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

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30 Abstract

31 River gravel bars are dynamic and heterogeneous habitats standing on transition between
 32 aquatic and terrestrial environment. Periodical flooding, low nutrient content, frost, missing safe
 33 sites, drought, and heat on the ground surface significantly influence life in these habitats.
 34 Mutualistic symbiosis may be a successful strategy for organisms to survive and to proliferate under
 35 harsh conditions. The lichen genus *Stereocaulon* was selected as a model symbiotic system among
 36 the organisms living on river gravel bars. The aim of our work was to determine effect of this
 37 dynamic environment on a phycobiont (i.e., green eukaryotic photobiont) community structure. We
 38 analysed 147 *Stereocaulon* specimens collected in the Swiss Alps using Sanger sequencing (fungal ITS
 39 rDNA, algal ITS rDNA, algal actin type I gene) and 8 selected thalli and 12 soil samples using Illumina
 40 metabarcoding (ITS2 rDNA). We performed phytosociological sampling on each study plot (n=13).
 41 Our analyses of communities of phycobionts, lichens, bryophytes, and vascular plants indicated an
 42 ongoing colonisation by phycobionts and gradual change of phycobiont community along to the
 43 successional gradient. We recovered great phycobiont diversity associated with *Stereocaulon*
 44 mycobionts including algae reported as phycobionts for the first time. Each of two *Stereocaulon*
 45 mycobiont OTUs has distinct pool of predominant phycobionts in the study area. Finally, all thalli
 46 selected for Illumina metabarcoding contained a wide range of additional intrathalline algae, i.e.,
 47 showed algal plurality. In general, succession process on newly emerged or recently disturbed
 48 localities also takes place within a community of microscopic symbiotic organisms, such as
 49 phycobionts.

50 Key words: selectivity, lichen phycobionts, succession, community composition, metabarcoding, algal
 51 plurality

52 Introduction

53 River gravel bars are dynamic and heterogeneous habitats ranging from glacial floodplains and
 54 alpine wide river valleys to piedmont (Montgomery and Buffington 1998; Malard et al. 2006; Tockner
 55 et al. 2006; Hohensinner et al. 2018). Periodical floods together with variation of speed and intensity
 56 of the water current, model richly braided channels with a mosaic of channels, pools, bars and
 57 islands (Junk et al. 1989; Tockner et al. 2000; Ward et al. 2002). Destruction and reformation of the
 58 river gravel bars by floods results in a wide structural change and a cyclic vegetation succession.
 59 Gravel bar vegetation is characterized by early-successional pioneer vegetation and subsequent,
 60 mainly shrub, vegetation stages in the colonization sequence (Pettit and Froend 2001; Prach et al.
 61 2016). These vegetation types form a mosaic of units according to microhabitat environmental
 62 conditions and disturbance level (Müller 1996; Tockner et al. 2000; Wellstein et al. 2003; Gilvear et

al. 2008). Gravel bars in alpine zones contribute significantly to the regional diversity of an otherwise harsh alpine environment (Tockner and Malard 2003). Early- to mid-successional stands are often occupied by relatively species rich communities of vascular plants of high evenness and relatively low vegetation cover. In later successional stages, the evenness and species richness decrease as organic matter and nutrients accumulate and the competition from established dominant species increase. This pattern was shown gravel bars, for example, by Corenblit et al. (2009), Prach et al. (2014), Kalníková et al. (2018).

Alpine rivers with glacier source are highly influenced by daily flooding, due to melting of glaciers which makes the environment even more extreme (e.g. Milner and Petts 1994; Tockner et al. 2000; Malard et al. 2006). Low nutrient content in a substrate surface represents the most limiting environmental factor in the glacier floodplains. Especially the recently unglaciated terrain is characterized by bare soils which do not contain any organic matter and the soil seed bank is initially not present. In addition, frost, missing safe sites, drought, and heat on the ground surface significantly influence life in these habitats (Stöcklin and Bäumler 1996; Tockner et al. 2006; Marcante et al. 2014). Common strategies of the gravel bar species are usually high diasporas dispersability, fast growth, disturbance tolerance, clonal growth and ability to grow on poor soils (Jeník 1955; Vitt et al. 1986; Muotka and Virtanen 1995; Stöcklin 1999; Karrenberg et al. 2003; Ellenberg and Leuschner 2010).

Under harsh conditions of river gravel bars, mutualistic symbiosis may be a successful strategy for the organisms to survive and to proliferate (Doty et al. 2016). Since lichens represent one of the oldest known and recognizable examples of mutualistic symbiosis living under stressful conditions (Seckbach and Grube 2010), we selected the *Stereocaulon* lichens, among the organisms living on river gravel bars, as a model symbiotic system. The *Stereocaulon* genus is widespread and ecologically successful and is a pioneer lichen growing in harsh conditions on newly formed substrates (Stretch and Viles 2002; Meunier et al. 2014). Moreover, previous studies confirmed its ability to survive episodic submersion (Sadowsky et al. 2012), even though it is not natively aquatic. Recently, an exceptional diversity of phycobionts was discovered to be associated with *Stereocaulon*, including three ecologically diversified trebouxioophycean genera *Asterochloris*, *Vulcanochloris* and *Chloroidium* (Vančurová et al. 2015, 2018).

The lichen host ecological amplitude may be greatly influenced by its specificity towards the photobionts (Rolshausen et al. 2017; Vančurová et al. 2018). Symbiotic interactions vary along the environmental gradients (Godschalx et al. 2019) and could be affected by stressful environments (Romeike et al. 2002; Engelen et al. 2010). Therefore, the aim of our work was to determine the

phycobiont diversity pattern of *Stereocaulon alpinum* in the gradient of the vegetation succession. We applied both Sanger sequencing (n=147) and Illumina metabarcoding (n=8) to *Stereocaulon alpinum* specimens collected in 13 study plots to address the following questions: 1) Is the phycobiont diversity influenced by succession, water regime, or alternatively other biotic and abiotic conditions?; 2) Do *Stereocaulon* lichens use their ability to co-operate with various phycobionts to cope with extreme conditions on river gravel bars of glacial floodplains?; 3) Do *Stereocaulon* lichens show algal plurality in this habitat?

Material and Methods

Study area and field sampling

The sampling was carried out in August 2017. The study area covered three glacial valleys (1) Morteratsch valley and 2) Roseg valley in the Bernina range and 3) Lötschental valley of the Lonza River in the Bernese Alps in the Swiss Alps. Four localities (Morteratsch, Roseg I, Roseg II and Lonza) situated on the river gravel bars of glacial floodplains (1995–2070 m a. s. l.) were sampled. We carried out 13 vegetation plots of 4 × 4 m. A detailed description of the location of each plot is provided in Online Resource 1/Table S1. Study plots in the localities belong to three succession stages: first (early-successional), second (defined by shrub layer), and third (the most developed usually with tree layer). In the locality Roseg II, only first and second stages were present. Examples of study plots belonging to different succession stages are given in Online Resource 2/Fig. S1.

Coordinates of each plot were recorded using a portable GPS device (WGS-84 coordination system). We recorded all lichen, bryophyte and vascular plant species within the vegetation plots. Lichens and bryophytes were collected from the ground and stones. In each plot we estimated visually: a) the cover of each species according to the extended Braun-Blanquet cover scale (Westhoff and Van Der Maarel 1978), b) the total vegetation cover and the cover of each layer (tree, shrub, herb, moss and lichen). We measured the elevation of the gravel bar (as a distance from its highest point to the actual water level with a tape) as well as distance from the river. One soil sample per plot was taken. Data on vegetation structure for each plot are listed in the Online Resource 1/Table S2. The substrate of all localities is rather acidic (in several cases with basic fractions), since the *Stereocaulon* lichens do not occur on calcareous substrata (Lamb 1951).

