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Research Article

The guilds in green algal lichens—an insight into the life of terrestrial symbiotic communities

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One sentence summary: Lichen fungi form highly complex and extensive photobiont-mediated system composed of various and differently overlapping guilds that are established across the basic ecological groups of saxi-, terricolous and epiphytic lichens.
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Abstract

Lichenized algae and cyanobacteria are known to be shared and selected by unrelated lichen-forming fungi coexisting in so-called photobiont-mediated guilds. Life in such a guild could be crucial for the survival of a large group of lichen fungi dependent on horizontal transmission of photobionts. Here, we investigate frequent lichen phycobionts of the genus *Trebouxia* in rock-dwelling lichen communities. We found intensive and repeated sharing of specific *Trebouxia* assemblages by co-occurring lichens across distant localities. Rock chemistry, expressed as pH, determined the composition of photobiont pools and separated three saxicolous lichen guilds, sharing environmentally specific photobiont groups. Moreover, unlike the majority of lichen fungi, many *Trebouxia* photobionts represented opportunists in the choice of general substrate form (soil-rock-tree bark/wood), maintaining their pH preferences. Thus, saxicolous communities form just a part of a complex guild system that is in principle mediated by environmentally conditioned groups of naturally co-occurring photobionts. The complexity of the system is influenced by diverse photobiont life strategies, including also dispersal style. The findings of photobionts strictly or predominantly associated with sexually reproducing fungi stimulated us to emphasize the role of free-dispersing photobionts in the establishment and maintenance of lichen guilds.

Keywords: communities, dispersal, lichen guilds, pH, photobiont, rock chemistry

Introduction

Organisms have a natural tendency to interact with other organisms occupying the same environment and geographical area, forming ecological communities. Symbiosis represents a basic type of interaction among co-occurring species and constitutes the foundation of many globally crucial communities or ecosystems, e.g. the cnidarian–algal symbioses of coral reefs (Stanley 2006) or plant–fungal mycorrhizae of various terrestrial vegetation types (Johnson, Gehring and Jansa 2017). Fungal–algal or fungal–cyanobacterial symbiosis in lichens also plays an important role at a global scale, occurring in almost all terrestrial biotopes from the tropics to polar regions (Galloway 2008), as well as some aquatic habitats (Thüs and Schultz 2009).

Within a climatically uniform region, each particular substrate usually hosts a characteristic and often remarkably uniform lichen vegetation (James, Hawksworth and Rose 1977). This phenomenon has been studied using phytosociological methods, describing many species assemblages as distinct communities or syntaxa (Klement 1955, Barkman 1958, Wirth 1972, James, Hawksworth and Rose 1977, Bültmann 2012). In fact, such classification sorts only the assemblages of lichen-forming fungi (mycobionts), neglecting other lichen components such as lichen-forming algae and cyanobacteria (photobionts).

The species diversity in photobiont assemblages as such is little known, as well as the relationships between photobionts and their hosts within a community. Nevertheless, spatially limited fungal assemblages can host photobionts belonging to various genera (Beck, Kasalicky and Rambold 2002, Engelen, Convey and Ott 2010, Moya *et al.* 2015, Vaiglová 2017, Vančurová *et al.* 2020) or to several photobiont species or genotypes belonging to a single genus (Beck 1999, Yahr, Vilgalys and Depriest 2004, Doering and Piercey-Normore 2009, Bačkor *et al.* 2010, Vargas Castillo and Beck 2012, Leavitt *et al.* 2013, Dal Grande *et al.* 2014). Anyhow, lichen fungi within the assemblage usually share the same photobiont genotype or closely related genotypes. Photobiont sharing has been revealed among species from a single study site (citations above) and even from separated sites within certain geographic area hosting the same lichen community (Yahr, Vilgalys and Depriest 2004, Myllys *et al.* 2007, Leavitt *et al.* 2013). Finally, specific photobiont can be shared by fungal species across lichen communities and large area, e.g. *Trebouxia incrustata* has been detected in at least 10 saxicolous lichen-forming fungi across the Europe (Blaha, Baloch and Grube 2006, Guzow-Krzemińska 2006, Peksa and Škaloud 2008, Gasulla, Guéra and Barreno 2010, Voytsekhovich and Beck 2016) and individual *Trebouxia* operational taxonomic units (OTUs) are shared by many species and genera across the North America (Leavitt *et al.* 2015).

As photobionts constitute basic resource for the life of mycobionts, the groups of lichen-forming fungi exploiting the same autotrophic partner or partners can be considered as basic structural units of communities—the guilds (Root 1967). The guild concept predicts temporally and spatially stable guild patterns within communities based on certain assembly rules (Korňan and Kropil 2014). In lichen symbiosis, such a rule was proposed by Rikki-

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nen (1995, 2003) who emphasized the central role of photobionts and suggested the concept of functional arrangement in lichen communities—the photobiont-mediated species guilds. The guild concept provides another perspective on the issue of lichen 'communities' than the syntaxonomy (Rikkinen 1995). It does not comprise only an external aspect, i.e. the co-occurrence of lichen taxa; however, it emphasizes the relationships between mycobionts and photobionts. The phenomenon has been described based on the example of boreal and temperate forest cyanolichens, forming two horizontally linked systems differing in associated groups of related trnL *Nostoc* genotypes, known as the *Nephroma* and *Peltigera* guilds (Rikkinen, Oksanen and Lohtander 2002). Within a lichen guild, individual fungal hosts usually exhibit different styles of reproduction and acquisition of photobionts. Lichen-forming fungi disperse either via sexual or asexual spores or via symbiotic vegetative propagules (see the 'Materials and methods' section). In the case of aposymbiotic dispersal, a fungal spore must find a suitable autotrophic partner in order to form a lichen thallus. Rikkinen (1995, 2003) suggested horizontal transmission of photobionts as a key mechanism ensuring the survival of exclusively sexual members of the guild. He hypothesized that symbiotic propagules of vegetatively reproducing lichens (core species) provide a source of suitable photobionts for spores of sexually reproducing fungi (fringe species). If the compatible photobiont is not able to live aposymbiotically in the substrate, the occurrence of some sexual fungi can be absolutely dependent on the presence of asexually reproducing species hosting the same photobiont, as described for *Pectenia plumbea* (Cardós *et al.* 2019).

For green-algal lichens, the existence of lichen guilds has been also suggested (Rikkinen 1995) and later confirmed. Dal Grande *et al.* (2014) found *Symbiochloris*-mediated guilds and pointed out to the existence and relevance of guilds formed by lichen fungi highly specialized on a single shared photobiont. Peksa and Škaloud (2011) revealed that the group of *Asterochloris* photobionts shared within a fungal guild is characterized on environmental basis, not on phylogenetic relationships. The distribution/availability of particular green-algal photobionts was found to be affected by climate (Helms 2003, Peksa and Škaloud 2011, Ruprecht, Brunauer and Printzen 2012, Vargas Castillo and Beck 2012) and substrate (Helms 2003, Guzow-Krzemińska 2006, Beiggi and Piercey-Normore 2007, Peksa and Škaloud 2011, Voytsekhovich and Beck 2016). The variability of habitats occupied by green-algal lichens is enormous and many different lichen communities have been described (e.g. Klement 1955, Wirth 1972). One can therefore ask how would the potential guilds be structured or which groups of lichen-forming fungi would be able to effectively cooperate in selection and dispersal of photobionts. Moreover, in contrast to forest cyanolichens (Rikkinen, Oksanen and Lohtander 2002, Kaasalainen *et al.* 2021), only ~30% of green-algal lichens produce symbiotic propagules (see the 'Discussion' section). If the theory of core and fringe species holds true, the establishment and maintenance of such guilds is dependent on very low specificity and selectivity of a vegetatively dispersing minority whose symbiotic propagules should contain the wide photobiont pool in order to effectively supply other species. However, vegetatively reproducing fungi associated with green algae tend to be rather highly specific (Cao *et al.* 2015, Singh *et al.* 2015, Steinová *et al.* 2019; but see Leavitt *et al.* 2015). So, it is questionable how do these guilds work. Sexually reproducing hosts may be supplied with photobionts from other sources, e.g. by capturing free-living algae or stealing photobionts from other community members (Friedl 1987, Ott 1987, Ott, Meier and Jahns 1995, Rikkinen 1995, Wedin *et al.* 2016). However, all these mechanisms are difficult to study and thus remain poorly understood.

In the geologically heterogeneous region of Central Europe, very different lichen assemblages can be found growing close to each other, with various rock types, including sandstones, granites, shales, serpentinites, basalts and limestones, hosting dozens of lichen taxa with unknown photobiont identity and unclear interspecific relationships. The general goal of this study was to elucidate the relationships between lichen-forming fungi and their associated algae in these rock-dwelling, species-rich lichen communities. We examined photobiont diversity among substrate-specific fungal assemblages to answer the following questions:

(1) Do saxicolous lichen-forming fungi share their photobionts? If yes, does the sharing respect the assemblage borders or does it follow different rules?
(2) How does substrate, as the key environmental factor, influence the occurrence of individual species and the interactions among lichen-forming fungi and algae?
(3) Do saxicolous lichen fungi form the guilds mediated by photobionts? If yes, do the guilds maintain their exclusivity in the context of neighbouring epiphytic and terricolous lichen communities?
(4) Do lichens reproducing by symbiotic propagules fulfil the role of photobiont distributors (core species) in mycobiont assemblages?

In total, we analysed almost 400 lichen thalli. Our data revealed that most of the saxicolous lichen-forming fungi were associated with *Trebouxia* algae, with many of unrelated lichens sharing a common pool of photobionts. Therefore, we consider these *Trebouxia*-hosting groups as potentially good model for the study of lichen guilds.

