier foreland species in the growth chamber and in the field, Plant Ecol. 215 (2014) 747–758, https://doi.org/10.1007/s11258-014-0361-8.
[19] J. Stöcklin, E. Bäumler, Seed rain, seedling establishment and clonal growth strategies on a glacier foreland, J. Veg. Sci. 7 (1996) 45–56, https://doi.org/10.2307/3236415.
[20] H. Ellenberg, C. Leuschner, Vegetation Mitteleuropas mit den Alpen, Eugen Ulmer, Stuttgart, 2010.
[21] J. Jeník, Sukcese rostlin na náplavech řeky Belé v Tatrách (Succession of plants on gravel bars of the Belá River in the Tatra Mountains), Acta Univ Carolinae 4 (1955) 1–59.
[22] S. Karrenberg, J. Kollmann, P.J. Edwards, et al., Patterns in woody vegetation along the active zone of a near-natural Alpine river, Basic Appl Ecol 4 (2003) 157–166, https://doi.org/10.1078/1439-1791-00123.
[23] T. Muotka, R. Virtanen, The stream as a habitat templet for bryophytes: species' distributions along gradients in disturbance and substratum heterogeneity, Freshw. Biol. 33 (1995) 141–160, https://doi.org/10.1111/j.1365-2427.1995.tb01156.x.
[24] J. Stöcklin, Differences in life history traits of related Epilobium species: clonality, seed size and seed number, Folia Geobot 34 (1999) 7–18, https://doi.org/10.1007/BF02803073.
[25] D.H. Vitt, J.M. Glime, C. Lafarge England, Bryophyte vegetation and habitat gradients of montane streams in western Canada, Hikobia 9 (1986) 367–386.
[26] S.L. Doty, A.W. Sher, N.D. Fleck, et al., Variable nitrogen fixation in wild *Populus*, PLoS One 11 (2016) e0155979, , https://doi.org/10.1371/journal.pone.0155979.
[27] N.A. Moran, Symbiosis as an adaptive process and source of phenotypic complexity, Proc. Natl. Acad. Sci. 104 (2007) 8627–8633, https://doi.org/10.1073/pnas.0611659104.
[28] J. Seckbach, M. Grube (Eds.), Symbioses and Stress, Springer Netherlands, Dordrecht, 2010.
[29] J.D. Meunier, S. Kirman, D. Strasberg, et al., Incipient weathering by *Stereocaulon vulcani* at Réunion volcanic island, Chem. Geol. 382 (2014) 123–131, https://doi.org/10.1016/j.chemgeo.2014.05.033.
[30] R. Stretch, H. Viles, The nature and rate of weathering by lichens on lava flows on Lanzarote, Geomorphology 47 (2002) 87–94, https://doi.org/10.1016/S0169-555X(02)00143-5.
[31] A. Sadowsky, A. Hussner, S. Ott, Submersion tolerance in a habitat of *Stereocaulon paschale* (Stereocaulaceae) and *Cladonia stellaris* (Cladoniaceae) from the high mountain region Rondane, Norway, Nova Hedwigia 94 (2012) 323–334, https://doi.org/10.1127/0029-5035/2012/0014.
[32] T. Spribille, V. Tuovinen, P. Resl, et al., Basidiomycete yeasts in the cortex of ascomycete macrolichens, Science 353 (2016) 488–492, https://doi.org/10.1126/science.aaf8287.
[33] C. Lavoie, M. Renaudin, R.T. McMullin, et al., Extremely low genetic diversity of *Stigonema* associated with *Stereocaulon* in eastern Canada, Bryologist 123 (2020) 188–203, https://doi.org/10.1639/0007-2745-123.2.188.
[34] R. Lücking, J.D. Lawrey, M. Sikaroodi, et al., Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria, Am. J. Bot. 96 (2009) 1409–1418, https://doi.org/10.3732/ajb.0800258.
[35] L. Vančurová, L. Muggia, O. Peksa, et al., The complexity of symbiotic interactions influences the ecological amplitude of the host: a case study in *Stereocaulon* (lichenized Ascomycota), Mol. Ecol. 27 (2018) 3016–3033, https://doi.org/10.1111/mec.14764.
[36] L. Vančurová, O. Peksa, Y. Němcová, P. Škaloud, *Vulcanochloris* (Trebouxiales, Trebouxiophyceae), a new genus of lichen photobiont from La Palma, Canary Islands, Spain, Phytotaxa 219 (2015) 118–132, https://doi.org/10.11646/phytotaxa.219.2.2.