On each plot, at minimum 10 *Stereocaulon* samples were collected. For each *Stereocaulon* sample the type of substrate and affiliation to the plot was noted. 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 Herbarium collection of the Charles University in Prague (PRC). Vouchers of accompanying lichens

were deposited in the personal herbarium of J. Malíček. Vascular plants and bryophytes which were not identified in the field (unambiguous cases) were collected and identified in the lab. All records on vegetation plots were stored in the Gravel bar vegetation database – ID: EU-00-025 (Kalníková and Kudrnovsky 2017), which is included in the European Vegetation Archive (Chytrý et al. 2016). Nomenclature follows Euro+Med PlantBase (2006–2019) for vascular plants, Hill et al. (2006) for mosses, Grolle & Long (2000) for liverworts, and Nimis et al. (2018) for lichens.

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 (Cubero et al. 1999). 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; Weber and Kabsch 1994) were PCR amplified using primers listed in Online Resource 1/Table S3. PCRs were performed as described in Vančurová et al. (2018). All PCR were performed in 20 µl using Red Taq Polymerase (Sigma) as described by Peksa and Škaloud (2011) 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 xxx (Online Resource 1/Table S4).

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 sequences and 60 previously published sequences from *Stereocaulon* and other lichens retrieved from GenBank. The actin type I dataset consisted of 68 sequences: 7 newly obtained sequences, and 60 previously published sequences. The alignment was automatically performed by MAFFT v.7 software (Katoh and Standley 2013) under the Q-INS-I strategy and manually edited according to the published secondary structures of ITS2 rDNA (Škaloud and Peksa 2010) using MEGA v.6 (Tamura et al. 2013). The actin type I sequences were aligned using MAFFT v.7 software (Katoh and Standley 2013) 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 *Stereocaulon* (mycobiont) ITS rDNA dataset comprised 171 sequences: 145 newly obtained sequences and 26 representative sequences selected to cover all main clades 1–8 published by Högnabba (2006). The alignment was automatically performed by MAFFT v.7 software (Katoh and

Standley 2013) 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 Bayesian Inference (BI) carried out in MrBayes v.3.2.2 (Huelsenbeck and Ronquist 2001), maximum likelihood (ML) analysis implemented in GARLI v.2.0 (Zwickl 2006), and maximum parsimony (MP) analysis using PAUP v.4.0b10 (Swofford 2003). BI and ML analyses were carried out on a partitioned dataset to differentiate among ITS1, 5.8 S and ITS2 rDNA, actin intron 206, actin intron 248, and actin exon regions. The best-fit substitution models (Online Resource 1/Table S5) were selected using the Bayesian information criterion (BIC) implemented in JModelTest2 (Guindon and Gascuel 2003; Darriba et al. 2012). ML analysis was carried out using default settings, five search replicates, and the 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.

Statistical analyses

From the total of 147 samples with successfully sequenced phycobiont, two were excluded because of the absence of the mycobiont sequence. The phylogeny of *Stereocaulon alpinum* showed two lineages (OTU35 and OTU2; see below). Since the mycobiont identity affects the phycobiont diversity (Vančurová et al. 2018) 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 photobionts.

The relationship between species richness of *Stereocaulon alpinum* (OTU35) phycobionts and overall lichen species richness was inspected as a correlation between the number of phycobiont species-level lineages and number of lichen species. Since the number of samples per plot varied, the number of phycobiont species-level lineages were down-sampled to the smallest sample size in the data set which was 5 samples (Online Resource 2/Fig. S2). After excluding the study plot number 11 (with sample size of 5 samples), the smallest sample size in the data set increased to 10 samples. The rarefaction was performed using the *rarefy* function in *vegan* R package (Oksanen et al. 2019). The linear regression was performed separately for dataset including all plots and for dataset restricted to plots with sample size ≥ 10 . Since the parametric regression analyses can be significantly biased in small sample sizes, we performed the Bayesian linear regression instead. We constructed a regression model where we modelled the number of phycobionts (X_i) as $X_i \sim \text{Normal}(\mu_i, \sigma)$, where μ_i was determined as $a + b * \text{number of lichen species}_i$ (a = intercept, b = slope of the regression line)

and σ as the variance of the residuals. The priors were set as follows: $a \sim \text{Normal}(0, 0.001)$, $b \sim \text{Normal}(0, 0.001)$, $\sigma \sim \text{Uniform}(0, 100)$.

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 succession stage (coded 1, 2, 3). We constructed a regression model where we modelled the proportion of the most abundant phycobionts (X_i) as $X_i \sim \text{Normal}(\mu_i, \sigma)$, where μ_i was determined as $a + b * \text{succession stage}_i$ (a = intercept, b = slope of the regression line) and σ as the variance of the residuals. The priors were set as described above. We ran three chains of the model for 1,000,000 iterations, discarding the initial 100,000 as burnin. We fit the regression model in program JAGS v. 4.2.0 (Plummer 2003) through the R2JAGS package (Su and Yajima 2009) in R.

To inspect the dynamics of phycobiont communities in the context of the river gravel bar habitats, two PCoA ordinations were created. We used two different proxies to explain the vegetation succession on river gravel bars: the cover of shrub layer and the cover of crustose lichens. For this purpose, successional status of study plots was classified into two groups (early successional and developed). This classification provides two alternative views on successional processes. Both ordination analyses were based on the square-rooted Bray-Curtis dissimilarity. The original Braun-Blanquet cover-abundance values were transformed to percentage and square-root transformed in order to calculate the dissimilarity index. The first ordination was computed based on vascular plant communities and the second on bryophytes + lichens. In both ordination plots, Ellenberg-type indicator values (Ellenberg et al. 1991) for light and temperature were passively superimposed. In the plot based on bryophytes + lichens, indicator values for bryophytes were used (Hill et al. 2006). We also compared if the variation of different taxa groups corresponds to similar environmental conditions, by calculating procrustes distances between taxa groups. The output was then used as input distance matrix for PCoA ordination, visualizing a relative dissimilarities of community composition gradients of the taxa groups.

The vegetation plot data were stored in Turboveg for Windows v.2 database (Hennekens and Schaminée 2001) and further managed with JUICE software (Tichý 2002) and in the R environment (R Core Team 2017) with the help of the *vegan* R package (Oksanen et al. 2019).

Bipartite association network between study plots and phycobiont species-level lineages was produced using *bipartite* R package (Dormann et al. 2008).

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 selected thalli (four assigned to mycobiont OTU35 and four to OTU2) were rehydrated with Milli-Q sterile water one day before being processed and stored in a growth chamber at 20°C under a 12h/12h light/dark cycle (15 $\mu\text{mol}/\text{m}^2/\text{s}$). Thalli were cleaned under a stereomicroscope to remove soil particles and then superficially sterilized following Arnold et al. (2009). 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 from study plots (one sample per plot) was sampled. Soil samples were sieved to remove all possible contaminations. Total genomic DNA was isolated and purified using the Soil DNA Isolation Plus Kit® (Norgen Biotek Corp.), following the manufacturer's instructions.

Chlorophyta algal communities associated with the eight thalli and twelve soil samples were assayed using Illumina high-throughput sequencing of ITS2 of the rRNA operon, proposed as a universal barcode across eukaryotic kingdoms (Coleman 2009). High-coverage PCR primers at conserved sites were designed using a custom database for the algal phylum Chlorophyta (Online Resource 1/Table S3).