Materials and methods

Sampling

Our sampling covered the majority of common assemblages growing in Central European open rock habitats represented by the main lichen syntaxa *Rhizocarpetea geographici* Wirth 1972 (acidic, intermediate rocks), *Verrucarietea nigrescentis* Wirth 1980 and *Aspicilietalia calcareae* Roux 2009 (calcareous rocks) (see Table S1, Supporting Information). Each lichen community was sampled at several sites with the same type of rock, including 16 natural rock outcrops and two mine-spoil heaps, across the Czech Basin, Moravia and western Slovakia (Fig. S1 and Table S2, Supporting Information). To better assess the substrate sensitivity of photobionts and their ability to disperse, we also sampled lichen assemblages from small isolated islands comprising specific substrates occurring within a large homogenous area of quite different substrate: (i) a small quartz vein (locality QL) in a limestone area (Li4) and (ii) the concrete feet railings on a tourist viewpoint (Co1, Co2, Co3) situated on sandstone rocks in completely acidic sandstone areas (Sa1, Sa2, Sa3). In total, 22 sites were sampled, falling within the range 220–760 m a.s.l., with most of the sites located from 220 to 455 m a.s.l. (Table S2, Supporting Information). At each locality, we selected a sample of 9 m^2 of open habitat (without the influence of trees), with each site having a similar exposure (SW, S or SE). As we used fungal morphospecies as our sampling unit, one healthy thallus of each species was collected at each sample site. The dataset was finally enriched by 23 individual samples of sorediate or isidiate lichens from additional localities within the study area (Table S2, Supporting Information).

Chemical analysis

The major elemental composition of the rock features sampled were assessed using total digestion in mineral acids (HF-$HClO_4$) and/or sintering at the laboratories of the Geological Institute at the Faculty of Science of Charles University (Prague) and the Laboratories of the Czech Geological Survey (Prague). Al_2O_3, Fe_2O_3, FeO, MgO, CaO, Na_2O, K_2O, MnO and TiO_2 were determined by flame atomic absorption spectrometry and/or inductively coupled plasma-optical emission spectroscopy, while SiO_2 was determined gravimetrically and P_2O_5 by phosphorus management tool following protocols used by the labs—for results see Table S2 (Supporting Information). To measure pH, 10 g of crushed rock were soaked in 100 ml of distilled water for 24 h, after which pH was measured using a Cyberscan pH11 pH meter (Eutech Instruments Pte Ltd, Singapore) with a standard glass electrode .

Molecular analysis

Sanger sequencing was used to reveal the dominant photobiont in each lichen sample (Paul *et al.* 2018). We used ITS rDNA as it is a highly variable DNA barcode frequently used for delimitation of *Trebouxia* species-level lineages (Leavitt *et al.* 2015). We considered the ITS marker as sufficient for the purposes of our study as we did not aspire to an evolutionary study. Other less commonly used markers exhibit either lower discriminating power (*rbcL*) or are known for problematic amplification (*cox2*, see Muggia *et al.* 2020; actin type I locus, our observations).

Total genomic DNA was isolated following the standard cetyl trimethylammonium bromide protocol—CTAB (Doyle and Doyle 1987). Amplification of ITS rDNA was performed as described by Peksa and Škaloud (2011), using the primers nr-SSU-1780 (Piercey-Normore and DePriest 2001) and ITS4 (White *et al.* 1990). The polymerase chain reaction (PCR) products were purified using the JetQuick PCR Purification kit (Genomed, USA). The purified amplification products were sequenced using the PCR primers above on an Applied Biosystems ABI 3730XL automated sequencer (Thermo Fischer Scientific, USA) at the Macrogen Corp. (Seoul, Korea).

Total genomic DNA was extracted from 358 lichen thalli of 118 lichen taxa from 22 sampling sites, and from 23 lichen thalli of 20 lichen taxa from additional localities.

While, photobiont identity was resolved by Sanger sequencing of ITS rDNA loci, fungal hosts were determined morphologically as the study focused primarily on functional reproductive traits, which may not correspond to genetically characterized lineages, even where multi-locus phylogenies were used (Lohtander *et al.* 1998, Myllys, Lohtander and Tehler 2001, Articus *et al.* 2002, Wirtz, Printzen and Lumbsch 2012, Tehler, Ertz and Irestedt 2013, Altermann *et al.* 2014).

Phylogenetic analysis

To reveal relationships between photobionts from different main substrate forms (rock, soil, tree bark) and identify potential core species, we enriched the basic dataset from 22 saxicolous communities using a further 164 *Trebouxia* ITS rDNA sequences, using (i) 23 new *Trebouxia* sequences from vegetatively reproducing lichens collected at additional localities in the study region, (ii) unique GenBank *Trebouxia* sequences from vegetatively reproducing lichens and (iii) unique *Trebouxia* sequences from sexually reproducing epiphytic and terricolous lichens (Table S3, Supporting Information). All GenBank sequences originated from Europe, except for 19 sequences used for identification of particular clades/OTUs. Samples from other continents (including the Canary Islands and Svalbard) were avoided due to the high probability of including geographically restricted photobionts incapable of participating in the Central European lichen communities sampled (cf. Leavitt *et al.* 2013, Řídká *et al.* 2014, Singh *et al.* 2017).

The final dataset included a total of 433 *Trebouxia* nrITS sequences, including 250 newly obtained sequences and 183 GenBank sequences. The new sequences from this study were deposited in GenBank under the accession numbers MW694953–MW695202 (Table S3, Supporting Information).

The sequences were aligned using MAFFT v.6 software (Katoh *et al.* 2002) under the Q-INS-i strategy and checked for obvious sequencing errors. Alignment was improved by eliminating ambiguously aligned regions using Gblocks v. 0.91b (Castresana 2000). The resulting alignment is freely available on Mendeley Data (Peksa, Gebouska, Vancurova, Skvorova, & Skaloud, 2021).

Maximum likelihood (ML) analysis was performed using GARLI, v. 2.01. Three search replicates were applied with automatic termination set to 5 000 000. ML bootstrapping (consisted of rapid heuristic searches (100 pseudo-replicates) with automatic termination set to 100 000. The analyses were performed using the GTR+G substitution model selected by jModelTest v. 2.1.4. (Darriba *et al.* 2012). The phylogenetic trees were inferred by Bayesian inference (BI) using MrBayes version 3.2.6 (Ronquist *et al.* 2012). Two parallel Markov chain Monte Carlo (MCMC) runs were carried out for 8 000 000 generations, each with one cold and three heated chains. Trees and parameters were sampled every 100 generations. Convergence of the two cold chains was assessed during the run by calculating the average standard deviation of split frequencies (SDSF), with the burn-in value determined using the 'sump' command. The Bayesian implementation of the Poisson tree processes approach (bPTP; Zhang *et al.* 2013) was used to infer putative species boundaries based on DNA sequence data. The analysis was run on the bPTP web Server (http://species.h-its.org/ptp/) for 200 000 generations, with computation based on an unrooted phylogenetic tree inferred by MrBayes.

Based on our previous experience (Peksa and Škaloud 2011), closely related lineages can exhibit very different ecologies; hence, we decided to work with smaller units in subsequent ecological analyses including, in some cases, clades having lower statistical support (e.g. clades *I01a*, *S02a*, *S02b*; see Fig. S2, Supporting Information).

The incomplete taxonomy and nomenclature for the genus *Trebouxia* resulted in problems naming particular species-level lineages. For the purposes of this paper, we adopted the OTU (clade) numbering proposed by Leavitt *et al.* (2015) and clarified by Muggia *et al.* (2020). Where OTUs were split between several clades, we used a letter after the OTU number (e.g. *I01a*, *I01b*). OTUs not included in their phylogenies were coded with a letter instead of a number (e.g. *Ax*, *Ay*).

Species network

The network of associated mycobionts and photobionts was explored and visualized using the Gephi interactive platform (Bastian, Heymann and Jacomy 2009), using the Force Atlas 2 continuous layout algorithm.

Multivariate analysis

The main gradients in community composition and relationships between samples and mycobiont and photobiont species were assessed using detrended correspondence analysis (DCA) with down-weighting of rare species and also using non-metric multidimensional scaling (NMDS). The significant environmental variables (rock composition and pH) were added to DCA as supple-

mentary variables, being previously tested using Monte Carlo permutation test within canonical correspondence analysis (CCA). A forward selection procedure was also used to find the best predictors of species composition. CCA ordination was also performed to assess the influence of particular photobiont species (as explanatory variables) on the mycobiont distribution and on the pattern of lichen guilds (response variables), being also previously tested using Monte Carlo permutation test within CCA. Geographical distances were included to analyses as covariables to factor out the effect of spatial autocorrelation. The distances were transformed to the principal coordinates of neighbour matrices (PCNM; Dray, Legendre and Peres-Neto 2006) in R v. 3.3.3 (R Development Core Team), using the packages *BoSSA* (Lefeuvre 2018) and *vegan* (Oksanen *et al.* 2020), applying a threshold of 5000 km to get appropriate PCNM scores. All analyses were based on 22 samples containing a total of 62 fungal morphospecies and 16 algal clades (all species with a single occurrence were excluded from the dataset) and the results depicted as ordination plots. All multivariate analyses were calculated using Canoco for Windows 5.10 (ter Braak and Šmilauer 2018).

Variation partitioning

The relative effects of substrate (content of basic chemical compounds and pH), climate, geographic distance (latitude and longitude) and mycobiont and photobiont species composition on variation in three lichen guild pattern were analysed using redundancy analysis variation partitioning, using the varpart function in the R v. 3.3.3, using the package *vegan*. The affiliation of samples into three designated lichen guilds (siliceous, calcareous and volcanic rock guilds) was used as the response variable, coded as three dummy variables (i.e. each variable was coded either as '0' or '1'). Climatic data were obtained from the WorldClim v. 2 database (Hijmans *et al.* 2005) using a resolution of 2.5 arc minutes. At each sampling site, 19 bioclimatic variables were obtained by applying a 1 km buffer to limit the effects of spatial bias. In addition, we included altitudes and retrieved annual evapo-transpiration values for each sampling site using the Global Potential Evapo-Transpiration (Global-PET) dataset provided at a resolution of 30 arc sec. The Global-PET values were buffered in a similar way to the WorldClim variables. Substrate (pH and 11 chemical rock features), mycobiont and photobiont species, were coded as dummy variables. Rare mycobiont and photobiont species (with single occurrence) were excluded, resulting in a total of 62 mycobiont and 16 photobiont dummy variables. Since the collinearity among the variables was detected, all explanatory variables were condensed into principal components (PCs) by the principal component analyses (PCA). The Broken-stick distribution (Jackson 1993) was used to select which PCs to include in variation partitioning analysis. Accordingly, three climatic, three substrate, six photobiont and 23 mycobiont PCs were selected. Significance of the net effect of explanatory variables was tested using the function anova applied on partial ordination models defined by redundancy analyses with one variable as explanatory and the other as covariables. All analyses were performed using R v. 4.1.0 (R Development Core Team), using the packages *vegan* (Oksanen *et al.* 2020), *rgdal* (Bivand, Keitt and Rowlingson 2021), *raster* (Hijmans 2021) and *venneuler* (Wilkinson 2011). The R script has been uploaded into Mendeley Data (http://dx.doi.org/10.17632/95t3rzv6rs.2)

Other statistics

The bar charts in Fig. 1 (made in MS Excel within Microsoft Office Professional Plus 2013) are based on information about particular fungal morphospecies specimens (Table S3, Supporting Information; depicted in detail in Fig. S2, Supporting Information).

