Myco-, photobiont and bacterial microbiome associations along an elevational gradient in the Hohe Tauern, Salzburg, Austria

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Lichens are among the few organisms capable of thriving in extreme environments, forming miniature ecosystems composed of diverse fungal, algal, and bacterial communities. To characterize their symbiotic community structure along climatic gradients, the composition, distribution, and interactions of their primary symbiotic partners (mycobiont [fungus]/ photobiont [algae]) and the bacterial microbiomes were studied. Using molecular techniques, including Sanger and high-throughput amplicon sequencing, we analyzed saxicolous lichens along an elevational gradient (1168–2562 m a.s.l.) in the Grossglockner region, Salzburg, Austria.

The following research questions were addressed: (i) Do the symbiotic partners of lichens – mycobionts, photobionts, and bacterial communities – vary in composition and diversity along an elevational gradient? (ii) Do lichens at the same site share locally available symbiotic partners, such as photobionts, and bacterial microbiomes?

All three partners of lichen communities, i.e. mycobionts, photobionts, and bacteria, varied in diversity and composition along the elevational gradient. Neighboring lichens within microhabitats shared photobionts, indicating local availability as a key factor in partner selection. However, bacterial microbiomes showed substantial heterogeneity across and within sites, influenced by mycobiont-photobiont interactions. The formation of local guilds drives new symbiotic combinations that may improve the adaptability of lichens to environmental changes and the colonization of new niches.

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Flechten gehören zu den wenigen Organismen, die in der Lage sind, auch in extremen Umgebungen zu gedeihen und formen Miniatur-Ökosysteme, die aus verschiedenen Pilz-, Algen- und Bakteriengemeinschaften bestehen. Zur Charakterisierung ihrer Gemeinschaftsstruktur entlang klimatischer Gradienten analysierten wir die Zusammensetzung, Verteilung und Interaktionen ihrer Symbiosepartner und Mikrobiome mit molekularen Techniken wie Sanger- und Next-Generation-Sequenzierung. Die Studie fokussierte sich auf felsenbewohnende Flechten entlang eines Höhengradienten (1168–2562 m ü. NN) in der Großglocknerregion, Österreich.

Im Rahmen der vorliegenden Studie wurden folgende Forschungsfragen untersucht:

(i) Lassen sich Veränderungen in der Zusammensetzung der Symbiosepartner von Flechten – Mykobionten, Photobionten und bakterielle Mikrobiome – entlang eines Höhengradienten beobachten? (ii) Teilen sich Flechten am selben Standort die lokal verfügbare symbiotische Partner, wie Mykobionten, Photobionten und Mikrobiome?

Die Ergebnisse zeigen deutliche Veränderungen der Flechtengemeinschaften und ihrer Symbiosepartner entlang des Höhengradienten. Innerhalb einzelner Mikrohabitate teilen benachbarte Flechten häufig Photobionten, was auf lokale Verfügbarkeit als entscheidenden Faktor hinweist. Mikrobiome zeigen jedoch beträchtliche Heterogenität zwischen und innerhalb der beprobten Areale, beeinflusst durch Mykobionten-Photobionten-Assoziationen. Die Bildung lokaler Gildenstrukturen erlaubt die Etablierung neuartiger symbiotischer Kombinationen von Flechten und ihren assoziierten Partnern. Dies könnte ihre für ihre Adaptionsfähigkeit an Umweltveränderungen sowie für die Besiedlung neuer ökologischer Nischen von Vorteil sein.

Keywords: Saxicolous crustose lichens, symbiotic associations, photobionts, mycobionts, bacterial microbiomes, molecular ecology, elevational gradient.

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Introduction

Lichens represent intricate symbiotic systems including fungi and photosynthetic partners, such as algae or cyanobacteria, as well as other fungi, algae and bacteria which interact closely, enabling them to thrive in a wide range of environmental conditions (Aschenbrenner et al. 2016; Hawksworth & Grube 2020; Goodenough & Roth 2021; Morillas et al. 2022). Over the past 600 million years, lichens have demonstrated remarkable persistence, colonizing some of the most extreme environments on Earth, from arid deserts to polar areas (Galloway 2008; Thüs & Schultz 2009). Understanding the association patterns of lichens in relation to environmental conditions is fundamental for identifying the drivers of lichen biodiversity across all symbiotic components (Briggs & Walters 2016; Spribille et al. 2022).