[37] G. Rolshausen, F. Dal Grande, A.D. Sadowska-Deś, et al., Quantifying the climatic niche of symbiont partners in a lichen symbiosis indicates mutualist-mediated niche expansions, Ecography (Cop) (2017) 1–12, https://doi.org/10.1111/ecog.03457.

[38] A.L. Godschalx, G. Rodríguez-Castañeda, S. Rasmann, Contribution of different predator guilds to tritrophic interactions along ecological clines, Curr Opin Insect Sci 32 (2019) 104–109, https://doi.org/10.1016/j.cois.2019.01.002.

[39] A. Engelen, P. Convey, S. Ott, Life history strategy of *Lepraria borealis* at an Antarctic inland site, Coal Nunatak, Lichenol 42 (2010) 339–346, https://doi.org/10.1017/S0024282909990600.

[40] J. Romeike, T. Friedl, G. Helms, S. Ott, Genetic diversity of algal and fungal partners in four species of *Umbilicaria* (lichenized Ascomycetes) along a transect of the Antarctic Peninsula, Mol. Biol. 19 (2002) 1209–1217.

[41] C.A. Burga, B. Krüsi, M. Egli, et al., Plant succession and soil development on the foreland of the Morteratsch glacier (Pontresina, Switzerland): straightforward or chaotic? Flora 205 (2010) 561–576, https://doi.org/10.1016/j.flora.2009.10.001.

[42] V. Westhoff, E. van der Maarel, The Braun-Blanquet approach, in: R.H. Whittaker (Ed.), Classification of Plant Communities, Springer Netherlands, Dordrecht, 1978, pp. 287–399.

[43] H. Ellenberg, H.E. Weber, R. Düll, et al., Zeigerwerte von Pflanzen in Mitteleuropa, Scr Geobot 18 (1991) 1–248.

[44] M.O. Hill, J.O. Mountford, D.B. Roy, R.G.H. Bunce, Ellenberg's Indicator Values for British Plants. ECOFACT Volume 2 Technical Annex, Institute of Terrestrial Ecology, 1999.

[45] M.O. Hill, C.D. Preston, S.D.S. Bosanquet, D.B. Roy, Bryoatt: Attributes of British and Irish Mosses, Liverworts and Hornworts, NERC Centre for Ecology & Hydrology & Countryside Council for Wales, 2007.

[46] V. Kalníková, H. Kudrnovsky, Gravel bar vegetation database, Phytocoenologia 47 (2017) 109–110, https://doi.org/10.1127/phyto/2017/0177.

[47] M. Chytrý, S.M. Hennekens, B. Jiménez-Alfaro, et al., European Vegetation Archive (EVA): an integrated database of European vegetation plots, Appl. Veg. Sci. 19 (2016) 173–180, https://doi.org/10.1111/avsc.12191.

[48] Euro + Med, Euro + Med PlantBase – the information resource for Euro-Mediterranean plant diversity, http://ww2.bgbm.org/EuroPlusMed/, (2006–2019).

[49] M.O. Hill, N. Bell, M.A. Bruggeman-Nannenga, et al., An annotated checklist of the mosses of Europe and Macaronesia, J. Bryol. 28 (2006) 198–267, https://doi.org/10.1179/174328206X119998.

[50] R. Grolle, D.G. Long, An annotated check-list of the Hepaticae and Anthocerotae of Europe and Macaronesia, J. Bryol. 22 (2000) 103–140, https://doi.org/10.1179/jbr.2000.22.2.103.