Amplicons for Illumina MiSeq sequencing were generated from nested PCR: in the first PCR the forward 1378-Chlorophyta (newly designed; Online Resource 1/Table S3) and the reverse ITS4 primers (White et al. 1990) were used (27 amplification cycles were run), in the second PCR three replicates were amplified using the primers 5.8F-Chlorophyta (newly designed; Online Resource 1/Table S3) 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'; 22 cycles). These three replicates were then pooled together. PCR reactions were performed as described in Moya et al. (2017).

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, United States) 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 2x 300 bp), at STAB Vida, Lisbon, Portugal and Genomics Core Facility at the University of Valencia, Spain.

Bioinformatic 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; Bolyen et al. 2018). Demultiplexed paired-end sequence reads were preprocessed using DADA2 (Callahan et al. 2016), 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. Solely algal sequences were further analyzed. Phylogenetic tree (Online Resource 2/Fig. S3) was inferred with Bayesian Inference (BI) using MrBayes v.3.2.2 (Huelsenbeck and Ronquist 2001) as described above. Euler diagrams were produced using *eulerr* R package (Larsson 2019).

Results

Vegetation on study plots

In total, we noted 88 taxa of vascular plants, 19 taxa of bryophytes and 45 taxa of lichens within 13 study plots. Online Resource 1/Table S1 contains summary of species richness for particular plots.

Phycobiont and mycobiont phylogenetic analysis

The predominant phycobiont detected in *Stereocaulon* thalli by Sanger sequencing of ITS rDNA, belong in a 97% of samples to the genus *Asterochloris* and only five specimens represent other trebouxiphycean algae. In the case of these five specimens, coded as A574, A574.1, A633, A634 and A634V the identity of the phycobiont was confirmed by Blast search against the GenBank database. Significant matches of 99% to 87% were obtained with *Coccomyxa viridis* HG973000 for A574, *Elliptochloris reniformis* LT560354 for A574.1 and uncultured Trebouxiphyceae FJ554399 for A633, A634 and A634V. These three sequences formed a well-supported clade with more distantly related sequence KF907701 (86% sequence similarity; Online Resource 2/Fig. S3) previously assigned to clade URa28 (Ruprecht et al. 2014). We follow this nomenclature hereafter for these sequences.

The phylogenetic hypothesis resulting from Bayesian analysis of the ITS rDNA and actin type I sequences of *Asterochloris* (Fig. 1) is congruent with previous studies (Peksa and Škaloud 2011; Gauslaa et al. 2013; Škaloud et al. 2015; Moya et al. 2015; Vančurová et al. 2018). The species

boundaries delimited in Vančurová et al. 2018 and nomenclature used *ibidem* were maintained. We recovered a total of 14 lineages, including one novel lineage here referred to as StA9. Eleven of these lineages were previously found as phycobionts of *Stereocaulon* (Vančurová et al. 2018 and references therein), namely, *Asterochloris 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 (*Asterochloris echinata* and *A. leprarii*) were found in association with *Stereocaulon* mycobiont for the first time here. The most frequently occurring phycobionts linked with the lineages *Asterochloris* StA5 and *A. phycobiontica*.

A phylogram resulting from Bayesian analysis of ITS rDNA sequences of *Stereocaulon* mycobiont is shown in Fig. 2. The majority of the recovered mycobiont sequences formed a well-supported lineage delimited as OTU35 by Vančurová et al. (2018). Five sequences matched with a distant related OTU2 (sister to DQ396973 and DQ396974), despite the morphological similarity of all studied samples. Both OTU35 and OTU2 fall into Group 8b *sensu* Högnabba (2006).

Phycobiont community structure and its changes

Stereocaulon alpinum OTU2 was sampled only in two study plots (numbers 8 and 11) and its phycobionts were successfully sequenced in four cases. One sequence was assigned to *Asterochloris italiana* and three sequences to trebouxiophycean lineage termed URa28. None of them was shared with the predominant *Stereocaulon* mycobiont (OTU35) in the study area.

Stereocaulon alpinum OTU35 (n=141) associated with 15 species-level lineages of phycobionts in the study area: 13 lineages of *Asterochloris*, one *Coccomyxa* and one *Elliptochloris*. Phycobionts from two to six species level lineages were recorded in each of 13 study plots (Fig. 3). When the sample size was reduced to five samples, 2-3.7 phycobiont species per plot were estimated. For the sample size of 10 (plot 11 with only five samples was excluded), 2.8-5.3 phycobiont species per plot were estimated.

Species richness of *Stereocaulon alpinum* OTU35 phycobionts increased significantly with the species richness of all lichens recorded at a study plot (Fig. 4). Moreover, gradual change of phycobiont community composition was proved using Bayesian linear regression (Fig. 5). The proportion of two most abundant phycobiont species-level lineages (*Asterochloris phycobiontica* and *Asterochloris* StA5) decreased significantly with the succession stage in favor of other species recovered in lower frequency.

The gradient of succession in both ordinations (vascular-plants-based and non-vascular-plant-based; Fig. 6) positively correlated with the diversity of lichens and phycobionts and the number of locally rare phycobionts (defined as all species with the exception of the species *Asterochloris* StA5

and *A. phycobiontica* highly prevalent in the study area; Fig. 3). Phycobiont communities of early-successional stages were relatively species poor and mostly consisted of species generally abundant in the study area (thus, the species composition was similar within study sites). With ongoing succession, the number of locally rare phycobiont species increased altogether with the total number of phycobiont species and the number of lichen species. The diversity of vascular plants and bryophytes was not associated with this gradient. Higher moss layer cover and higher amount of light demanding species of both bryophytes and vascular plants were typical of the early-successional stages.

As indicated by the PCoA based on procrustes distances of taxa groups (Fig. 7), the variation of species composition of photobiont communities responded to the environment similarly as that of bryophyte communities, while clearly differing from vascular plants and lichens (Online Resource 1/Table S2). Note that the gradient of species diversity of taxa groups did not correspond to that of their community composition.

Microalgal metabarcoding

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

Abundances of recovered algal clades by sample are shown in Fig. 8. The predominant phycobiont covered from 52.4 to 98.8% reads. Thalli A523M, A554M, A563M, and A570M (assigned to the mycobiont OTU35) contained various *Asterochloris* species as the predominant phycobiont: lineage StA9 (sample A523M), *A. irregularis* (A563M) and *A. phycobiontica*/StA4/StA5 (A554M and A570M). The species-level lineages StA4, StA5 and *A. phycobiontica* are undistinguishable using ITS2 rDNA marker. Relative frequency of ASVs linked to *Asterochloris* spp. by sample is shown in Online Resource 2/Fig. S4. Thalli A597M, A598M, A633M, and A634M (assigned to mycobiont OTU2) contained predominant phycobiont from the trebouxioephycean lineage URa28.

Diversity of soil algae

Phycobiont diversity in twelve 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 a mean of 73,049 (median 63,827) algal reads per sample, with a minimum of 18,255 and a maximum of 164,643 reads per sample. Filtered metabarcoding dataset consisted of 427 hits (4 to 89 per sample). The phylogenetic hypothesis resulting from Bayesian analysis of the ITS2 rDNA sequences obtained by Illumina metabarcoding of soil samples, selected lichen samples

and reference sequences from GenBank is shown in Online Resource 2/Fig. S3. We recovered sequences in 44 well-supported clades, 27 of which contain exclusively soil algae, two contain exclusively phycobionts and 15 are shared by these two groups. Occurrence of particular clades of soil algae on each study plot is depicted in Online Resource 2/Fig. S5.