While lichens can thrive in a broad range of environmental conditions, their actual occurrence is scale-dependent, shaped by microhabitat conditions at fine scales as well as regional climate factors at broader scales. Thus, some lichen guilds show similar community structures across sites with comparable microhabitat conditions, regardless of differences in broader climatic settings (Peksa et al. 2022). Despite current gaps in comprehensive knowledge of lichen diversity and the relationships between the mycobiont (fungus) and the photobiont (algae), documented regional occurrences indicate that climate, with its main factors such as temperature, precipitation patterns, wind, and solar radiation, also significantly influences the distribution of photobionts and thus the ability to adapt to external stress factors (Wagner et al. 2020; Rolshausen et al. 2023).

The core of the lichen symbiosis consists of a dominant mycobiont and photobiont partner, the latter typically green algae and/or cyanobacteria, embedded in a bacterial layer with biofilm-like structures (Grube et al. 2009; Bates et al. 2011; Aschenbrenner et al. 2014). In addition to these main partners, lichens host a multitude of further symbionts, including diverse communities of endolichenic and lichenicolous fungi, basidiomycetous yeasts (Lawrey & Diederich 2003; Arnold et al. 2009; Spribille et al. 2022), several green algal (Guzow-Krzemińska 2006; Peksa & Škaloud 2011) as well as diverse bacterial communities (Grube & Berg 2009; Bates et al. 2011). This assemblage forms a highly integrated miniature ecosystem with complex interactions among fungal, algal, and bacterial components (Aschenbrenner et al. 2014). Recent studies describe lichens as holobionts, integrated biological units comprising a host and microbiota. Mycobionts, typically Ascomycota, sometimes Basidiomycota, directly expose their mycelium to environmental conditions. They contribute to structural integrity and stress protection, while photobionts, through photosynthesis, supply energy-rich compounds. Bacterial components, often forming biofilms

on fungal surfaces, perform key functions such as nitrogen fixation, pathogen defense, and growth regulation (Grube et al. 2015; Auður Sigurbjörnsdóttir et al. 2016). In addition, the poikilohydric lifestyle of lichens enables them to survive in prolonged drought, which allows for their persistence in challenging habitats such as alpine regions or the extreme cold areas of the Antarctic Continent (Schroeter et al. 2010). Furthermore, these complex microbial consortia appear to be shaped by both environmental filtering, co-dispersal, and co-evolutionary processes (Fernández-Mendoza et al. 2011; Aschenbrenner et al. 2014; Spribille et al. 2022).

The phenomenon of anthropogenic climate change has been observed to accelerate several environmental transformations, including geographic range shifts, alterations to species interactions, and a reduction in biodiversity (Thomas et al. 2004; Tylianakis et al. 2008; Pecl et al. 2017). Alpine ecosystems are particularly susceptible to the effects of climate change, with rising temperatures driving vegetation shifts to higher altitudes and reducing the availability of niches for species at lower elevations, thereby increasing extinction risks (Pörtner et al. 2022). Understanding these dynamics is essential to predicting and mitigating biodiversity loss in the face of rapid environmental change (Leclère et al. 2020; Pörtner et al. 2022; Pfenning-Butterworth et al. 2024; Götz et al. 2026).

Elevational gradients provide a valuable framework for studying lichen ecology and the impacts of climate change. These gradients serve as proxies for climatic variability, revealing insights into species distribution patterns and their adaptive capacities. For instance, lichens exhibit significant turnover in myco-/photobiont associations along elevational transects, suggesting that environmental filtering and niche specialization play crucial roles in shaping community assemblages (Dal Grande et al. 2017; Rolshausen et al. 2023). Observations from genomic and community studies indicate that compositional shifts in lichen symbioses often coincide with regional climatic transition zones (Rolshausen et al. 2023).

The Großglockner Mountain range in the Austrian Alps represents a valuable site for investigating biodiversity and climate interactions. Spanning diverse climatic zones, from montane beech-spruce forests to nival zones of snow and ice, this region provides distinct ecological niches shaped by factors such as temperature, precipitation, and microclimatic variability (Grabherr et al. 2010). Recent studies have highlighted significant temperature increases and altered precipitation patterns in the region, influencing species composition and ecosystem structure (Taucher et al. 2009).

This study aims to assess the distribution and diversity of the dominant mycobiont and photobiont as well as bacterial microbiome associations in rock-dwelling lichens along an elevational gradient (1168–2562 m a.s.l.) along the Grossglockner High Alpine Road (Salzburg, Austria), to analyze the influence of climatic factors on lichen communities and evaluate the potential of lichens as bioindicators for climate change. This work will contribute to understanding the dynamics of biodiversity under changing climatic conditions, with implications for conservation and management strategies.