[51] P.L. Nimis, J. Hafellner, C. Roux, et al., The lichens of the Alps - an annotated checklist, MycoKeys 31 (2018) 1–634, https://doi.org/10.3897/mycokeys.31.23658.

[52] O.F. Cubero, A. Crespo, J. Fatehi, P.D. Bridge, DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi, Plant Syst. Evol. 216 (1999) 243–249, https://doi.org/10.1007/BF01084401.

[53] K. Weber, W. Kabsch, Intron positions in actin genes seem unrelated to the secondary structure of the protein, EMBO J. 13 (1994) 1280–1286.

[54] O. Peksa, P. Škaloud, Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae), Mol. Ecol. 20 (2011) 3936–3948, https://doi.org/10.1111/j.1365-294X.2011.05168.x.

[55] M.D. Piercey-Normore, P.T. DePriest, Algal switching among lichen symbioses, Am. J. Bot. 88 (2001) 1490–1498, https://doi.org/10.2307/3558457.

[56] M. Gardes, T.D. Bruns, ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts, Mol. Ecol. 2 (1993) 113–118.

[57] T.J. White, T. Bruns, S. Lee, J.W. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White (Eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, 1990, pp. 315–322.

[58] P. Škaloud, O. Peksa, Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris*, Mol. Phylogenet. Evol. 54 (2010) 36–46, https://doi.org/10.1016/j.ympev.2009.09.035.

[59] K. Katoh, D.M. Standley, MAFFT multiple sequence alignment software version 7: improvements in performance and usability, Mol. Biol. Evol. 30 (2013) 772–780, https://doi.org/10.1093/molbev/mst010.

[60] K. Tamura, G. Stecher, D. Peterson, et al., MEGA6: Molecular Evolutionary Genetics Analysis version 6.0, Mol. Biol. Evol. 30 (2013) 2725–2729, https://doi.org/10.1093/molbev/msm092.

[61] F. Högnabba, Molecular phylogeny of the genus *Stereocaulon* (Stereocaulaceae, lichenized ascomycetes), Mycol. Res. 110 (2006) 1080–1092, https://doi.org/10.1016/j.mycres.2006.04.013.

[62] J.P. Huelsenbeck, F. Ronquist, MRBAYES: Bayesian inference of phylogenetic trees, Bioinformatics 17 (2001) 754–755, https://doi.org/10.1093/bioinformatics/17.8.754.

[63] D.J. Zwickl, Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion, The University of Texas at Austin, 2006.

[64] D.L. Swofford, PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4, Sinauer Associates, Sunderland, Massachusetts, 2003.

[65] D. Darriba, G.L. Taboada, R. Doallo, D. Posada, jModelTest 2: more models, new heuristics and parallel computing, Nat. Methods 9 (2012) 772, https://doi.org/10.1038/nmeth.2109.

[66] S. Guindon, O. Gascuel, A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood, Syst. Biol. 52 (2003) 696–704, https://doi.org/10.1080/10635150390235520.

[67] J. Oksanen, F.G. Blanchet, M. Friendly, et al., vegan: Community Ecology Package, (2019).

[68] M. Plummer, JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling, Proc Int Workshop Distrib stat Comput 124 (2003) 125.

[69] Y.-S. Su, M. Yajima, R2jags: using R to Run 'JAGS'. R package version 0.5-6, http://CRAN.r-project.org/package = R2jags, (2015).

[70] S.M. Hennekens, J.H.J. Schaminée, TURBOVEG, a comprehensive data base management system for vegetation data, J. Veg. Sci. 12 (2001) 589–591, https://doi.org/10.2307/3237010.

[71] L. Tichý, JUICE, software for vegetation classification, J. Veg. Sci. 13 (2002) 451–453, https://doi.org/10.1111/j.1654-1103.2002.tb02069.x.

[72] R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, 2017https://www.R-project.org.

[73] C.F. Dormann, B. Gruber, J. Fruend, Introducing the bipartite package: analysing ecological networks, R News 8 (2008) 8–11.