We also analyzed the occurrence of particular algal ITS2 haplotypes, including whole dataset obtained by Sanger sequencing (probably predominant phycobionts; Fig. 9a). Nine haplotypes were obtained by Sanger sequencing, in common with Illumina sequencing of soil and lichens. Vast majority of haplotypes was unique for soil (n=256) or Illumina lichen (n=79) datasets. On the contrary, 27 haplotypes were shared by soil and lichens but were not detected by Sanger sequencing of *Stereocaulon* in the study area. Same analysis restricted to haplotypes with frequency ≥ 1000 (in order to eliminate possible bias produced by errors from PCR and sequencing; Huse et al. 2010) showed a similar pattern (Fig. 9b).

Discussion

Change of community structure along a succession gradient

The succession on river gravel bars is an important driver of both species' composition and diversity. It is well documented in case of vascular plants (e. g., Gilvear et al. 2008; Prach et al. 2014), but also applies to the microorganisms, such as soil bacteria or mycorrhizal fungi (Li et al. 2014; Sheng et al. 2017). However, different taxa groups response differently to this gradient. For example, vascular plants are known to follow a nested-community pattern when the highest species diversity is connected to the early- to mid-successional stages and the community is being impoverished with the ongoing succession (e. g., Walker and del Moral 2003; Corenblit et al. 2009; Chytrý et al. 2015). This corresponds to the pattern of vascular-plant-species-richness observed within this study.

On the other hand, the pattern observed for the phycobiont communities of *Stereocaulon* lichens differed. The phycobiont communities of early-vegetation stages were composed of relatively few species lineages such as *Asterochloris phycobiontica* or *Asterochloris* StA5 (Fig. 3), which are alpine and psychrophilic (Peksa and Škaloud 2011; Vančurová et al. 2018). As locally adapted, they are probably frequent also in source populations in the surroundings of the study plots. Therefore, newly emerged river gravel bars are easily colonized by *A. phycobiontica* and/or *Asterochloris* StA5 lineage. In subsequent stages, the observed species richness of phycobiont algae was mostly higher and these two species-level lineages were gradually substituted by others (Fig. 5). We hypothesize that some of them could be specialized to slightly different microhabitat conditions within particular plots. For example, clades 8, 12 and StA3 tolerate higher pH (Piercey-Normore and DePriest 2001; Bačkor et al. 2010; Vančurová et al. 2018; Steinová et al. 2019). On the river gravel bars of glacial

floodplains, organisms with various substrate optima could coexist together because of heterogeneity of substrate transported by a river or glacier from more distant localities and various substrate layers. In the study area, acidophilic vascular plants and bryophytes dominated. However, occurrence of basophilic species such as *Didymodon fallax*, *Lophozia excisa*, *Syntrichia ruralis*, and *Veronica fruticans* is considered as indication of basic fractions in substrate.

The species richness of terricolous lichens on glacier forelands in Alps were positively correlated with time since deglaciation (Nascimbene et al. 2017) analogically to lichen species richness on deglaciated plots in maritime Antarctica (Favero-Longo et al. 2012). In both cases, most of species, once established, persisted to the oldest successional stages. On the river gravel bars the species richness of phycobionts was positively correlated with that of lichens (Fig. 4) but less with that of vascular plants and bryophytes (Fig. 6). This correlation probably indicates colonization of the localities by phycobionts and mycobionts using the similar dispersal strategies. On the other hand, we observed that the community composition variation (summarized by the procrustes tests; Fig. 7) of lichens and phycobiont communities of *Stereocaulon* lichens differed.

We hypothesized ongoing colonization of river gravel bars by the phycobionts, or alternatively, the acquisition of the phycobionts from soil *in situ* (Dal Grande et al. 2012; Fontaine and Beck 2012; Ohmura et al. 2019). We found several algal clades both in soil and lichens (Online Resource 2/Fig. S3), but the phycobiont pool seems to be independent of soil algae (Fig. 10). For example, the most frequent ITS2 rDNA haplotype among Sanger sequences (*A. phycobiontica*/StA4/StA5 recovered in 69% of all samples) was present only in two soil samples in a very low amount (212 and 135 reads, which is 2.4% and 3.1% of algal reads, respectively). This result supports our hypothesis that phycobionts originating from the surroundings colonize the recently emerged plots without substantial contribution of “soil seed bank”. Still, these results should be perceived as the basis for future research. The number of soil samples is rather limited, and some taxa could be undetected (Rippin et al. 2018). However, the taxonomic composition of algae occurring on river gravel bars is comparable to the pool of soil algae detected in the forefield of Damma glacier in the Swiss Alps (Frey et al. 2013). Notably, a significantly different algal community was found in an early-successional stage, which is in their case represented by a bare soil near to a receding glacier. The lichen phycobionts, including *Asterochloris*, were reported from soil in transitional and developed stage. Besides others, they found the lineage StA9, firstly reported as lichen phycobiont in the present study.

Beck et al. (2019) found two haplotypes *Stichococcus antarcticus*, phycobiont of *Placopsis* lichen in maritime Antarctica, exclusively in areas deglaciated for a long time with more developed

soil and lichen community, unlike to other haplotypes occurring in whole area. The succession of vegetation causes numerous physical and chemical changes in soil. Therefore, the correlation between species richness of various organisms and successional stage (or age of the sample site) is frequently connected with changing soil characteristics, such as nutrient concentrations (Li et al. 2014; Sheng et al. 2017). However, according the Ellenberg indication values (Online Resource 1/Table S1), we did not detect such gradients along successional gradient in the study area. Contrarily, the gradients of distance from a river and the elevation above the water surface, are detectable in our data. Since the succession is blocked by river on gravel bars; succession gradient and distance from the river are naturally correlated. Nevertheless, no algae known from aquatic or humid environment (e.g. *Chloroidium*; Vančurová et al. 2018) were found as predominant phycobiont in samples growing in proximity to a river. Probably, the river disturbs the community occasionally but does not favor individuals tolerating submersion.

Low specificity as an adaptive strategy

The specificity (i. e., the taxonomic range of acceptable partners; Rambold et al. 1998; Yahr et al. 2004, 2006) of both mycobiont and phycobiont has been considered as crucial characteristic of lichen interactions. The lower specificity towards symbiotic partners has been frequently reported as advantageous strategy in harsh environments (Romeike et al. 2002; Engelen et al. 2010), because it enables association with phycobionts adapted to a variety of conditions.

Two species-level lineages of *Stereocaulon* were recorded in the study area (Fig. 2). Both lineages are morphologically identical, also indistinguishable in the field. Overwhelming majority of samples belonged to OTU35, which has been reported to be low-specific towards its phycobionts (Vančurová et al. 2018). Frequently, the mycobionts belonging to this lineage adopted algae known generally as phycobionts of *Lepraria* lichens (*A. phycobiontica*, *A. echinata*, *A. leprarii*; Škaloud et al. 2015), in the study area. We hypothesize that such a low specificity (OTU35 associated with 13 species-level lineages of *Asterochloris* and in two cases also with representatives of other Trebouxiothacean phycobionts in study area) could facilitate colonizing heterogeneous and harsh habitat of river gravel bars of glacial floodplains.

On the other hand, none of the samples of *S. alpinum* OTU2 associated with the same phycobionts as the mycobiont OTU35, despite growing in the same environment. The OTU2 mycobiont mostly associated with trebouxiothacean alga Ura28 as a phycobiont. 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 place (Hartmann et al. 2009).