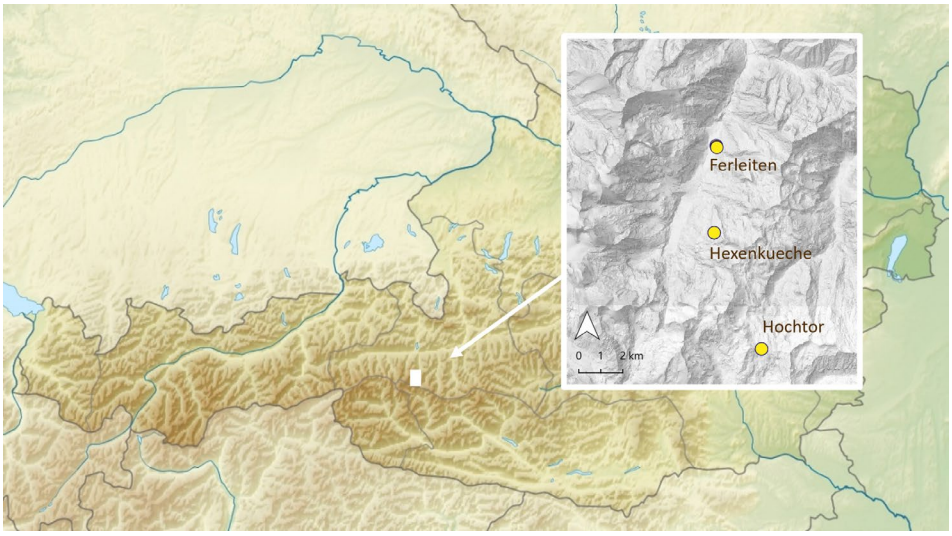


Fig. 1: Topographic Map of Austria. The rectangle indicates the position of the study area within Austria. The insert shows a detailed topographic view of the sampling region in Salzburg, Hohe Tauern, with individual sampling sites marked by yellow circles: Ferleiten, 1168 m a.s.l., Hexenküche, 2153 m a.s.l., Hochtor, 2562 m a.s.l. Source: CC / Uwe Dedering. | **Abb. 1:** Topographische Übersichtskarte von Österreich mit Markierung und Detailansicht des Untersuchungsgebiets in Salzburg, Hohe Tauern. Die Untersuchungsgebiete sind durch gelbe Kreise gekennzeichnet: Ferleiten, 1168 m Seehöhe, Hexenküche, 2153 m, Hochtor, 2562 m. Quelle: CC / Uwe Dedering.

Materials and Methods

Long Term Lichen Diversity Experiment (LTLDE)

This work is the basic survey of all existing lichens at the LTLDE observation sites and provides baseline data for tracking succession processes over a ten-year period. The long-term project "LTLDE: Long Term Lichen Diversity Experiment," initiated in 2017, aims to monitor the succession and recolonization dynamics of lichen-associated organism groups annually after complete removal. As part of the project, a comprehensive molecular evaluation of the internal diversity of 103 lichen samples was conducted at T0, the project start, as shown in Fig. S1. These samples were collected from three sites along an elevational gradient on the Grossglockner High Alpine Road in Austria, see Fig. 1, focusing on the diversity of the dominant myco-, photobionts and the bacterial microbiome and their associations.

Sampling

Lichen community samples were collected at three sampling sites along an elevation gradient during the month of August 2017. For each site, three approx. 30 x 20 cm rocks were removed and transported to the laboratory to assign the lichen samples present on the rock with molecular techniques. All sites were located on the Grossglockner High Alpine Road, Austria.

At 1168 m a.s.l. Ferleiten (F), N 47.16536/ E 012.81457, represents the lowest site along the elevational gradient. The site is located below the treeline and frequent agricultural use for grazing may result in elevated nitrogen levels. As with the first sampling site, three rock fragments were taken from the second sampling site, Hexenküche (He), at 2153 m above sea level, N 47.12968/ E 012.81369. It is located approximately at the tree line and is rarely used as pasture for sheep and cattle. Hochtor (H), N 47.08228/ E 012.84254, is the highest site in this study at 2562 m above sea level, Tab. S1. As with the previous sampling sites, three rock fragments were collected here. The vegetation is dominated by biological soil crusts and saxicolous plant and lichen communities. Livestock grazing is relatively rare. Mean annual temperature and precipitation data for the three sampling sites are available at GeoSphere Austria (formerly the Central Institute for Meteorology and Geodynamics, ZAMG; https://www.geosphere.at).

A total of 103 lichen samples were collected across all rock replicates by scraping organic material from the center of each thallus into (1) Eppendorf tubes for the mycobiont and photobiont analyses and (2) tubes for stabilizing the bacterial communities, using a sterilized scalpel. All samples were stored at minus 20° Celsius.