[74] F. Paul, J. Otte, I. Schmitt, F. Dal Grande, Comparing Sanger sequencing and high-throughput metabarcoding for inferring photobiont diversity in lichens, Sci. Rep. 8 (2018) 8624, https://doi.org/10.1038/s41598-018-26947-8.

[75] A.E. Arnold, J. Miadlikowska, K.L. Higgins, et al., A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? Syst. Biol. 58 (2009) 283–297, https://doi.org/10.1093/sysbio/syp001.

[76] A.W. Coleman, Is there a molecular key to the level of "biological species" in eukaryotes? A DNA guide, Mol. Phylogenet. Evol. 50 (2009) 197–203, https://doi.org/10.1016/j.ympev.2008.10.008.

[77] P. Moya, A. Molins, F. Martinez-Alberola, et al., Unexpected associated microalgal diversity in the lichen *Ramalina farinacea* is uncovered by pyrosequencing analyses, PLoS One 12 (2017) 1–21, https://doi.org/10.1371/journal.pone.0175091.

[78] E. Bolyen, J.R. Rideout, M.R. Dillon, et al., QIIME 2: reproducible, interactive, scalable, and extensible microbiome data science, PeerJ Prepr (2018), https://doi.org/10.7287/peerj.preprints.27295.

[79] B.J. Callahan, P.J. McMurdie, M.J. Rosen, et al., DADA2: high-resolution sample inference from Illumina amplicon data, Nat. Methods 13 (2016) 581.

[80] J. Larsson, eulerr: Area-Proportional Euler and Venn Diagrams With Ellipses, (2019).

[81] U. Ruprecht, G. Brunauer, R. Türk, High photobiont diversity in the common European soil crust lichen *Psora decipiens*, Biodivers. Conserv. 23 (2014) 1771–1785, https://doi.org/10.1007/s10531-014-0662-1.

[82] Y. Gauslaa, M. Bidussi, K.A. Solhaug, et al., Seasonal and spatial variation in carbon based secondary compounds in green algal and cyanobacterial members of the epiphytic lichen genus *Lobaria*, Phytochemistry 94 (2013) 91–98, https://doi.org/10.1016/j.phytochem.2013.04.003.

[83] P. Moya, P. Škaloud, S. Chiva, et al., Molecular phylogeny and ultrastructure of the lichen microalga *Asterochloris mediterranea* sp. nov. from Mediterranean and Canary Islands ecosystems, Int. J. Syst. Evol. Microbiol. 65 (2015) 1838–1854, https://doi.org/10.1099/ijs.0.000185.

[84] P. Škaloud, J. Steinová, T. Řídká, et al., Assembling the challenging puzzle of algal biodiversity: species delimitation within the genus *Asterochloris* (Trebouxiophyceae, Chlorophyta), J. Phycol. 51 (2015) 507–527, https://doi.org/10.1111/jpy.12295.

[85] T. Řídká, O. Peksa, H. Rai, D.K. Upreti, Photobiont diversity in Indian *Cladonia* lichens, with special emphasis on the geographical patterns, in: H. Rai, D.K. Upreti (Eds.), Terricolous Lichens in India, Springer New York, 2014, pp. 53–71.

[86] M.P. Nelsen, A. Gargas, Actin type I introns offer potential for increasing phylogenetic resolution in *Asterochloris* (Chlorophyta: Trebouxiophyceae), Lichenol 38 (2006) 435–440, https://doi.org/10.1017/S0024282906005779.

[87] S.M. Huse, D.M. Welch, H.G. Morrison, M.L. Sogin, Ironing out the wrinkles in the rare biosphere through improved OTU clustering, Environ. Microbiol. 12 (2010) 1889–1898, https://doi.org/10.1111/j.1462-2920.2010.02193.x.

[88] Y. Li, H. Wen, L. Chen, T. Yin, Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and re-vegetation on coal mine spoils in China, PLoS One 9 (2014), https://doi.org/10.1371/journal.pone.0115024.

[89] M. Sheng, X. Chen, X. Zhang, et al., Changes in arbuscular mycorrhizal fungal attributes along a chronosequence of black locust (*Robinia pseudoacacia*) plantations can be attributed to the plantation-induced variation in soil properties, Sci. Total Environ. 599–600 (2017) 273–283, https://doi.org/10.1016/j.scitotenv.2017.04.199.