Algal plurality

The occurrence of more phycobiont species in a single lichen thallus (i.e., algal plurality) is a common but overlooked phenomenon (e. g. Bačkor et al. 2010; Moya et al. 2017; Onuț-Brännström et al. 2018) known also in *Stereocaulon* (Vančurová et al. 2018). However, various lichen species differ in the prevalence of algal plurality. Dal Grande et al. (2017) proved an occurrence of more phycobionts in 49.2% of *Lasallia hispanica* thalli but only in 1.7% of *L. pustulata* thalli. The algal plurality is mostly undetectable by the Sanger sequencing. Using the Illumina metabarcoding, we found more than one phycobiont in all selected samples from both mycobiont species-level lineages (Fig. 8). However, these samples were selected on the ground of difficulties with Sanger sequencing which could indicate the algal plurality (Paul et al. 2018). Illumina metabarcoding as well as the Sanger sequencing uncovered URa28 as a predominant phycobiont of *Stereocaulon alpinum* OTU2. In most cases, the phycobiont determined by the Sanger sequencing corresponds with a predominant phycobiont according to the high-throughput sequencing (Molins et al. 2018; Paul et al. 2018).

Even though the two mycobiont species-level lineages OTU2 and OTU35 differ in predominant phycobiont pool, they share the pool of other intrathalline algae, unlike two *Circinaria* spp. collected at the same location shared the predominant phycobiont, but showed a completely different pool of other intrathalline algae (Molins et al. 2018). The *Stereocaulon* OTUs differ significantly in the frequency of intrathalline algae (Fig. 8). Above all, most of the OTU35 (with *Asterochloris* as the predominant phycobiont) thalli contain also a small amount of URa28 algae and *vice versa*. 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 *Ramalina farinacea* lichen is assumed to bring physiological benefits to that symbiotic system (Casano et al. 2011; Centeno et al. 2016). Alternatively, the minority phycobionts could occur in thalli without direct effects to the lichen. They might be used as a source of algal symbionts for other lichens in the locality.

Conclusions

The diversity of phycobionts shifts along the succession gradient on the river gravel bars. We revealed positive correlation between the species richness of phycobionts and the species richness of the accompanying lichens in the locality indicating ongoing colonization of this habitat by both groups. The phycobiont communities of early-successional stages comprised relatively few species lineages. In subsequent stages, the observed species richness of phycobionts was mostly higher and the species-level lineages typical for early-successional stages were gradually substituted by others probably adapted to heterogenous microhabitat conditions of the river gravel bars.

A substantial phycobiont diversity (including 14 *Asterochloris* species-level lineages and three additional trebouxiphycean algae) recovered on the river gravel bars suggested low specificity of *Stereocaulon* mycobionts. This range of phycobionts could help to cope with heterogenous and dynamic conditions on river gravel bars of glacial floodplains.

We found more than one phycobiont in all selected samples belonging to both mycobiont OTUs (OTU2 and OTU35). *Asterochloris* phycobionts were recovered as the predominant phycobionts of OTU35 and trebouxiphycean lineage URa28 was the predominant phycobiont of OTU2. Although, broad community of other intrathalline algae was shared by both mycobionts.

This study provides novel insights into community structure of symbiotic microorganisms under harsh and dynamic conditions of river gravel bars. Additionally, it poses several challenging questions concerning cryptic lichen species and specificity towards the photobionts, dispersal of microscopic symbionts, an ecological function of additional intrathalline algae and an observed discrepancy between communities of soil and lichen algae.

References

- Arnold AE, Miadlikowska J, Higgins KL, et al (2009) A phylogenetic estimation of trophic transition networks for ascomycetous Fungi: Are lichens cradles of symbiotrophic Fungal diversification? Syst Biol 58:283–297. doi: 10.1093/sysbio/syp001
- Bačkor M, Peksa O, Škaloud P, Bačkorová M (2010) Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences. Ecotoxicol Environ Saf 73:603–12. doi: 10.1016/j.ecoenv.2009.11.002
- Beck A, Bechteler J, Casanova-Katny A, Dzhilyanova I (2019) The pioneer lichen *Placopsis* in maritime Antarctica: Genetic diversity of their mycobionts and green algal symbionts, and their correlation with deglaciation time. Symbiosis 79:1–24. doi: 10.1007/s13199-019-00624-4
- Bolyen E, Rideout JR, Dillon MR, et al (2018) QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. PeerJ Prepr. doi: 10.7287/peerj.preprints.27295
- Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581
- Casano LM, del Campo EM, García-Breijo FJ, et al (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? Environ Microbiol 13:806–818. doi: 10.1111/j.1462-2920.2010.02386.x
- Centeno DC, Hell AF, Braga MR, et al (2016) Contrasting strategies used by lichen microalgae to cope with desiccation-rehydration stress revealed by metabolite profiling and cell wall analysis. Environ Microbiol 18:1546–1560. doi: 10.1111/1462-2920.13249
- Chytrý M, Dražil T, Hájek M, et al (2015) The most species-rich plant communities in the Czech Republic and Slovakia (with new world records). Preslia 87:217–278
- Chytrý M, Hennekens SM, Jiménez-Alfaro B, et al (2016) European Vegetation Archive (EVA): an integrated database of European vegetation plots. Appl Veg Sci 19:173–180. doi: 10.1111/avsc.12191

529 Coleman AW (2009) Is there a molecular key to the level of “biological species” in eukaryotes? A DNA
530 guide. *Mol Phylogenet Evol* 50:197–203. doi: 10.1016/j.ympev.2008.10.008

531 Corenblit D, Steiger J, Gurnell AM, et al (2009) Control of sediment dynamics by vegetation as a key
532 function driving biogeomorphic succession within fluvial corridors. *Earth Surf Process*
533 *Landforms* 34:1790–1810. doi: 10.1002/esp.1876

534 Cubero OF, Crespo A, Fatehi J, Bridge PD (1999) DNA extraction and PCR amplification method
535 suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Syst Evol* 216:243–249.
536 doi: 10.1007/BF01084401

537 Dal Grande F, Rolshausen G, Divakar PK, et al (2017) Environment and host identity structure
538 communities of green algal symbionts in lichens. *New Phytol* 217:277–289. doi:
539 10.1111/nph.14770

540 Dal Grande F, Widmer I, Wagner HH, Scheidegger C (2012) Vertical and horizontal photobiont
541 transmission within populations of a lichen symbiosis. *Mol Ecol* 21:3159–72. doi:
542 10.1111/j.1365-294X.2012.05482.x

543 Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and
544 parallel computing. *Nat Methods* 9:772. doi: 10.1038/nmeth.2109

545 Dormann CF, Gruber B, Fruend J (2008) Introducing the bipartite Package: Analysing Ecological
546 Networks. *R news* 8:8–11

547 Doty SL, Sher AW, Fleck ND, et al (2016) Variable nitrogen fixation in wild *Populus*. *PLoS One*
548 11:e0155979. doi: 10.1371/journal.pone.0155979

549 Ellenberg H, Leuschner C (2010) Vegetation Mitteleuropas mit den Alpen. Eugen Ulmer, Stuttgart

550 Ellenberg H, Weber HE, Düll R, et al (1991) Zeigerwerte von Pflanzen in Mitteleuropa. *Scr Geobot*
551 18:1–248

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

554 Euro+Med (2006–2019) Euro+Med PlantBase – the information resource for Euro-Mediterranean
555 plant diversity. <http://ww2.bgbm.org/EuroPlusMed/>