DNA extraction of mycobiont, photobiont and the bacterial microbiome

For extraction and isolation of genomic DNA of the lichen specimens, the DNeasy® Plant Mini Kit (Qiagen) was used according to the manufacturer's instructions. To disrupt the tissue, the Eppendorf tubes containing the samples were ground with micro pestles in liquid nitrogen. All bacterial samples were extracted and provided by the LTLDE project group 2017 using the Xpedition Fungal/Bacterial DNA MiniPrep following the manufacturer's instructions (Zymo Research).

Sanger Sequencing (Myco-/photobiont) and amplicon generation/sequencing (bacterial microbiome)

The widely used barcode marker Internal Transcribed Spacer (ITS) was amplified and sequenced for both symbiotic partners in order to detect the available species. For the mycobiont the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) and for the photobiont ITS1T and ITS4T (Trebouxia, Kroken & Taylor 2000) and ITS-sense-A and ITS-antisense-A (Ruprecht et al. 2014), as well as nr-SSU-1780-5' (Piercey-Normore & DePriest 2001) and ITS4 were used according to Ruprecht et al. (2020), as shown in Tab. S3. For the bacteria the v3v4 region of the bacterial rRNA gene was amplified with standard primers provided by Eurofins. Sanger sequencing as well as Illumina sequencing and analysis of the bacterial microbiome composition was done at Eurofins Genomics in Germany.

Taxonomic identification

The final sequences of the mycobionts' and photobionts' nuclear ITS region were assembled and edited with Geneious version 6.1.8 (Kearse et al. 2012) and aligned with Geneious Alignment and MAFFTv7.017 (Katoh et al. 2002). The Basic Local Alignment Search Tool (BLAST) of the National Library of Medicine/National Centre of Biotechnology

and Information (NCBI) database (Altschul et al. 1990) was employed to facilitate the comparison of each mycobiont sequence with the international database for the purpose of identification. Further mycobiont sequences of the genera *Lecidea* Ach. and *Porpidia* Körb. as well as the photobiont sequences of the genera *Asterochloris* Tschermak-Woess and *Trebouxia* Puymaly were compared with those previously generated by Ruprecht et al. (2020) and Muggia et al. (2020), which formed the basis for the corresponding taxonomic classification.

Diversity indices

Alpha, beta and gamma diversity of mycobionts, photobionts and bacterial microbiomes were calculated as Hill numbers of order q = 1, equivalent to the exponential of Shannon entropy, using the R package entropart (Marcon & Hérault 2015). This measure weights species by their relative abundance and is less sensitive to rare species than species richness (q = 0). Estimation bias was corrected using the "Best" estimator as implemented in entropart. Prior to diversity calculation, bacterial count data were rarefied to a minimum sequencing depth of 1040 reads using the R package rtk (Saary et al. 2017), to account for unequal sequencing effort across samples. Diversity was calculated separately for each of the three symbiotic components, mycobionts, photobionts and bacterial microbiomes, and compared across the three sampling sites (Ferleiten, Hexenküche, Hochtor).

Statistical analyses

Statistical analyses were performed using R version 4.3.2. To visualize differences in the species composition and the associated partners we used non-metric multidimensional scaling (NMDS) plots produced with the metaMDS function in R package *vegan* (Oksanen et al. 2022) based on Bray-Curtis dissimilarity values with a maximum of 100 random starts and three dimensions. The ggvenn function in the R package *ggvenn* (Yan 2025) was used to graphically represent species distributions for mycobionts, photobionts, and bacteria based on the number of species shared between sites. As an unconstrained ordination approach based on a dissimilarity matrix, several calculations were performed using a distance-based redundancy analysis (db-RDA) with the capscale-function in R package vegan. A network of all associated Taxa and OTUs of the mycobionts and photobionts was conducted using the Gephi interactive platform and the Force Atlas continuous layout algorithm (Bastian et al. 2009). A complete species list is provided in the Supplementary Information: (Tab. S2 and App. 1/2).

Bipartite network and network indices

To visualize the ecological network patterns between mycobiont, photobiont, and the bacterial microbiome of the dataset, bipartite diagrams were created using the plotweb() function provided in the R-package *bipartite* (Dormann et al. 2008). The network descriptive indices H_2' and d' (Blüthgen et al. 2006) were calculated using the function H2fun() and dfun(). When determining the level of specialization H_2' describes the complementary specialization of all interacting groups. H_2' becomes larger with increasing selectivity of a species. d' can range from zero to one, where one means a complete specialist in terms of available association partners at the higher level. It determines how divergent a species group is compared to a random sample of available interaction or association partners.