[90] L.R. Walker, R. del Moral, Primary Succession and Ecosystem Rehabilitation, Cambridge University Press, Cambridge, 2003.

[91] M. Chytrý, T. Dražil, M. Hájek, et al., The most species-rich plant communities in the Czech Republic and Slovakia (with new world records), Preslia 87 (2015) 217–278.

[92] M. Bačkor, O. Peksa, P. Škaloud, M. Bačkorová, Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences, Ecotoxicol. Environ. Saf. 73 (2010) 603–612, https://doi.org/10.1016/j.ecoenv.2009.11.002.

[93] J. Steinová, P. Škaloud, R. Yahr, et al., Reproductive and dispersal strategies shape the diversity of mycobiont-photobiont association in *Cladonia* lichens, Mol. Phylogenet. Evol. 134 (2019) 226–237, https://doi.org/10.1016/j.ympev.2019.02.014.

[94] J. Nascimbene, H. Mayrhofer, M. Dainese, P.O. Bilovitz, Assembly patterns of soil-dwelling lichens after glacier retreat in the European Alps, J. Biogeogr. 44 (2017) 1393–1404, https://doi.org/10.1111/jbi.12970.

[95] S.E. Favero-Longo, M.R. Worland, P. Convey, et al., Primary succession of lichen and bryophyte communities following glacial recession on Signy Island, South Orkney Islands, Maritime Antarctic, Antarct. Sci. 24 (2012) 323–336, https://doi.org/10.1017/S0954102012000120.
[96] A. Beck, J. Bechteler, A. Casanova-Katny, I. Dzhilyanova, The pioneer lichen *Placopsis* in maritime Antarctica: genetic diversity of their mycobionts and green algal symbionts, and their correlation with deglaciation time, Symbiosis 79 (2019) 1–24, https://doi.org/10.1007/s13199-019-00624-4.
[97] F. Dal Grande, I. Widmer, H.H. Wagner, C. Scheidegger, Vertical and horizontal photobiont transmission within populations of a lichen symbiosis, Mol. Ecol. 21 (2012) 3159–3172, https://doi.org/10.1111/j.1365-294X.2012.05482.x.
[98] K. Fontaine, A. Beck, Photobiont relationships and phylogenetic history of *Dermatocarpon luridum var. luridum* and related *Dermatocarpon* species, Plants 1 (2012) 39–60, https://doi.org/10.3390/plants1020039.
[99] Y. Ohmura, S. Takeshita, M. Kawachi, Photobiont diversity within populations of a vegetatively reproducing lichen, *Parmotrema tinctorum*, can be generated by photobiont switching, Symbiosis 77 (2019) 59–72, https://doi.org/10.1007/s13199-018-0572-1.
[100] M. Rippin, N. Borchhardt, L. Williams, et al., Genus richness of microalgae and Cyanobacteria in biological soil crusts from Svalbard and Livingston Island: morphological versus molecular approaches, Polar Biol. 41 (2018) 909–923, https://doi.org/10.1007/s00300-018-2252-2.
[101] B. Frey, L. Bühler, S. Schmutz, et al., Molecular characterization of phototrophic microorganisms in the forefield of a receding glacier in the Swiss Alps, Environ. Res. Lett. 8 (2013) 15033, , https://doi.org/10.1088/1748-9326/8/1/015033.
[102] K.M. Quigley, L.K. Bay, B.L. Willis, Temperature and water quality-related patterns in sediment-associated *Symbiodinium* communities impact symbiont uptake and fitness of juveniles in the genus *Acropora*, Front. Mar. Sci. 4 (2017), https://doi.org/10.3389/fmars.2017.00401.
[103] A. Ali, N.G. Kriefall, L.E. Emery, et al., Recruit symbiosis establishment and Symbiodiniaceae composition influenced by adult corals and reef sediment, Coral Reefs 38 (2019) 405–415, https://doi.org/10.1007/s00338-019-01790-z.