556 Favero-Longo SE, Worland MR, Convey P, et al (2012) Primary succession of lichen and bryophyte
557 communities following glacial recession on Signy Island, South Orkney Islands, Maritime
558 Antarctic. *Antarct Sci* 24:323–336. doi: 10.1017/S0954102012000120

559 Fontaine K, Beck A (2012) Photobiont relationships and phylogenetic history of *Dermatocarpon*
560 *luridum* var. *luridum* and related *Dermatocarpon* species. *Plants* 1:39–60. doi:
561 10.3390/plants1020039

562 Frey B, Bühler L, Schmutz S, et al (2013) Molecular characterization of phototrophic microorganisms
563 in the forefield of a receding glacier in the Swiss Alps. *Environ Res Lett* 8:15033. doi:
564 10.1088/1748-9326/8/1/015033

565 Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to
566 the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118

567 Gauslaa Y, Bidussi M, Solhaug KA, et al (2013) Seasonal and spatial variation in carbon based
568 secondary compounds in green algal and cyanobacterial members of the epiphytic lichen genus
569 *Lobaria*. *Phytochemistry* 94:91–8. doi: 10.1016/j.phytochem.2013.04.003

570 Gilvear D, Francis R, Willby N, Gurnell AMA (2008) Gravel bars: a key habitat of gravel-bed rivers for

571 vegetation. In: Habersack H, Piégay H, Rinaldi M (eds) Gravel-bed rivers VI: from process
572 understanding to river restauration, Developmen. Elsevier, Amsterdam, pp 677–700

573 Godschalx AL, Rodríguez-Castañeda G, Rasmann S (2019) Contribution of different predator guilds to
574 tritrophic interactions along ecological clines. *Curr Opin Insect Sci* 32:104–109. doi:
575 10.1016/j.cois.2019.01.002

576 Grolle R, Long DG (2000) An annotated check-list of the Hepaticae and Anthocerotae of Europe and
577 Macaronesia. *J Bryol* 22:103–140. doi: 10.1179/jbr.2000.22.2.103

578 Guindon S, Gascuel O (2003) A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by
579 Maximum Likelihood. *Syst Biol* 52:696–704. doi: 10.1080/10635150390235520

580 Hartmann M, Lee S, Hallam SJ, Mohn WW (2009) Bacterial, archaeal and eukaryal community
581 structures throughout soil horizons of harvested and naturally disturbed forest stands. *Environ*
582 *Microbiol* 11:3045–3062. doi: 10.1111/j.1462-2920.2009.02008.x

583 Hennekens SM, Schaminée JHJ (2001) TURBOVEG, a comprehensive data base management system
584 for vegetation data. *J Veg Sci* 12:589–591. doi: 10.2307/3237010

585 Hill MO, Bell N, Bruggeman-Nannenga MA, et al (2006) An annotated checklist of the mosses of
586 Europe and Macaronesia. *J Bryol* 28:198–267. doi: 10.1179/174328206X119998

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

589 Hohensinner S, Hauer C, Muhar S (2018) River Morphology, Channelization, and Habitat Restoration

590 Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees.
591 *Bioinformatics* 17:754–755. doi: 10.1093/bioinformatics/17.8.754

592 Huse SM, Welch DM, Morrison HG, Sogin ML (2010) Ironing out the wrinkles in the rare biosphere
593 through improved OTU clustering. *Environ Microbiol* 12:1889–1898. doi: 10.1111/j.1462-
594 2920.2010.02193.x

595 Jeník J (1955) Sukcese rostlin na náplavech řeky Belé v Tatrách (Succession of plants on gravel bars of
596 the Belá River in the Tatra Mountains). *Acta Univ Carolinae* 4:1–59

597 Junk WJ, Bayley PB, Sparks RE (1989) The Flood Pulse Concept in River-Floodplain Systems. *Can J Fish*
598 *Aquat Sci* 106:110–127

599 Kalníková V, Chytrý K, Chytrý M (2018) Early vegetation succession on gravel bars of Czech
600 Carpathian streams. *Folia Geobot* 53:317–332. doi: 10.1007/s12224-018-9323-6

601 Kalníková V, Kudrnovsky H (2017) Gravel Bar Vegetation Database. *Phytocoenologia* 47:109–110. doi:
602 10.1127/phyto/2017/0177

603 Karrenberg S, Kollmann J, Edwards PJ, et al (2003) Patterns in woody vegetation along the active
604 zone of a near-natural Alpine river. *Basic Appl Ecol* 4:157–166. doi: 10.1078/1439-1791-00123

605 Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements
606 in performance and usability. *Mol Biol Evol* 30:772–80. doi: 10.1093/molbev/mst010

607 Lamb IM (1951) On the morphology, phylogeny, and taxonomy of the lichen genus *Stereocaulon*. *Can*
608 *J Bot* 29:522–584. doi: 10.1139/b51-045

609 Larsson J (2019) eulerr: Area-Proportional Euler and Venn Diagrams with Ellipses

610 Li Y, Wen H, Chen L, Yin T (2014) Succession of bacterial community structure and diversity in soil

611 along a chronosequence of reclamation and re-vegetation on coal mine spoils in China. PLoS
612 One 9:1. doi: 10.1371/journal.pone.0115024

613 Malard F, Uehlinger U, Zah R, Tockner K (2006) Flood-Pulse and Riverscape Dynamics in a Braided
614 Glacial River. Ecology 87:704–716. doi: 10.1890/04-0889

615 Marcante S, Erschbamer B, Buchner O, Neuner G (2014) Heat tolerance of early developmental
616 stages of glacier foreland species in the growth chamber and in the field. Plant Ecol 215:747–
617 758. doi: 10.1007/s11258-014-0361-8

618 Meunier JD, Kirman S, Strasberg D, et al (2014) Incipient weathering by *Stereocaulon vulcani* at
619 Réunion volcanic island. Chem Geol 382:123–131. doi: 10.1016/j.chemgeo.2014.05.033

620 Milner AM, Petts GE (1994) Glacial rivers: physical habitat and ecology. Freshw Biol 32:295–307. doi:
621 10.1111/j.1365-2427.1994.tb01127.x

622 Molins A, Moya P, García-Breijo FJ, et al (2018) Molecular and morphological diversity of *Trebouxia*
623 microalgae in sphaerothalloid *Circinaria* spp. lichens. J Phycol 54:494–504. doi:
624 10.1111/jpy.12751

625 Montgomery DR, Buffington JM (1998) Channel Processes, Classification, and Response. In: Naiman R,
626 Bilby R (eds) River Ecology and Management. Springer-Verlag New York, pp 13–42

627 Moya P, Molins A, Martinez-Alberola F, et al (2017) Unexpected associated microalgal diversity in the
628 lichen *Ramalina farinacea* is uncovered by pyrosequencing analyses. PLoS One 12:1–21. doi:
629 10.1371/journal.pone.0175091

630 Moya P, Škaloud P, Chiva S, et al (2015) Molecular phylogeny and ultrastructure of the lichen
631 microalga *Asterochloris mediterranea* sp. nov. from Mediterranean and Canary Islands
632 ecosystems. Int J Syst Evol Microbiol 65:1838–54. doi: 10.1099/ijs.0.000185

633 Müller N (1996) River dynamics and floodplain vegetation and their alterations due to human impact.
634 Large Rivers 9:477–512. doi: 10.1127/lr/9/1996/477

635 Muotka T, Virtanen R (1995) The stream as a habitat template for bryophytes: species' distributions
636 along gradients in disturbance and substratum heterogeneity. Freshw Biol 33:141–160. doi:
637 10.1111/j.1365-2427.1995.tb01156.x