Results

Taxonomic classification and spatial distribution

A total of 103 lichen specimens were sampled, of which 80 had sequence data available for all three symbiotic partners (mycobiont, photobiont, and bacterial microbiome). Altogether 32 dominant mycobiont species belonging to ten families were determined, with associated photobionts distributed among six operational taxonomical units (OTUs) of the genera *Trebouxia* and *Asterochloris*, hereafter abbreviated as *Tr* and *Ast*, respectively. In total, bacteria belonging to 114 families were detected, of which 110 were retained after CSS-normalization

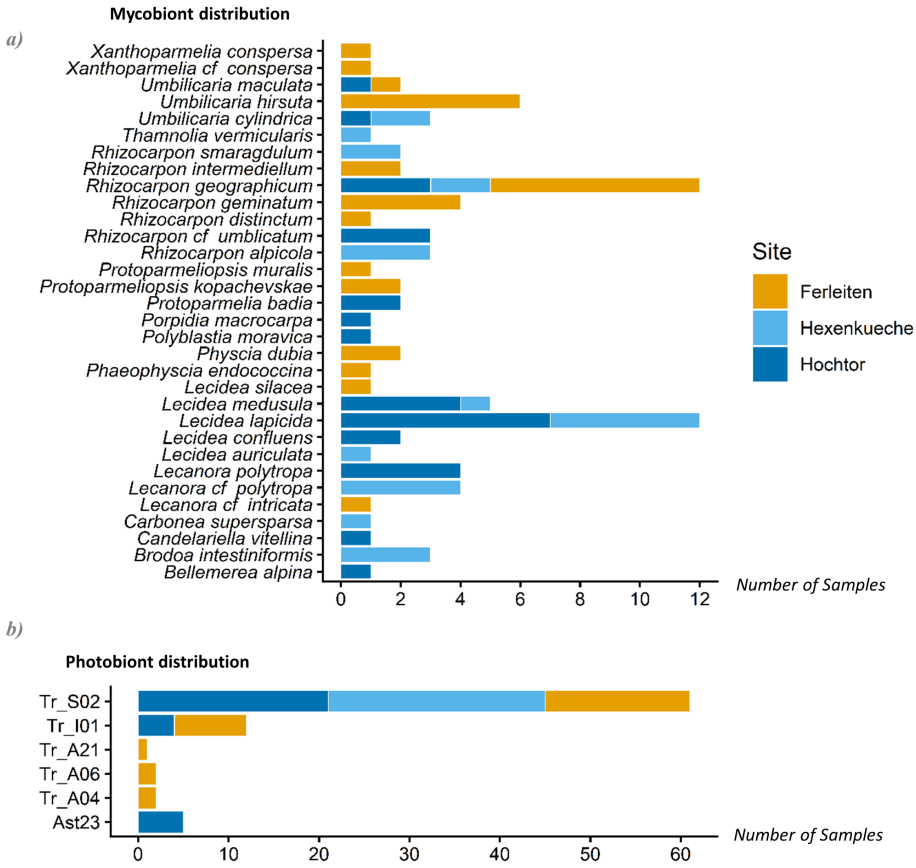


Fig. 2: Distribution of mycobiont species and photobiont OTUs across the sites Ferleiten, Hexenküche and Hochtor along the Großglockner High Alpine Road, Austria. Bars show the number of samples of each identified species/OTU, colour-coded by site: **a)** mycobionts, **b)** photobionts. | **Abb. 2:** Verteilung der Mykobionten-Arten und Photobionten-OTUs in den Untersuchungsgebieten Ferleiten, Hexenküche und Hochtor entlang der Großglockner Hochalpenstraße, Österreich, basierend auf insgesamt 103 saxicolen Flechtenproben. Dargestellt ist die summierte Häufigkeit pro Art bzw. OTU an den farbcodierten Standorten für **a)** Mykobionten und **b)** Photobionten.