The correlations between the sample scores derived from the mycobiont and photobiont species scores (DCA, NMDS) as well as the samples scores derived from independent rock composition analysis (PCA, NMDS) were calculated in Statistica v. 8 (Statsoft Inc.; Hill and Lewicki 2007). The same software was used for visualization of differences in pH ranges of *Trebouxia* clades by boxplots. Species response curves of photobionts against pH were fitted using generalized additive models (GAMs) with Poisson response distribution in Canoco for Windows 5.10 (ter Braak and Šmilauer 2018).

Reproduction mode

The complex phenomenon of lichen reproduction and dispersal has been described several times (e.g. Büdel and Scheidegger 2008). However, we frequently use a number of terms in this study that may need further clarification:

Sexual reproduction: Mycobionts—production of meiotic fungal spores; *Trebouxia*—production of gametes, formation of zygotes (rarely observed, e.g. Ahmadjian 1960).

Asexual reproduction: Mycobionts—aposymbiotic conidia, thallospores etc.; *Trebouxia*—aposymbiotic zoospores, autospores, aplanospores; both partners—symbiotic diaspores in the form of (i) thallus fragments or (ii) specialized symbiotic vegetative propagules (soredia, isidia, phyllidia, schizidia, etc.).

In the following text, we use the terms 'sexual reproduction' for production and dispersal by fungal spores and 'vegetative reproduction' (i.e. multiplication) for dispersal by symbiotic diaspores, including thallus fragments. Thallus fragmentation is potentially possible in all lichens; however, it is most frequently used in fruticose lichens (Büdel and Scheidegger 2008). As such, we assigned this characteristic to several lichens usually lacking apothecia and specialized symbiotic propagules (*Bryoria*, *Cetraria*, *Flavocetraria*; see Table S3, Supporting Information).

While the role of conidia is still not fully understood, we assume that they are more likely to have a function in the dikaryon formation process, rather than in re-establishment of the lichen thallus (Honegger and Scherrer 2008).

To find out the proportion of reproduction mode among *Trebouxia*-hosting European lichens, we excerpted the work of Wirth, Hauck and Schultz (2013), which contains a significant part of lichens growing in Europe, especially in its central part representing our sampling area. The type of reproduction was also checked in Smith *et al.* (2009). The synthesis of Miadlikowska *et al.* (2014) provided the information whether the lichen-forming fungus is associated with *Trebouxia*.

Results

Phylogenetic analysis of photobionts

Amplification provided a total of 250 *Trebouxia* sequences (Table S3, Supporting Information), along with 48 sequences belonging to other photobiont genera (*Asterochloris*, *Diplosphaera*, *Myrmecia*, etc.—data not shown).

Based on ML phylogeny (Fig. 1 and Fig. S2, Supporting Information) and bPTP analysis (Fig. S3, Supporting Information), 44 *Trebouxia* species-level lineages (hereinafter clades) were recovered from the European samples analysed. Among these, 23 *Trebouxia* clades were associated with 91 fungal morphospecies, which formed the basic dataset of 22 sampled rock-dwelling assemblages. The majority of photobionts recorded belong to previ-

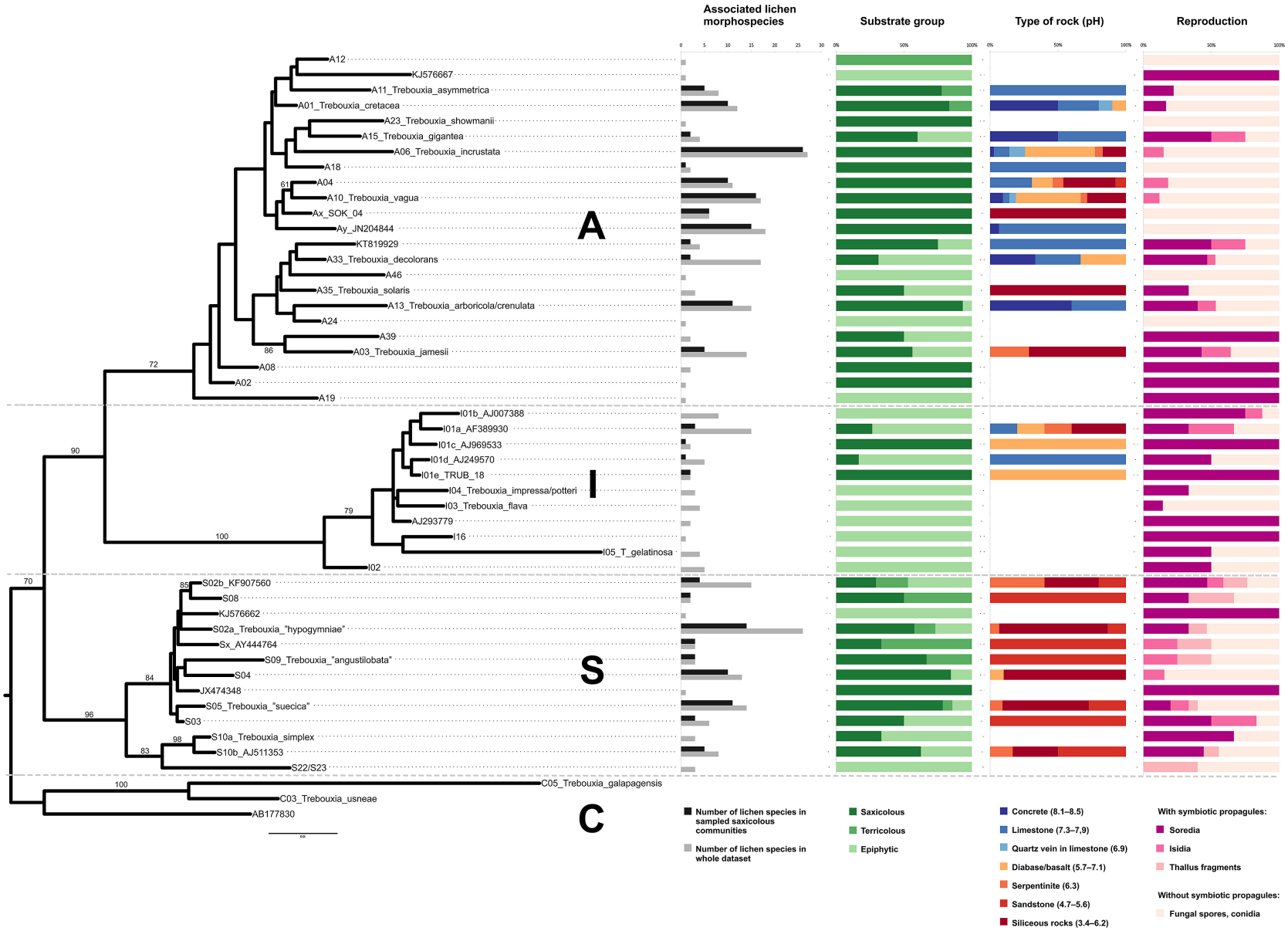

Figure 1. Visualization of important aspects of photobionts' life, including substrate preference and dispersal style (i.e. lichen reproduction mode). From left to right: internal transcribed spacer nuclear ribosomal DNA (ITS rDNA) phylogeny of *Trebouxia* based on unique sequences; bar charts showing the number of associated lichen morphospecies; proportional bar charts based on number of associated saxicolous, terricolous and epiphytic lichen species; proportional bar charts based on number of saxicolous samples from different rock types; and proportional bar charts based on number of associated lichen species having or lacking vegetative reproduction (the 'thallus fragment' category includes some fruticose lichens with fragile thalli lacking specialized symbiotic diaspores). Bar charts in the third column are based on our saxicolous samples, while bar charts in the second and fourth columns are based on all lichen morphospecies in the dataset. For details of particular associated fungal morphospecies and their characters, see Fig. S2 and Table S3, Supporting Information).

ously known species or OTUs within three main *Trebouxia* clades A, I and S (*sensu* Muggia *et al.* 2020).

Sharing of photobionts

Frequent sharing of photobionts was observed in the saxicolous assemblages sampled, with identical photobiont clades often detected in the thalli of two or more fungal morphospecies at a single study site. The highest degree of sharing at the same site involved as much as 12 different fungal morphospecies of eight genera sharing one photobiont clade (clade *A06* at loc. Vo2). On average, 8.6 fungal morphospecies associated with one photobiont clade across the whole dataset. The highest number of mycobionts were recorded in the most common photobionts, i.e. clade *A06* (associated with 26 fungal morphospecies), *A10* (16), *Ay* (16), *S02a* (14) and *A04* (10). Consequently, many fungal morphospecies associated with several *Trebouxia* clades. This intensive symbiont sharing provides a network of relationships among mycobionts and their photobionts to constitute a huge interconnected system (Fig. 2). Several species remained out of this network (clades *A18*, *A33*, *A35*, *I01a*, *I01d* and *Sx* with their mycobionts); however, further sampling is needed to confirm that they really form separate clusters. Numerous *Trebouxia* clades identified in saxicolous lichens also associate with epiphytic and terricolous lichens (Fig. 1 and Fig. S2, Supporting Information). As such, the network appears to be open for further members from the communities investigated.