638 Nascimbene J, Mayrhofer H, Dainese M, Bilovitz PO (2017) Assembly patterns of soil-dwelling lichens
639 after glacier retreat in the European Alps. J Biogeogr 44:1393–1404. doi: 10.1111/jbi.12970

640 Nelsen MP, Gargas A (2006) Actin type I introns offer potential for increasing phylogenetic resolution
641 in *Asterochloris* (Chlorophyta: Trebouxiophyceae). Lichenol 38:435–440. doi:
642 10.1017/S0024282906005779

643 Nimis PL, Hafellner J, Roux C, et al (2018) The lichens of the Alps - An annotated checklist. MycoKeys
644 31:1–634. doi: 10.3897/mycokeys.31.23658

645 Ohmura Y, Takeshita S, Kawachi M (2019) Photobiont diversity within populations of a vegetatively
646 reproducing lichen, *Parmotrema tinctorum*, can be generated by photobiont switching.
647 Symbiosis 77:59–72. doi: 10.1007/s13199-018-0572-1

648 Oksanen J, Blanchet FG, Friendly M, et al (2019) vegan: Community Ecology Package

649 Onuț-Brännström I, Benjamin M, Scofield DG, et al (2018) Sharing of photobionts in sympatric
650 populations of *Thamnolia* and *Cetraria* lichens: evidence from high-throughput sequencing. Sci
651 Reports 2018 8:4406. doi: 10.1038/s41598-018-22470-y

652 Paul F, Otte J, Schmitt I, Dal Grande F (2018) Comparing Sanger sequencing and high-throughput

653 metabarcoding for inferring photobiont diversity in lichens. Sci Rep 8:8624. doi:
654 10.1038/s41598-018-26947-8

655 Peksa O, Škaloud P (2011) Do photobionts influence the ecology of lichens? A case study of
656 environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). Mol Ecol
657 20:3936–48. doi: 10.1111/j.1365-294X.2011.05168.x

658 Pettit NE, Froend RH (2001) Variability in flood disturbance and the impact on riparian tree
659 recruitment in two contrasting river systems. Wetl Ecol Manag 9:13–25. doi:
660 10.1023/A:1008471100136

661 Piercey-Normore MD, DePriest PT (2001) Algal Switching among Lichen Symbioses. Am J Bot
662 88:1490–1498. doi: 10.2307/3558457

663 Plummer M (2003) JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling

664 Prach K, Petřík P, Brož Z, Song JS (2014) Vegetation Succession on River Sediments along the Nakdong
665 River, South Korea. Folia Geobot 49:507–519. doi: 10.1007/s12224-014-9195-3

666 Prach K, Tichý L, Lencová K, et al (2016) Does succession run towards potential natural vegetation?
667 An analysis across seres. J Veg Sci 27:515–523. doi: 10.1111/jvs.12383

668 R Core Team (2017) R: A Language and Environment for Statistical Computing

669 Rambold G, Friedl T, Beck A (1998) Photobionts in Lichens: Possible Indicators of Phylogenetic
670 Relationships? Bryol 101:392–397

671 Řídká T, Peksa O, Rai H, Upreti DK (2014) Photobiont Diversity in Indian *Cladonia* Lichens, with Special
672 Emphasis on the Geographical Patterns. In: Rai H, Upreti DK (eds) Terricolous Lichens in India.
673 Springer New York, New York, NY, pp 53–71

674 Rippin M, Borchhardt N, Williams L, et al (2018) Genus richness of microalgae and Cyanobacteria in
675 biological soil crusts from Svalbard and Livingston Island: morphological versus molecular
676 approaches. Polar Biol 41:909–923. doi: 10.1007/s00300-018-2252-2

677 Rolshausen G, Dal Grande F, Sadowska-Deś AD, et al (2017) Quantifying the climatic niche of
678 symbiont partners in a lichen symbiosis indicates mutualist-mediated niche expansions.
679 Ecography (Cop) 1–12. doi: 10.1111/ecog.03457

680 Romeike J, Friedl T, Helms G, Ott S (2002) Genetic Diversity of Algal and Fungal Partners in Four
681 Species of *Umbilicaria* (Lichenized Ascomycetes) Along a Transect of the Antarctic Peninsula.
682 Mol Biol 19:1209–1217

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

685 Sadowsky A, Hussner A, Ott S (2012) Submersion tolerance in a habitat of *Stereocaulon paschale*
686 (Stereocaulaceae) and *Cladonia stellaris* (Cladoniaceae) from the high mountain region
687 Rondane, Norway. Nov Hedwigia 94:323–334. doi: 10.1127/0029-5035/2012/0014

688 Seckbach J, Grube M (eds) (2010) Symbioses and Stress. Springer Netherlands, Dordrecht

689 Sheng M, Chen X, Zhang X, et al (2017) Changes in arbuscular mycorrhizal fungal attributes along a
690 chronosequence of black locust (*Robinia pseudoacacia*) plantations can be attributed to the
691 plantation-induced variation in soil properties. Sci Total Environ 599–600:273–283. doi:
692 10.1016/j.scitotenv.2017.04.199

693 Škaloud P, Peksa O (2010) Evolutionary inferences based on ITS rDNA and actin sequences reveal
694 extensive diversity of the common lichen alga *Asterochloris*. Mol Phylogenet Evol 54:36–46. doi:

695 10.1016/j.ympev.2009.09.035

696 Škaloud P, Steinová J, Řídká T, et al (2015) Assembling the challenging puzzle of algal biodiversity:
697 species delimitation within the genus *Asterochloris* (Trebouxiophyceae, Chlorophyta). J Phycol
698 51:507–527. doi: 10.1111/jpy.12295

699 Steinová J, Škaloud P, Yahr R, et al (2019) Reproductive and dispersal strategies shape the diversity of
700 mycobiont-photobiont association in *Cladonia* lichens. Mol Phylogenet Evol 134:226–237. doi:
701 10.1016/j.ympev.2019.02.014

702 Stöcklin J (1999) Differences in life history traits of related *Epilobium* species: Clonality, seed size and
703 seed number. Folia Geobot 34:7–18. doi: 10.1007/BF02803073

704 Stöcklin J, Bäumler E (1996) Seed rain, seedling establishment and clonal growth strategies on a
705 glacier foreland. J Veg Sci 7:45–56. doi: 10.2307/3236415

706 Stretch R., Viles H. (2002) The nature and rate of weathering by lichens on lava flows on Lanzarote.
707 Geomorphology 47:87–94. doi: 10.1016/S0169-555X(02)00143-5

708 Su Y-S, Yajima M (2009) R Package “R2jags: A Package for Running jags from R”

709 Swofford DL (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)

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

712 Tichý L (2002) JUICE, software for vegetation classification. J Veg Sci 13:451–453. doi:
713 10.1111/j.1654-1103.2002.tb02069.x

714 Tockner K, Malard F (2003) Channel Typology. In: Ecology of a Glacial Flood Plain. Springer
715 Netherlands, Dordrecht, pp 57–73

716 Tockner K, Malard F, Ward J V (2000) An extension of the flood pulse concept. Hydrol Process
717 14:2861–2883

718 Tockner K, Paetzold A, Karaus U, et al (2006) Ecology of Braided Rivers. In: Smith GHS, Best JL,
719 Bristow CS, Petts GE (eds) Braided Rivers. Blackwell Publishing Ltd., Oxford, UK, pp 339–359