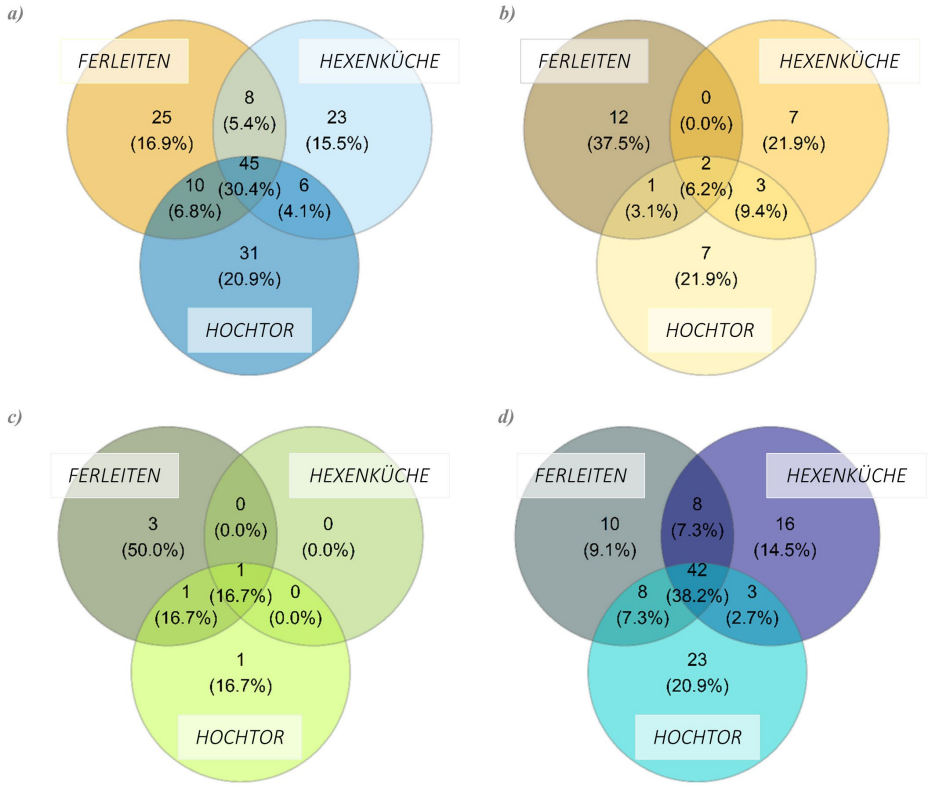


Fig. 3: Venn diagram showing species distribution by the numbers of species shared between the three sites, ranging from 1160 to 2750 m above sea level. **a)** All dominant symbiotic partners, **b)** mycobiont, **c)** photobiont, **d)** bacteria. | **Abb. 3:** Venn-Diagramme zur Artenverteilung zwischen den drei Untersuchungsstandorten entlang des Höhengradienten (1160–2750 m ü. d. M.). Dargestellt ist die Anzahl gemeinsam vorkommender Arten von **a)** allen dominanten symbiotischen Partnern, **b)** Mykobionten, **c)** Photobionten und **d)** bakteriellen Taxa.

and used in downstream analyses. All mycobiont species and photobiont OTUs present are listed in Fig. 2 and color-coded according to their location. Fig. 3a-d shows the species distribution between all three sampling sites at different elevations. The most common species among mycobionts were *Rhizocarpon geographicum* (L.) DC. and *Lecidea lapicida* (Ach.) Ach. and the most common photobiont was OTU *Tr*_S02. *Rhizocarpon geographicum* is also the only mycobiont species observed at all three sites as well as the photobiont OTU *Tr*_S02. Occurrences of mycobiont species and photobiont OTUs exclusively observed at one site are shown in Fig. 2a and Fig. 3b and c.

All three sampling sites along the altitudinal gradient did not differ significantly in their species/OTU composition of mycobionts, photobionts and bacteria. A permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity with 999 permutations showed that elevation accounted for approximately 32% of the variance in