Influence of rock characteristics on species composition

The PCA analysis of major elemental composition of rock samples showed the obvious chemical differences between sampled rock types, with exceptions of some siliceous and volcanic rocks that were similar in some aspects (Fig. S4, Supporting Information). The pH measurement revealed acidity gradient in the analysed substrates ranging from 3.4 (heavy-metal rich shales) to 8.5 (artificial concretes—see Table S2, Supporting Information).

Multivariate analysis of the fungal dataset confirmed differences between fungal assemblages growing on different substrates. Based on ordination space clustering (Fig. 3; Fig. S5, Supporting Information), we can distinguish several groups of assemblages characterized by similar species composition. Lichens on sandstones, limestones and concretes were clearly separated, while the remaining assemblages on quartz vein in limestone, siliceous rock (including heavy-metal rich rocks), serpentinite and also volcanic rock exhibited a higher degree of similarity, forming a heterogeneous group. The distribution was strongly affected by substrate chemistry, as lichen samples (assemblages) were distributed along a clear gradient from acidic siliceous rocks with high SiO_2 content to basic concretes and limestones correlated with CaO and pH (Fig. 3). The test within CCA analysis identified the amounts of CaO, Fe_2O_3, Al_2O_3, SiO_2, FeO, MnO and Na_2O, as well as pH as variables having the significant effect to lichen (mycobiont) distribution (Table S4a, Supporting Information).

Independent analysis of photobionts resulted in a sample distribution remarkably similar to that of lichen fungi. Photobiont assemblages were also distributed along the acidic to basic rock gradient. However, in contrast to the analyses of the fungal dataset, some photobiont assemblages exhibited different grouping according to DCA (Fig. 3) as well as NMDS (Fig. S5, Supporting Information), e.g. limestone assemblage Li4 was closer to volcanic ones due to high frequency of *A06* clade, pair of siliceous assemblages Si4 and Si6 was separated from each other due to high abundance of clades *S02a* and *Ax*. The test within CCA analysis identified the amounts of CaO, Fe_2O_3, SiO_2, Al_2O_3 and pH as variables with the most significant effect to photobiont distribution (Table S5a, Supporting Information).

To test the relationship of the rock composition, mycobionts and photobionts, we calculated the correlations between the sample scores derived from the mycobiont and photobiont species scores (DCA, NMDS) as well as the samples scores derived from independent rock composition analysis (PCA, NMDS). Positive correlations were revealed between all the pairs (rock chemistry-mycobiont, rock chemistry-photobiont, mycobiont-photobiont—see Table S6, Supporting Information), confirming a strong environmental conditionality of the mycobiont as well as photobiont assemblages distribution and a very similar response of lichen morphospecies and their photobionts to the rock chemistry.

Photobiont pH preferences

Thanks to chemical analysis of rock samples, including pH measurement, we could examine pH preferences for some *Trebouxia* clades (Fig. 4). We found out that individual lineages prefer clearly dissimilar pH ranges. Clades *A06*, *A10* and *A04*, for example, tolerated a very broad pH range (Fig. S6, Supporting Information) and occupied many different rock types (Fig. S7, Supporting Information). While *A06* and *A10* exhibited a clear subneutral pH optimum (around pH 6.5), however, clade *A04* appeared to be a real pH generalist species exhibiting no clear preference (Fig. 4).

Other *Trebouxia* lineages have more distinct pH preferences, occurring either on acidic or basic rocks. Algae of the clades *A01*, *A11*, *A13* and *Ay* occurred predominantly on substrates with a pH > 7, whereas members of clades *A03*, *Ax*, *S02b*, *S04*, *S05*, *S10b* and also *I01a* were mostly found on acidic to subneutral substrates with a pH < 6.5, with clade *S02a* being particularly acidophilic as it occurred strictly at a pH ≤ 4.5 (Figs S6 and S7, Supporting Information).

The striking preferences of the major *Trebouxia* lineages also suggest an importance of pH in *Trebouxia* evolution (Fig. 1, column 'Type of rock'). The preference of clade S for acidic substrates was particularly obvious, its members being associated with acidophilous saxi-, terricolous and epiphytic lichens. In comparison, algae of clade A were most often found on rocks with a higher pH, though with important exceptions of clades *A03* and *Ax*. Moreover, the *Trebouxia decolorans-solaris* subclade (*A33*/*A35*/*Az*) exhibited a clear preference to alkaline or lime-rich rock substrates or subneutral/eutrophic tree bark. Finally, clade I was predominantly associated with the family Physciaceae, known for many basiphilous and nitrophilous species.

Lichen guilds

The photobionts are regarded as resources for potential photobiont-mediated fungal guilds. Therefore, we performed the CCA analysis using photobionts as explanatory variables to analyse their influence on the distribution of mycobionts. The CCA plot (Fig. S8, Supporting Information) showed specific pattern that resembled the clustering inside the mycobiont-photobiont network (Fig. 2). The mycobiont assemblages exhibited distribution influenced by photobionts that formed three clearly separated groups: the first included *Trebouxia* lineages headed by *A03*, *S02b* and *S04*, in opposition to them appeared the group of photobionts *A01*, *A13*, *Ay* and also *I01d*, the last group contained clades *A06* and *A10* (all the mentioned *Trebouxia* clades are those having a significant influence on the mycobiont distribution—Table S7, Supporting Information). This grouping responded to

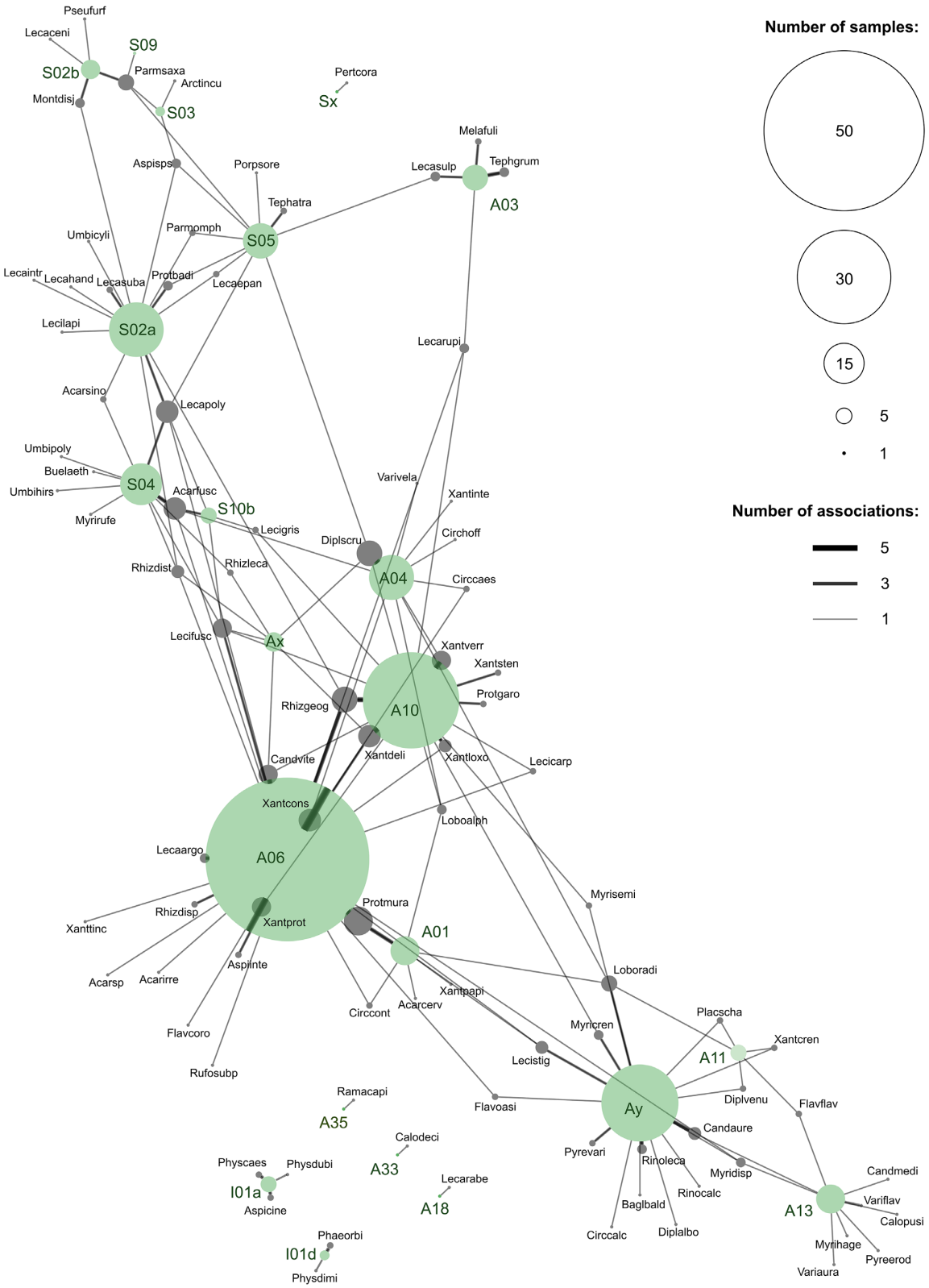

Figure 2. Species network uncovering relationships between lichen-forming fungi (grey circles) and their photobionts (green circles) in saxicolous lichen communities. The network is composed of 113 nodes (91 lichen morphospecies and 22 *Trebouxia* clades) and 224 edges. Node circle size is proportional to species abundance in the dataset. Edge line thickness is given by the number of repetitions in the association between two species (see scales on the right). For acronyms of fungal names, see Table S9 (Supporting Information).