[104] G. Rambold, T. Friedl, A. Beck, Photobionts in lichens: possible indicators of phylogenetic relationships? Bryol 101 (1998) 392–397.
[105] R. Yahr, R. Vilgalys, P.T. Depriest, Strong fungal specificity and selectivity for algal symbionts in Florida scrub *Cladonia* lichens, Mol. Ecol. 13 (2004) 3367–3378, https://doi.org/10.1111/j.1365-294X.2004.02350.x.
[106] R. Yahr, R. Vilgalys, P.T. DePriest, Geographic variation in algal partners of *Cladonia* subtenuis (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis, New Phytol. 171 (2006) 847–860, https://doi.org/10.1111/j.1469-8137.2006.01792.x.
[107] M. Hartmann, S. Lee, S.J. Hallam, W.W. Mohn, Bacterial, archaeal and eukaryal community structures throughout soil horizons of harvested and naturally disturbed forest stands, Environ. Microbiol. 11 (2009) 3045–3062, https://doi.org/10.1111/j.1462-2920.2009.02008.x.
[108] G. Helms, Taxonomy and Symbiosis in Associations of Physciaceae and *Trebouxia*, Georg-August Universität Göttingen, 2003.
[109] S.D. Leavitt, E. Kraichak, M.P. Nelsen, et al., Fungal specificity and selectivity for algae play a major role in determining lichen partnerships across diverse ecogeographic regions in the lichen-forming family Parmeliaceae (Ascomycota), Mol. Ecol. 24 (2015) 3779–3797, https://doi.org/10.1111/mec.13271.
[110] A. Beck, Selektivität der Symbionten schwermetalltoleranter Flechten, Ludwig-Maxmilians-Universität München, 2002.
[111] L. Muggia, L. Vančurová, P. Škaloud, et al., The symbiotic playground of lichen thalli - a highly flexible photobiont association in rock-inhabiting lichens, FEMS Microbiol. Ecol. 85 (2013) 313–323, https://doi.org/10.1111/1574-6941.12120.
[112] I. Onuț-Brännström, M. Benjamin, D.G. Scofield, et al., Sharing of photobionts in sympatric populations of *Thamnolia* and *Cetraria* lichens: evidence from high-throughput sequencing, Sci Reports 2018 (8) (2018) 4406, https://doi.org/10.1038/s41598-018-22470-y.
[113] H. Smith, F.D. Grande, L. Muggia, et al., Metagenomic data reveal diverse fungal and algal communities associated with the lichen symbiosis, bioRxiv (2020), https://doi.org/10.1101/2020.03.04.966853 (2020.03.04.966853).
[114] F. Dal Grande, G. Rolshausen, P.K. Divakar, et al., Environment and host identity structure communities of green algal symbionts in lichens, New Phytol. 217 (2017) 277–289, https://doi.org/10.1111/nph.14770.
[115] A. Molins, P. Moya, F.J. García-Breijo, et al., Molecular and morphological diversity of *Trebouxia* microalgae in sphaerothallioid *Circinaria* spp. lichens, J. Phycol. 54 (2018) 494–504, https://doi.org/10.1111/jpy.12751.
[116] L.M. Casano, E.M. del Campo, F.J. García-Breijo, et al., Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? Environ. Microbiol. 13 (2011) 806–818, https://doi.org/10.1111/j.1462-2920.2010.02386.x.
[117] D.C. Centeno, A.F. Hell, M.R. Braga, et al., Contrasting strategies used by lichen microalgae to cope with desiccation-rehydration stress revealed by metabolite profiling and cell wall analysis, Environ. Microbiol. 18 (2016) 1546–1560, https://doi.org/10.1111/1462-2920.13249.
[118] F. Gasulla, J.M. Barrasa, L.M. Casano, E.M. del Campo, Symbiont composition of the basidiolichen *Lichenomphalia meridionalis* varies with altitude in the Iberian Peninsula, Lichenol 52 (2020) 17–26, https://doi.org/10.1017/S002428291900046X.