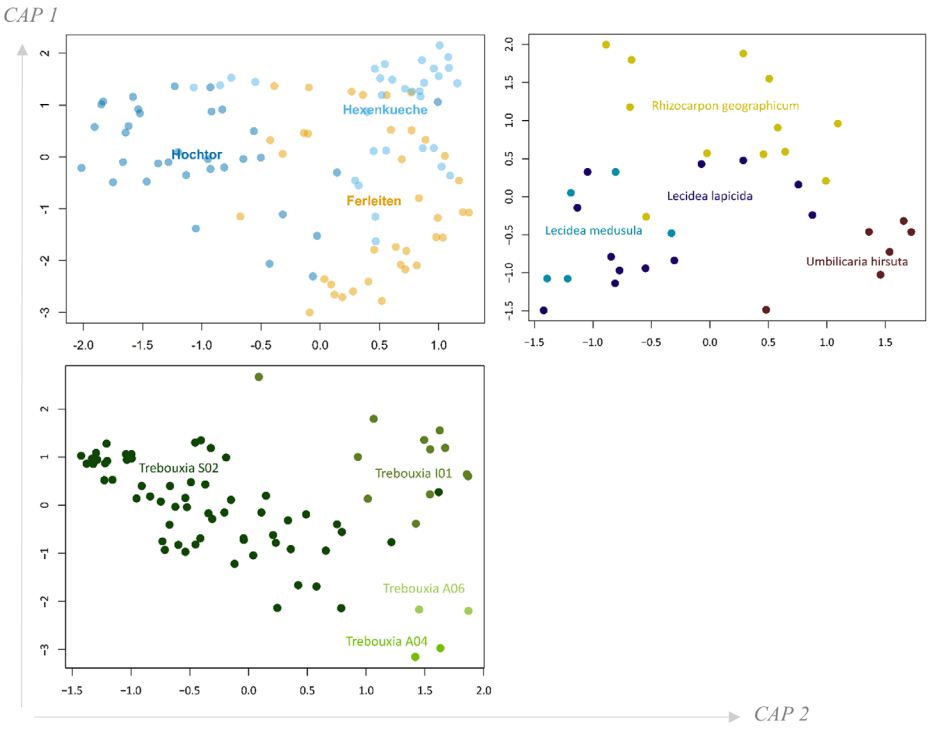


Fig. 4: CAP – Distance-based Redundancy Analysis. Permutation test (capscale under reduced model, anova: m1, by="term", permutations=1000) of the composition of the bacterial communities (OTUs on family level): **a)** along the elevational gradient, **b)** in relation to the dominant mycobiont species (n ≥ 5), **c)** the most common photobiont OTUs (n ≥ 2). | **Abb. 4:** CAP – Distanzbasierte Redundanzanalyse (db-RDA) zur Zusammensetzung des bakteriellen Mikrobioms (OTUs auf Familienebene), basierend auf einem Permutationstest (capscale, reduziertes Modell, anova: m1, by = „term", 1000 Permutationen): **a)** entlang des Höhengradienten, **b)** in Bezug auf die dominanten Mykobiontenarten (n ≥ 5), **c)** in Bezug auf die häufigsten Photobionten-OTUs (n ≥ 2).

overall community composition ($R^2 = 0.317$), an effect that was not statistically significant ($F_{2,6} = 1.392$, $p = 0.125$; see Tab. S4).

Because of the unconstrained ordination approach based on a dissimilarity matrix, several further calculations were performed using a distance-based redundancy analysis (db-RDA). The results show significant differences in bacterial microbiome composition between sampling sites ($F_{2,99} = 5.143$, $p < 0.001$; Fig. 4a). In addition, the composition of the bacterial microbiome was significantly influenced by the identity of the mycobiont species (Df = 3, F = 1.668, $p = 0.017$, n ≥ 5; see Fig. 4b) and the photobiont OTUs (Df = 3, F = 2.863, $p < 0.001$; see Fig. 4c; Tab. S5). The overall bacterial microbiome was dominated by *Acidobacteriaceae*, followed by *Acetobacteraceae* and *Solibacterales*. When testing individual rock replicates at each sampling site for differences in bacterial community composition, a significant effect of replicate identity was detected ($F_{8,93} = 3.657$, $p < 0.001$; Fig. 5, Tab. S6).