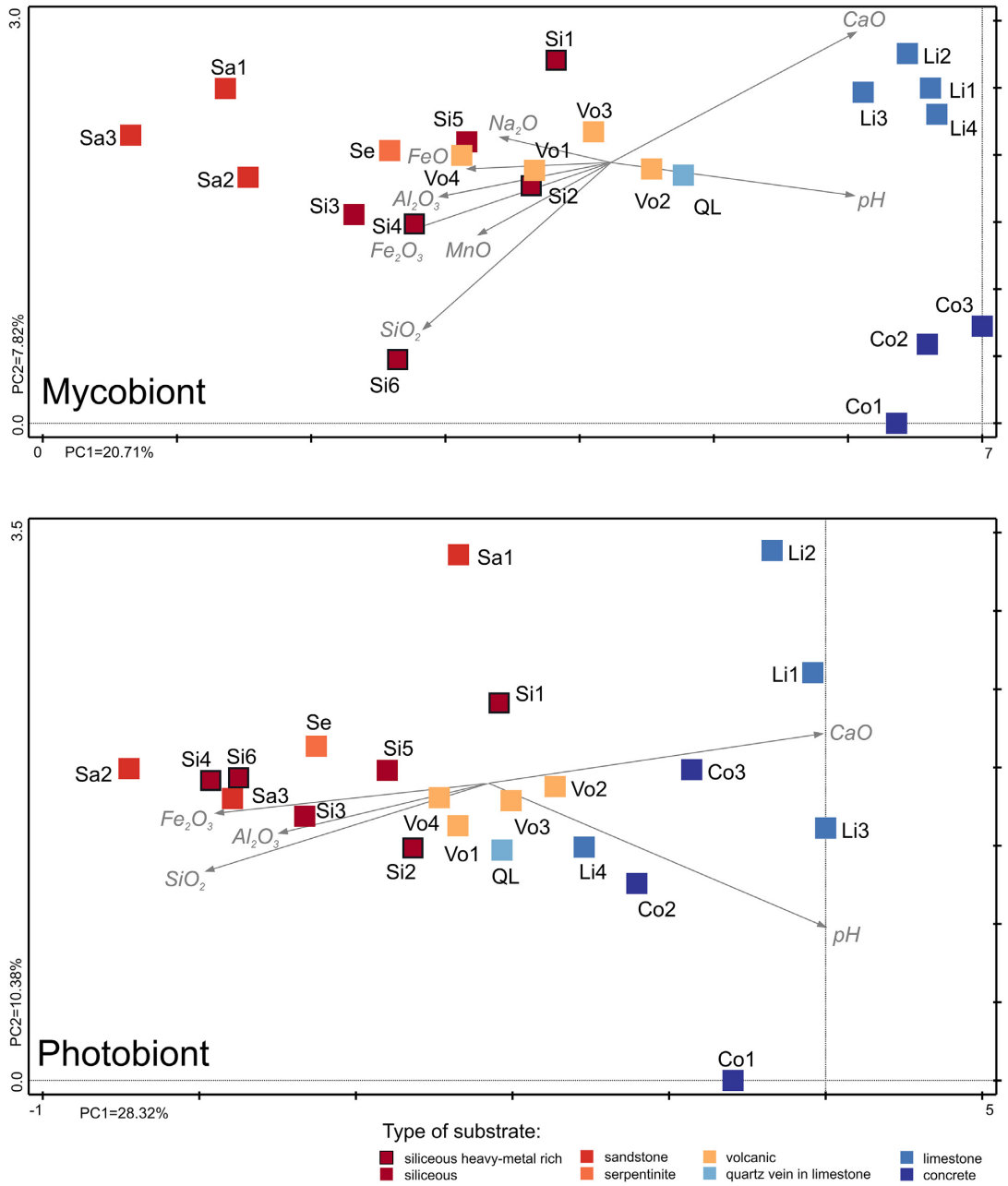

Figure 3. Ordination plots (DCA) for 22 samples (squares) based on mycobiont (lichen morphospecies) and photobiont (*Trebouxia* clades) species composition. Percentage of explained variation is given alongside each ordination axis. The environmental factors with significant influence ($P \leq 0.01$) for each dataset were added as supplementary variables (arrows). The distribution of assemblages (sampling sites) along the acidity gradient (pH, CaO vs other factors) is obvious for mycobionts as well as photobionts.

the arrangement of photobionts according to their pH preferences (Fig. 4), suggesting that the potential guilds could be photobiont-mediated, but consequently substrate-mediated, i.e. conditioned by substrate chemistry, mainly its pH. Nevertheless, DCA and NMDS as well as abovementioned CCA analysis suggested several possible hypotheses for partition of the mycobionts to the guilds (especially, the affiliation of volcanic and sandstone assemblages was questionable—see Fig. 3 and Figs S5 and S8, Supporting Information). We tested several different combinations of mycobiont clustering using photobionts as explanatory variables. From CCA ordinations performed, the partitioning into three specific guilds turned out to be the most significant (pseudo-F = 31.2, P = 0.002). The first calciphilous guild included the species growing on limestone and concrete, the second guild was formed by species on subneutral volcanic rocks and the third guild comprised acidophilous species on siliceous rocks, including serpentinite and sandstone assemblages (Fig. 5). The quartz vein in limestone community was placed on the border of the calciphilous and volcanic rock assemblages. Such 'calculated' pattern was clearly congruent with 'natural' pattern showed by CCA plot based on analysis of mycobiont dataset (compare Fig. 5 and Fig. S8, Supporting Information), revealing photobionts headed by *S02b* as resources for acidophilous guild, clades *A06* a *A10* for volcanic rocks guild and *A01*, *Ay* etc. for calciphilous guild (for the effect of individual photobionts on guild separation, see Table S8, Supporting Information).

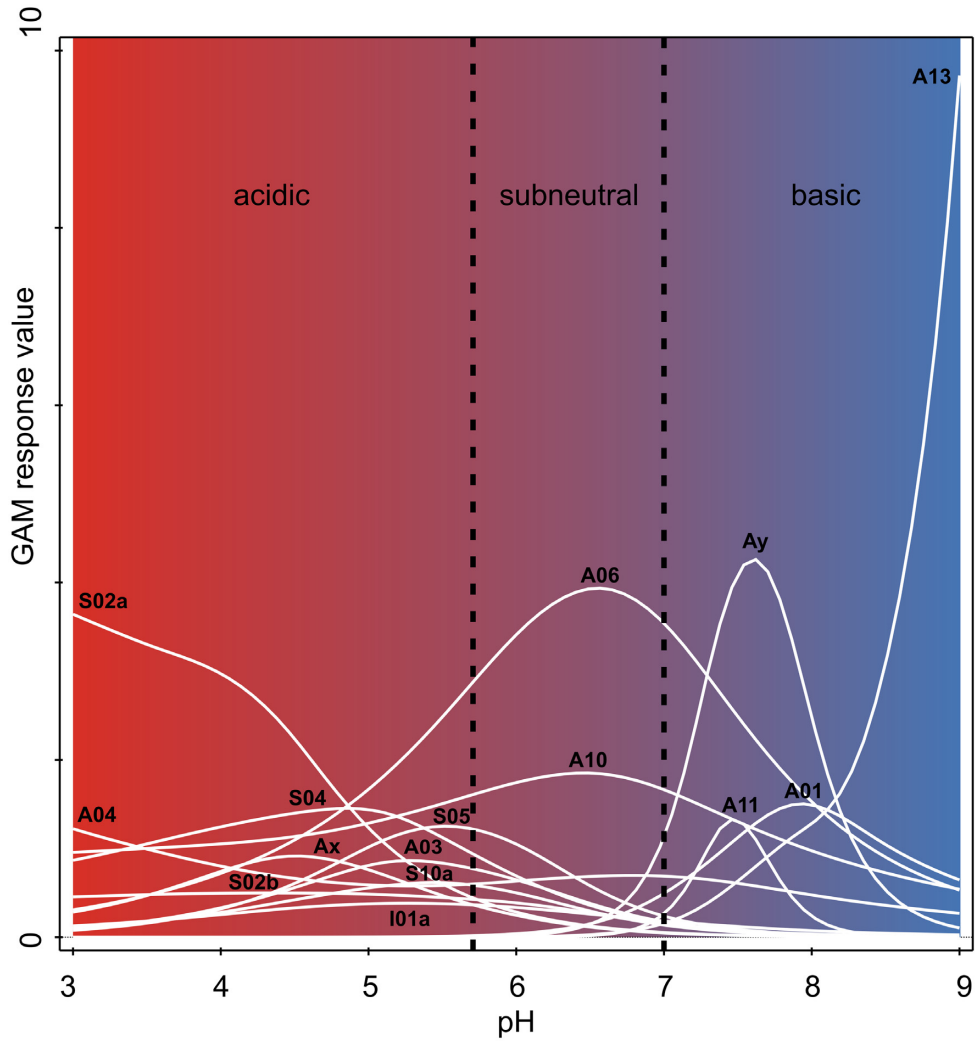

Figure 4. Response of photobionts to pH. The curves were fitted using GAMs. GAM response value represents predicted species pH response. Only *Trebouxia* clades with more than five samples from at least two localities were included. The pH zones follow Wirth *et al.* (2013).

Colour joining between associated mycobionts and photobionts in the DCA plot (Fig. 6) highlighted the affiliation of fungal species to the guilds as well as their distribution along the acidity gradient, while variation partitioning confirmed the clear influence of rock chemistry on the designated pattern of the three guilds. Substrate was shown to have the major effect on guild separation, explaining 22% of variability without interaction with another component, and additional 43% of variability with interactions. Photobionts and climate explained alone 3% and 2% of variability, respectively; mycobionts had a poor effect on guild separation (Fig. 7).

The interactions between photobionts and mycobionts in DCA plot clearly showed that some photobionts can constitute a resource for two ecologically close guilds (Fig. 6). An analogous pattern was also observed in another ecological context commonly used in lichen ecology (Seaward 2008), i.e. the separation of rock, soil and tree or wood dwelling lichens. Within 44 species-level lineages, photobionts from saxicolous lichens clustered in 32 clades, those from terricolous lichens belonged to nine clades and photobionts of corticolous/lignicolous lichens clustered in 28 clades. All three main substrate groups were intensively interconnected, with many photobionts shared by members of two (17 clades) or even all three (clades *S02a*, *S02b*, *S05*) groups (Fig. 1 and Fig. S2, Supporting Information).