720 Vančurová L, Muggia L, Peksa O, et al (2018) The complexity of symbiotic interactions influences the
721 ecological amplitude of the host: A case study in *Stereocaulon* (lichenized Ascomycota). Mol
722 Ecol 27:3016–3033. doi: 10.1111/mec.14764

723 Vančurová L, Peksa O, Němcová Y, Škaloud P (2015) *Vulcanochloris* (Trebouxiiales,
724 Trebouxiophyceae), a new genus of lichen photobiont from La Palma, Canary Islands, Spain.
725 Phytotaxa 219:118–132. doi: 10.11646/phytotaxa.219.2.2

726 Vitt DH, Glime JM, Lafarge England C (1986) Bryophyte vegetation and habitat gradients of montane
727 streams in western Canada. Hikobia 9:367–386

728 Walker LR, del Moral R (2003) Primary Succession and Ecosystem Rehabilitation. Cambridge
729 University Press, Cambridge

730 Ward J V, Tockner K, Arscott DB, Claret C (2002) Riverine landscape diversity. Freshw Biol 47:517–
731 539. doi: <https://doi.org/10.1046/j.1365-2427.2002.00893.x>

732 Weber K, Kabsch W (1994) Intron Positions in Actin Genes Seem Unrelated To the Secondary
733 Structure of the Protein. Embo J 13:1280–1286

734 Wellstein C, Uehlinger U, Zah R (2003) Terrestrial Floodplain Vegetation. In: Ward J V., Uehlinger U

- (eds) Ecology of a Glacial Flood Plain. Springer Netherlands, Dordrecht, pp 109–121
- Westhoff V, Van Der Maarel E (1978) The Braun-Blanquet Approach. In: Whittaker RH (ed) Classification of Plant Communities. Springer Netherlands, Dordrecht, pp 287–399
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp 315–322
- Yahr R, Vilgalys R, Depriest PT (2004) Strong fungal specificity and selectivity for algal symbionts in Florida scrub *Cladonia* lichens. Mol Ecol 13:3367–3378. doi: 10.1111/j.1365-294X.2004.02350.x
- Yahr R, Vilgalys R, DePriest PT (2006) Geographic variation in algal partners of *Cladonia subtenuis* (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. New Phytol 171:847–60. doi: 10.1111/j.1469-8137.2006.01792.x
- Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. The University of Texas at Austin

Figures

Fig. 1 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 (2010), A4, A9, A11 *sensu* Peksa and Škaloud (2011), URa14 *sensu* Ruprecht et al. (2014), I1, I2 *sensu* Řídká et al. (2014), S1, S3 *sensu* Nelsen and Gargas (2006), *A. aff. irregularis*, *A. aff. italiana* and StA1 – StA8 *sensu* Vančurová et al. (2018). StA9 lineage was identified as new in present study. Online Resource 1/Table S6 contains accession numbers of reference sequences retrieved from GenBank

Fig. 2 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 are belonging to Group 8b *sensu* Högnabba (2006), as marked

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

Fig. 4 Bayesian linear regression of number of accompanying lichen species as a predictor of the number of phycobionts associated with mycobiont OTU35 down-sampled to a sample size of 5, b sample size of 10 per plot. Dashed lines show the 95% CRI around the regression line

Fig. 5 Bayesian linear regression of number of a succession 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

Fig 6 Ordination diagrams of principle coordinate analyses of community composition of vascular plants (a, b, c) and bryophytes + lichens (d, e, f). Colors and symbols indicate the proxy of successional development (in case of a and d it is the cover of shrubs being higher than 10%; in case of b and e it is the cover of crustose lichens being higher than 10%). Plots c and f show the passively superimposed environmental and biological variables. Ellenberg-type indicator values (EIVs) for light

and temperature are calculated for vascular plants in case of plot c and for bryophytes in case of plot f. On axis labels the variation explained by particular ordination axis is given.

Fig 7 Ordination diagram (principle coordinate analysis) of procrustes distances between individual taxa groups. The diagram shows the difference in response of communities of individual taxa groups to ecological gradients covered by the sampled dataset. On axis labels the variation explained by particular ordination axis is given.

Fig. 8 Eight *Stereocaulon* thalli selected for Illumina metabarcoding and abundances of recovered algal clades. A523M, A554M, A563M, A570M belong to mycobiont OTU35. A597M, A598M, A633M, A634M belong to mycobiont OTU2. Clade affiliations: URa25, URa28 *sensu* Ruprecht et al. (2014). Clades VII and XVIII were identified as new in present study. Amplicon sequence variants (ASVs) were sorted into these clades based on phylogenetic hypothesis presented in Online Resource 2/Fig. S3. Online Resource 1/Table S7 describes relative abundance of each ASV in each sample

Fig. 9 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

Fig. 10 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. (2014). Clade XVIII was identified as new in present study. Amplicon sequence variants (ASVs) were sorted into these clades based on phylogenetic hypothesis presented in Online Resource 2/Fig. S3

Supplementary material

Table S1 Location, environmental and vegetation characteristics of study plots

Table S2 Species composition of gravel bar vegetation plots. Species are ranked by decreasing frequency of occurrence. The abundance of species is given on the nine-degree Braun-Blanquet scale. All relevés are stored in Gravel bar vegetation database (Kalníková and Kudrnovsky 2017)

Table S3 Primers used in this study

Table S4 GenBank accession numbers, phycobiont species-level lineage, mycobiont OTU, substrate and affiliation to study plot of *Stereocaulon* samples

Table S5 Substitution models selected for each partition of *Asterochloris* and *Stereocaulon* (mycobiont) datasets and for algal ITS2 rDNA dataset using the Bayesian information criterion (BIC) as implemented in JModelTest2 (Guindon & Gascuel 2003, Darriba et al. 2012)

Table S6 Accession numbers of *Asterochloris* reference sequences retrieved from GenBank

Table S7 Relative abundance of each ASV in each *Stereocaulon* sample

Fig. S1 Examples of study plots belonging to different succession stages: **a**, **b** succession stage 1 (plots 5 and 9), **c** succession stage 2 (plot 2), **d** succession stage 3 (plot 3)

Fig. S2 Rarefaction curves for 13 study plots. Vertical lines are drawn at sample size of n=5 and sample size of n=10

817 **Fig. S3** Phylogenetic hypothesis resulting from Bayesian analysis of the algal ITS2 rDNA sequences
 818 obtained by Illumina metabarcoding of soil samples, selected lichen samples and reference
 819 sequences from GenBank. Clade affiliations: URa25, URa28 *sensu* Ruprecht et al. (2014). Clades I–
 820 XVIII were identified as new in present study. Values at the nodes indicate the statistical supports of
 821 Bayesian posterior probability

822 **Fig. S4** Relative frequency of ASVs linked to various *Asterochloris* species. Solely samples with
 823 *Asterochloris* as a predominant phycobiont (>50% algal reads belonged to *Asterochloris*) were
 824 displayed

825 **Fig. S5** Frequency of ASVs linked to algal clades recovered from soil samples. Clade affiliations:
 826 URa25, URa28 *sensu* Ruprecht et al. (2014). Clades I–XVIII were identified as new in present study.
 827 Amplicon sequence variants (ASVs) were sorted into these clades based on phylogenetic hypothesis
 828 presented in Online Resource 2/Fig. S3

0.02

a

b

Plot 4
Soil 4

Plot 5
Soil 5

Plot 6
Soil 6

Plot 8
Soil 8

A554M

mycobiont OTU35

A563M

A570M

mycobiont OTU2

A597M

A598M