Reproduction mode of lichens vs photobionts

In the 22 saxicolous assemblages sampled, the group of lichens associated with *Trebouxia* photobionts was characterized by a low proportion of species forming symbiotic propagules (see Fig. 1 and Fig. S2, Supporting Information). The siliceous, volcanic and calcareous rock guilds included 34%, 29% and 19% of vegetatively reproducing species, respectively (27.3% in average). In this context, many *Trebouxia* lineages lacked a host lichen producing symbiotic propagules. The final dataset, intentionally enriched by specimens from vegetatively reproducing lichens (see the 'Materials and methods' section), included 44.5% of photobiont sequences originating from these lichens (representing 46% of all fungal morphospecies). The photobionts from sexually and vegetatively reproducing lichen fungi were distributed erratically throughout the phylogenetic tree (see Fig. 1, Reproduction column). Among the algal clades represented by high number of sequences, we distinguished some *Trebouxia* lineages clearly preferring lichen fungi producing symbiotic propagules (clades *A03*, *S02b*), and on the

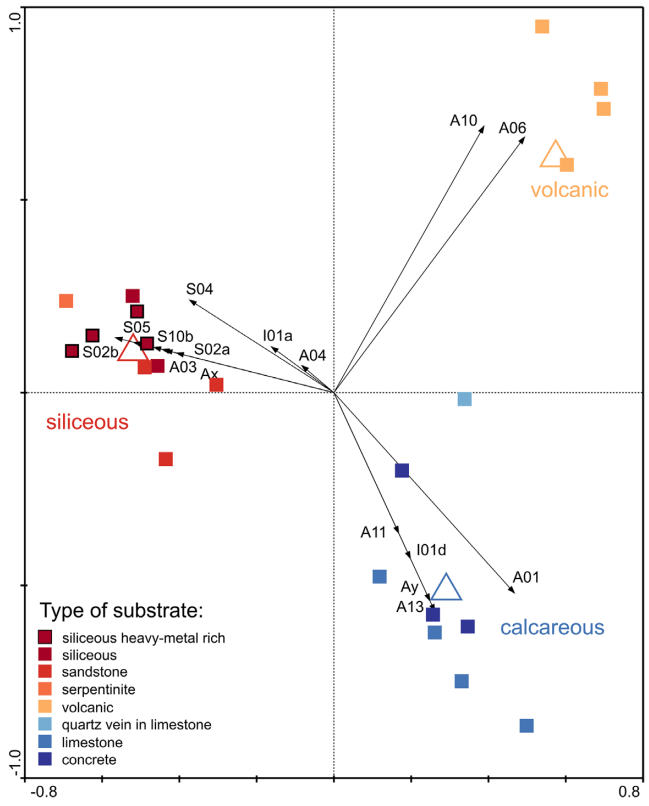

Figure 5. CCA using photobionts as explanatory variables (arrows), with the distribution of the 22 saxicolous samples (squares) shown and the affiliation to three lichen guilds (triangles) used as response variables. The percentage of variation explained by particular *Trebouxia* clades is given in Table S8 (Supporting Information).

other hand photobionts associated mainly with mycobionts utilizing spore dispersal (clades *A01*, *A06*, *A04*, *A10*, *A11* and *S04*). *Trebouxia* lineages *Ax* and *Ay* were only found in lichens completely lacking vegetative reproduction, and thus without possibility for dispersal by symbiotic propagules.

Discussion

Rock-dwelling guilds

In the case of symbiotic complexes, community formation is extremely interesting as particular components (symbionts) can react in different ways to environmental factors (Fernández-Mendoza *et al.* 2011). Helms (2003) suggested the ecological independence of the lichen photobiont from the mycobiont, as he found a close correlation of *Trebouxia* phylogeny with environmental factors and an apparent incongruence with the mycobiont phylogeny.

In our saxicolous dataset, we found photobionts generally showed similar preferences for distinct rock types as their mycobionts, forming ecologically congruent clusters of algal and fungal assemblages. The symbiotic network exhibited an internal structure, which was inhomogeneous and polarized due to pH gradient. Fungal and algal assemblages that occurred at two extreme ends did not communicate with each other (Figs 2 and 6), i.e. the fungi from acidic and basic substrates did not share any photobionts although they can grow side by side (e.g. sampling sites Sa and Co—see the 'Materials and methods' section). Moreover, the intermediary (volcanic) rock assemblages, occurring inside the network, did not form only a transition or conjunction between acidophilous and calciphilous lichens but they represented a significant cluster of fungi associated with photobionts *A06* and *A10*. These three groups of lichen fungi fulfil the definition of photobiont-mediated guilds, as they share specific groups of photobionts. However, in principle they are conditioned by substrate character, pH in particular, which determines the composition of photobiont pool as well as mycobiont–photobiont interactions.

Naturally, as each habitat has its unique features, each guild has local variants differing partly in species composition and exploited photobionts. Thus, the designated saxicolous guilds appear to be valid over a broadly conceived European temperate region, with similar lichen assemblages harbouring very close photobiont pools being inventoried at both nearby localities in Germany (Beck 2002) and Italy (Muggia, Grube and Tretiach 2008a) and at the relatively distant Crimean Peninsula (Voytsekhovich and Beck 2016).

Beyond rock habitats

To obtain a more complete insight into the photobiont-sharing fungal network, we also explored photobionts in epiphytic and tericolous lichens occurring in Europe. The results indicated that algal ecological demands could be much more complicated than inferred from the study of saxicolous communities alone. We found that *Trebouxia* algae exhibit four general life strategies relative to substrate:

(1) extreme specialists

a. only on rocks with a specific pH (acidophilous—clade *Ax*, basiphilous—*Ay*)
b. only on deciduous trees with nutrient-rich bark (*I05*, *I01b*, *I03*)

(2) rock specialists—only saxicolous, but occurring on a wide spectrum of rock types:

a. with clear preferences, dominating in distinct environments (*A06*, *A10*)
b. opportunists with no ecological optimum (*A04*);

(3) pH specialists—exhibiting clear preferences for particular pH values, but opportunistic in the choice of substrate form:

a. saxi-tericolous, basiphilous (*A01*, *A11*)
b. saxi-corticolous, basiphilous (*A13*, *I01d*) or acidophilous (*A03*, *S04*, *S10b*)
c. saxi-terri-corticolous, acidophilous (*S02a*, *S05*);

generalists—found on a wide range of substrates at differing pH values (*I01a*)

Trebouxia photobionts exhibiting strategies (3) or (4) are more ecologically plastic than the majority of their hosts as observed previously also by Helms (2003). The mycobionts generally show a much more rigid relationship to substrate type. Lichen fungi usually penetrate the substrate using various attachment organs, ranging from a loose web of hyphae to rhizines or holdfasts (Ozenda 1963, Büdel and Scheidegger 2008), which can lead to a strong adaptation to a particular substrate texture, porosity as well as chemistry (Brodo 1973). With the exception of some ecologically plastic species (e.g. *Amandinea punctata*—Helms 2003), the majority of lichen fungi represent specialists that alter the substrate either in somewhat accidental events (e.g. in harsh environmental conditions; Søchting 1989) or where the unusual substrate corresponds in chemistry and porosity to their preferred substrate (tree bark→specific siliceous rocks, siliceous rock→hard lignum, etc.). In comparison, many photo-

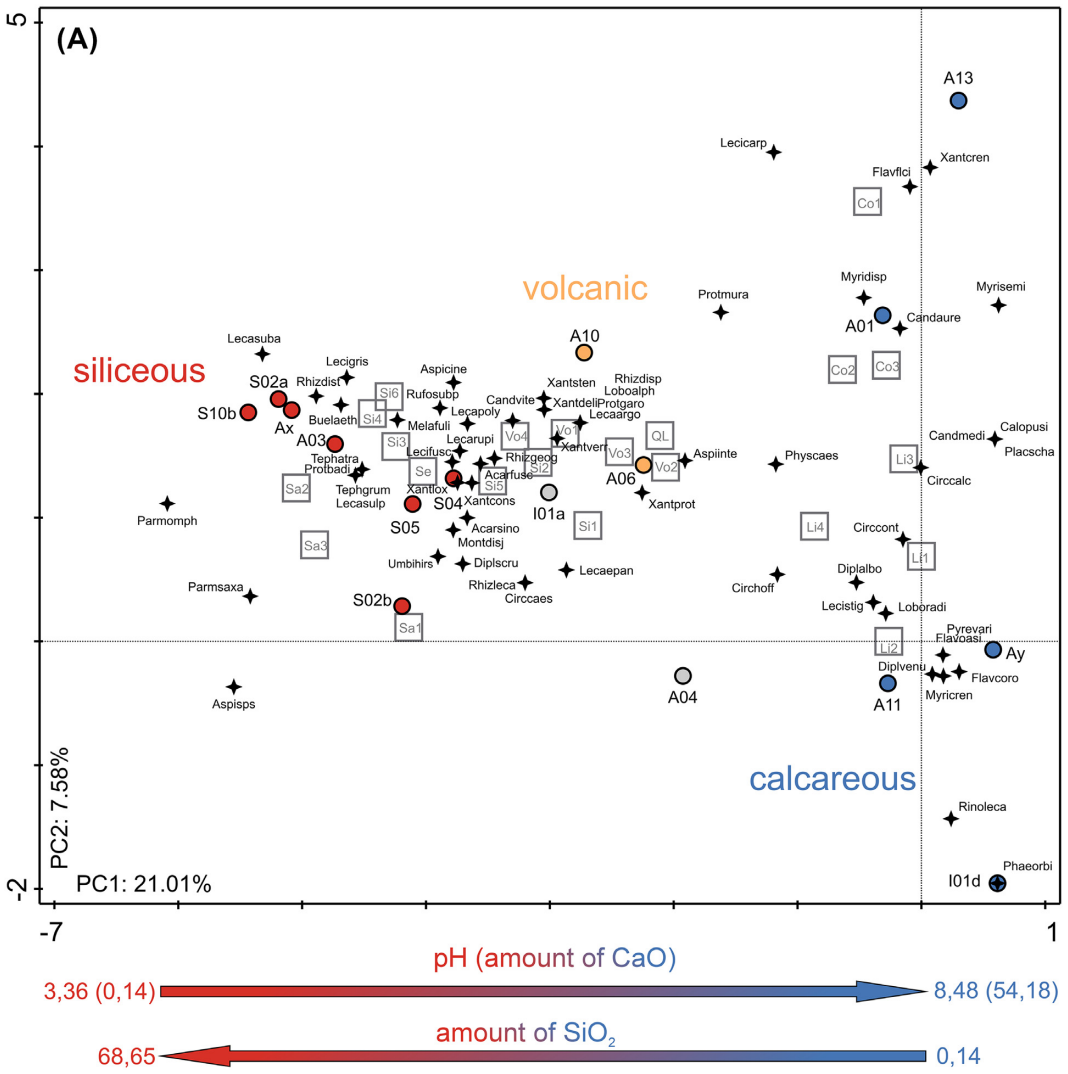

Figure 6. DCA of saxicolous lichen communities with three main rock types highlighted in colour. **(A)** Distribution of the 22 sampling sites (squares), 62 fungal taxa (black stars) and 16 *Trebouxia* clades (coloured dots) in the ordination space of the first and second ordination axes (percentage of explained variance is given alongside each axis). **(B)** The same plot as panel (A) with links between lichen-forming fungi and their photobionts (localities not shown). Connection colours indicate membership to a particular lichen guild (intermediary photobionts *A04* and *I01a* and their associations are in grey). Note the lack of sharpness in borders between neighbouring guilds, the intersections being related to ecologically plastic lichen fungi that are able to participate in different guilds. Arrows determine approximate gradients of pH, CaO and SiO_2 (in weight %). For fungal name acronyms, see Table S9 (Supporting Information).

bionts commonly live on different substrates; indeed, almost 75% of well-supported *Trebouxia* clades in this study represent opportunists in the choice of general substrate form. These photobionts were most frequently found on both rock and tree bark, or rock and soil (17 clades), with some clades revealed in lichens growing on all three substrate forms (Fig. 1). However, they always maintain their pH preferences and these different substrates are similar in acidity or basicity. As an example, *Trebouxia* clades *A03*, *S02a*, *S02b*, *S03*, *S05*, *S10a*, *S10b* and *Sx* interconnect European lichen species growing on acid soils, tree bark and siliceous rocks from boreal to temperate zone into a large 'cross-border' guild mediated by lower pH, while another guild includes basiphilous soil- and rock-dwelling communities (*A01*, *A11*) and basi-nitrophilous communities from deciduous trees and calcareous rocks or concretes (*A13*, *A33*, *T.* aff. *decolorans*, *I01a*). This concurs with similar behaviour previously reported in the genus *Asterochloris* (Peksa and Škaloud 2011, Škaloud *et al.* 2015, Škvorová *et al.* 2022).

Substrate chemistry, expressed as pH, appears to be the determining factor shaping algal pools in climatically congruent sites within continuous geographic regions. As acidity affects the availability of nutrients, the fundamental effect of pH is on algal ecophysiology (Brodo 1973, Smith 1982, Larcher 1984, Mortvedt *et al.* 1991, Bačkor *et al.* 1998, Hosier and Bradley 1999, Jensen 2010, Knez and Graham 2013). pH can also directly impact algal photosynthesis (Andrews and Lorimer 1987, Raven and Geider 2003), which is crucial for nutrition of the whole lichen. The mycobiont can partially regulate the water content and distribution within the thallus (Honegger 2007, 2009) and it can even partly protect the photobiont against the direct influence of substrate pH (Mollenhauer 1997). Nevertheless, the possibilities of lichen-forming fungi to protect their photobionts are necessarily limited due to poikilohydric nature of lichens, especially in primordial stage of the thallus. In possible free-living period (see later), the alga is actually directly exposed to the substrate. Therefore, it is not surprising that pH obviously correlates with *Trebouxia* phylogeny, as

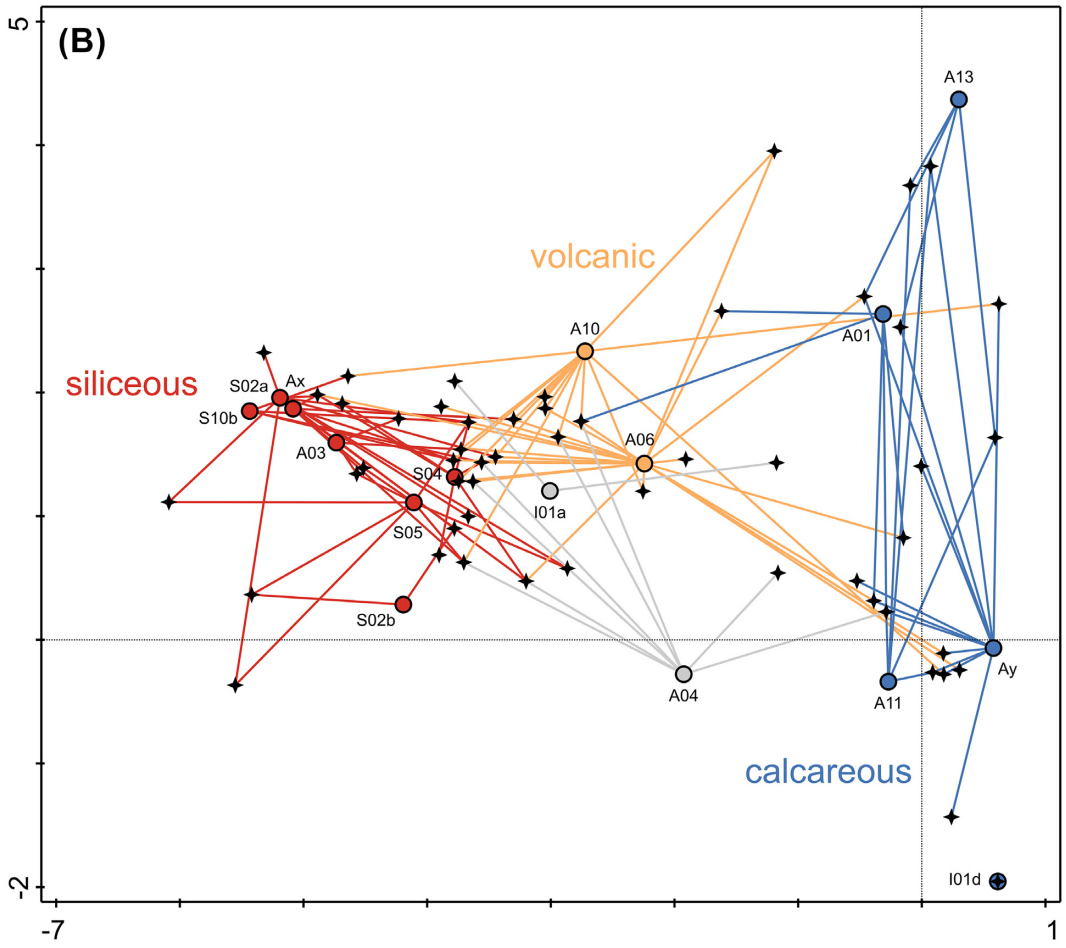

Figure 6. *Continued.*

found already by Helms (2003). The members of clade S are completely associated with acidic substrates; however, in the clade A, pH preferences differ between particular species-level lineages (Figs 1 and 4).

Although the traditional separation of saxicolous, terricolous and epiphytic communities can only be applied to lichen fungi, and not the holobionts, our concept of lichen guilds does not completely contradict the concept of lichen sociology. A number of lichen syntaxa are also determined by pH and nutrient composition of the substrate (Klement 1955, James, Hawksworth and Rose 1977, Bültmann 2012). Thus, they seem to form natural subunits of the abovementioned 'cross-border' guilds.

As fungal species often associate with several photobiont species or OTUs (Blaha, Baloch and Grube 2006, Guzow-Krzemińska 2006, Yahr, Vilgalys and Depriest 2006, Leavitt *et al.* 2016, Muggia *et al.* 2020) or even several genera (Engelen, Convey and Ott 2010, Vančurová *et al.* 2018, Osyczka *et al.* 2021), we assume common intertwining of the guilds at their edges, increasing with the number of little specific and ecological plastic guild members. Commonly, the guilds rather form major noda in the continuum of lichen symbiotic network than the separate units (cf. James, Hawksworth and Rose 1977). Similar to saxicolous assemblages (Figs 2 and 6), the whole *Trebouxia* lichen community probably forms a polarized network with modular structure, where the natural grouping of photobionts based on their environmental preferences arranges the basic partition of fungal guilds. Particular guilds can be further subdivided, as in the so-called *Peltigera* guild, which exhibits the modular/anti-nested structure where fungi form separate groups sharing specific *Nostoc* genotypes (Chagnon *et al.* 2018), even in homogenous environment of tropical mountain forests (Kaasalainen *et al.* 2021). Such structural character of the network may be a result of close phylogenetic relationship within modules (Magain *et al.* 2017) or photobiont ecology, including their ability to live aposymbiotically (Cardós *et al.* 2019, Kaasalainen *et al.* 2021).

Photobiont dispersal

The suggested concept of lichen guilds includes another important aspect, photobiont dispersal. The vegetative propagules of core species are hypothesized to be important vectors of photobionts in and out of the lichen guild (Rikkinen, Oksanen and Lohtander 2002). Our sampling, however, showed a low number of vegetatively reproducing species in the rock-dwelling assemblages examined (27% of all species on average). Such a deficiency would not be problematic if each algal lineage was associated with at least one strongly proliferating and abundant core species, which would allow effective dispersal over a particular locality or area. Within our basic set of saxicolous lichen samples, however, we found several photobionts (*A01*, *Ax*, *Ay*, *S04*, *S10b*) that never associated with sorediate or isidiate lichens, meaning that these photobionts are most likely to have entered the community from outside (Rikkinen 2003). Our tentative screening of 432 Central European *Trebouxia*-associated lichen species (see the 'Materials and

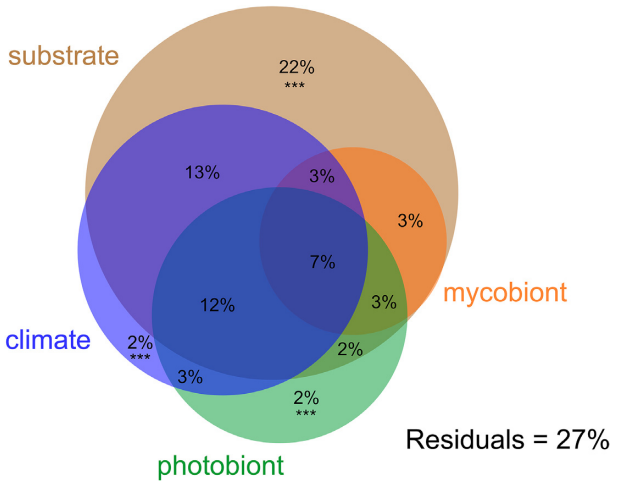

Figure 7. Venn diagrams showing variation in the pattern of three saxicolous lichen guilds explained by substrate (content of 11 basic chemical compounds and pH), climatic conditions (altitude, PCs for the analysis of 19 environmental variables) and photobiont and mycobiont species composition.

methods' section) confirmed a low number of taxa forming specialized symbiotic propagules in ombrophilic saxicolous and terricolous communities (25% and 1% of species, respectively). On the other hand, vegetatively reproducing species were strongly represented in epiphytic lichens (58%) or, more generally, in semi-ombrophobic and ombrophobic communities growing on steep or even overhanging surfaces. These lichens could serve as important suppliers of photobionts to other communities.

After the intentional addition of vegetatively reproducing lichen samples (see the 'Materials and methods' section), these comprised almost 45% of our final dataset. Further analysis indicated that the majority of *Trebouxia* lineages were associated with at least one of them (Fig. 1 and Fig. S2, Supporting Information). Nevertheless, some photobionts remained known predominantly (clades *A01*, *A04*, *A06*, *A10*, *S04*) or even exclusively (*Ax*, *Ay*) from lichens producing aposymbiotic spores only. While the primary reason for this may be undersampling, these clades are very abundant and are frequently sampled in many lichen taxa (Blaha, Baloch and Grube 2006, Muggia, Grube and Tretiach 2008b, Muggia *et al.* 2014, Voytsekhovich and Beck 2016; our data). Although the preference for sexually reproducing fungi mainly concerns basiphilous and subneutrophilous members of *Trebouxia* clade A (*A01*, *Ay*, *A06*, *A10*), it was detected also in acidophilous clades (*Ax*, *S04*). Thus, these findings rather indicate noticeable differences in lifestyle between particular *Trebouxia* clades. Basically, we distinguished two photobiont groups utilized by lichen guilds: (i) those predominantly co-dispersed with mycobionts in vegetative propagules (e.g. *A03*, *I01b*, *S02b*) and (ii) those mainly associated with fringe species completely lacking symbiotic diaspores (listed above). Interestingly, such partitioning appears not to be a particularity of *Trebouxia*-associated lichens. The same behaviour has been revealed in corals, which exhibit strong separation of algal symbionts in vertically and horizontally transmitting hosts. Fabina *et al.* (2012) showed that, on a global scale, 46% and 41% of *Symbiodinaceae* lineages are found exclusively in vertical or horizontal transmitters, respectively. Pochon *et al.* (2010) found that *Symbiodinium* types associated with vertical transmitters were firmly tied to their hosts. These algae did not occur as free-living in the sediments surrounding the corals, as is usual for horizontal transmitters (Ali *et al.* 2019). Thus, the importance of vegetative propagules in photobiont propagation and transmission may be limited, meaning that the sexually reproducing fungi associated with *Trebouxia* algae from the second group need to obtain photobionts elsewhere.

Photobiont sources

In addition to exploiting of disintegrating vegetative propagules or stealing from juvenile stages of lichen thalli (Rikkinen 1995, 2003), the spores of sexual species may obtain suitable algae from aposymbiotic forms of lichen photobionts. The non-lichenized *Trebouxia* cells under natural conditions have been rarely found on bark (Tschermak-Woess 1978, Bubrick, Galun and Frensdorff 1984) and soil surface (own unpublished observations) and on artificial substrate (Sanders 2005).

In lichen thalli, some algal cells are expected to be weakly wrapped by fungal hyphae or completely free of contact with the fungus (Slocum, Ahmadjian and Hildreth 1980). This is made possible by occasional aplanosporogenesis within the lichen thallus (Tschermak-Woess 1978), after which the fungus is unable to encase all daughter algal cells leaving the disrupting sporangium. Many lichens, including early colonizers, have simple thalli without a solid upper cortex from which these free algae could be easily released, e.g. in heavy rain or other erosion events. Moreover, the influence of animals feeding on lichens could also be considerable (Asplund *et al.* 2010, Boch *et al.* 2011), even in the case of saxicolous communities (Fröberg, Baur and Baur 1993). Disruption of thalli by feeders could facilitate the escape of photobionts; indeed, liberated, living algal cells have been observed in faecal pellets of mites (Meier, Scherrer and Honegger 2002) and snails (Fröberg *et al.* 2001). While possibly important, the potential for algal zoospores to escape from lichen thalli has also been understudied. Under certain conditions, some *Trebouxia* cells complete the process of zoosporogenesis within a natural thallus allowing motile zoospores to be released into the surrounding microenvironment (Slocum, Ahmadjian and Hildreth 1980). After escape from the thallus, the lichen algae can undergo an intensive reproduction phase (Tschermak-Woess 1978, Slocum, Ahmadjian and Hildreth 1980), which provides a sufficient number of small but available photobiont microcolonies hidden in micro-refuges in the vicinity of a lichen (Bubrick, Galun and Frensdorff 1984). Moreover, the exudates of fungal mycelia might be a sufficient attractant or stimulus for zoospores to settle close to them (Sanders 2005).

The occurrence of *Trebouxia* clades *A06*, *A10* and *Ay* in young assemblages of strictly sexual species growing on small islands of specific substrate, distant from the nearest related communities (study sites Co1, 2, 3, QL), supports such hypothesis. These photobionts were commonly found to be only (*Ay*) or predominantly hosted by fungi dispersing by spores (*A06*, *A10*)—see Fig. 1 and Fig. S2 (Supporting Information). Although the latter two can also associate with several vegetatively reproducing *Xanthoparmelia* species, these form big and heavy isidia unsuitable for long dispersal, and do not occur in the vicinity of Co and QL sampling sites. Thus, the presence of such photobionts indicates that an assemblage of strictly sexually reproducing lichen fungi do not have to develop only as a side-product of vegetative dispersal activity within neighbouring communities (Rikkinen 2003). In fact, this community is likely to have originated through a newly developed symbiosis between the arriving fungal spores and airborne algae. Moreover, thanks to the processes described above, such a community could even serve as a source of free-dispersing photobionts within as well as outside the community. One might even speculate about the 'intentional' release of algal zoospores from

the lichen, as an analogous process is known from assemblages of adult spawning corals that expel symbiotic algae into seawater and sediment, where they become available to juvenile corals (Adams, Cumbo and Takabayashi 2009, Cumbo, Baird and van Oppen 2013, Nitschke, Davy and Ward 2016).

So, the partition of lichen guild members to core and fringe species, as photobiont 'disseminators' and 'catchers', seems likely to us; however, it is not necessarily related to the division into the species forming symbiotic propagules and those dispersing by fungal spores, respectively. Sexually reproducing species can probably also serve as photobiont sources, e.g. the lichens with simple thalli allowing photobiont cells to escape. Such randomly or periodically expelling algae could represent a main source of *Trebouxia* lineages belonging to the group associated mainly with sexually reproducing fungi. The seasonality of algal release together with their ephemerality could explain why they are not frequently found in nature.

Conclusions

We found the guild system in *Trebouxia*-hosting lichens as environmentally conditioned. The lichen-forming fungi sense substrate properties differently from their photobionts, which mainly react to substrate chemistry, expressed as pH. In this way, the guilds are established across the classical ecological groups of saxi-, terricolous and epiphytic lichens. We consider this 'pH-mediated' system of lichen guilds as close to natural, because it divides the resources (i.e. photobionts) according to their real co-occurrence, which enables to create (and distinguish) a fully functioning guilds that share their resources intensively.

We revealed clear differences in life strategies of individual photobionts. In addition to environmental preference, mode of transmission (i.e. a predominant association with strictly sexually or vegetatively reproducing lichen fungi) appears to represent a stable lifestyle trait. The vegetative propagules most likely do not serve as a source of photobionts for all the other lichen fungi as many *Trebouxia* photobionts do not associate with a sufficient pool of vegetatively dispersing lichens that would be able to effectively supply the group of co-associated sexually reproducing species. Indeed, we believe that the role of free-dispersing photobionts in the establishment of some lichen guilds is important, however widely underestimated. We hope the future research including huge sampling and new methods such as DNA metabarcoding (Moya *et al.* 2021) combined with network-based analysis (Chagnon *et al.* 2018, Kaasalainen *et al.* 2021) and also thorough examination using microscopical and cultivation technics (Cardós *et al.* 2019) could deepen the knowledge of the rules in lichen systems.

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Supplementary data

Supplementary data are available at FEMSEC online.

Data accessibility

DNA sequences are available in GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/nuccore/ under accession numbers MW694953–MW695202. The alignment called 'Peksa, O. Gebouska, T., Vancurova, L., Skvorova, Z. & Skaloud, P. (2021). Photobiont-mediated guilds in green algal lichens—an insight into the life of terrestrial symbiotic communities is available in Mendeley Data at http://dx.doi.org/10.17632/95t3rzv6rs.1 The R script is available in Mendeley Data at http://dx.doi.org/10.17632/95t3rzv6rs.2.

Author contributions

OP designed the study, performed field work, identified and processed lichen samples, analysed the data and wrote the manuscript; TG, LV and ZS carried out laboratory works; PS performed phylogenetic analysis and variation partitioning; OP, LV, ZS and PS interpreted the data; and LV and PS revised the manuscript and gave critical input.

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