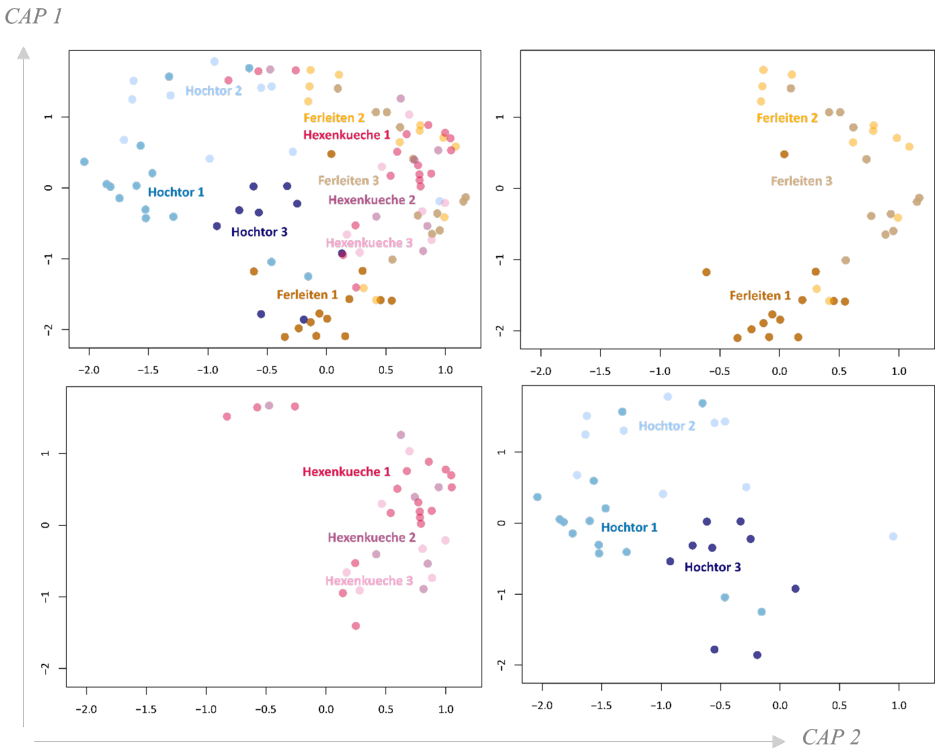


Fig. 5: CAP – Distance-based Redundancy Analysis for the single **replicates.** Permutation test (capscale under reduced model, anova: m1, by="term", permutations=1000) of the composition of the bacterial communities at **a)** all areas, **b)** Ferleiten, **c)** Hexenküche and **d)** Hochtor. | **Abb. 5:** CAP – Distanzbasierte Redundanzanalyse (db-RDA) zur Zusammensetzung des bakteriellen Mikrobioms auf Ebene einzelner Replikate, basierend auf einem Permutationstest (capscale, reduziertes Modell, anova: m1, by = „term", 1000 Permutationen): **a)** alle Untersuchungsgebiete kombiniert, **b)** Ferleiten, **c)** Hexenküche und **d)** Hochtor.

Diversity indices

Alpha diversity was highest at Ferleiten for both the photobiont and bacterial microbiome communities (photobiont: 3.555; bacterial microbiome: 7.127), based on Hill numbers calculated from OTU abundance data. For mycobionts, alpha diversity was comparable at Ferleiten (15.139) and Hochtor (15.603), with a marked decrease at the intermediate site Hexenküche (12.207). For the bacterial microbiome, alpha diversity decreased continuously with elevation (Ferleiten: 7.127; Hexenküche: 5.504; Hochtor: 4.576). The photobiont community at Hexenküche showed the lowest alpha diversity (1.000), consistent with only a single photobiont OTU recorded at this site (Fig. 2), with a slight increase at Hochtor (2.264). Beta diversity was higher for mycobionts (1.926) than for photobionts (1.252), indicating greater compositional turnover among mycobionts across sites, which partly reflects the substantially larger species pool of mycobionts compared to photobiont OTUs.

Tab. 1: Diversity indices – Alpha, Beta and Gamma Diversity – of the community composition of Lichen associated species, mycobiont, photobiont and bacterial microbiome along three elevational steps (Ferleiten, Hexenküche and Hochtor) at the Großglockner High Alpine Road, Austria. | **Tab. 1:** Diversitätsindizes – Alpha-, Beta- und Gamma-Diversität – der Artenzusammensetzung von Flechtenassoziierten Arten, Mykobionten, Photobionten und dem bakteriellen Mikrobiom entlang dreier Höhenstufen (Ferleiten, Hexenküche und Hochtor) entlang der Großglockner-Hochalpenstraße, Österreich.

	All	Mycobiont	Photobiont	Microbiome
Alpha Diversity (Hill numbers, q = 1)				
Ferleiten	7.132	15.139	3.555	7.127
Hexenküche	5.507	12.207	1.000	5.504
Hochtor	4.580	15.603	2.264	4.576
Beta Diversity	1.188	1.926	1.252	1.188
Gamma Diversity	6.709	21.176	2.393	6.704

Gamma diversity was similarly higher for mycobionts (21.176) than for photobionts (2.393), reflecting both a broader regional species pool and the higher total number of mycobiont species recorded (Tab. 1).

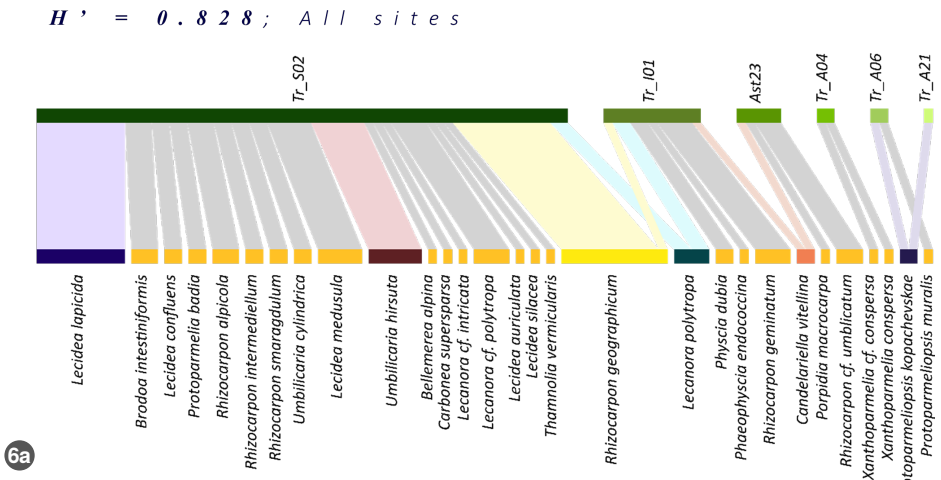
Network analysis

Interaction networks were analyzed for all symbiotic associations between mycobiont species (first level) and the corresponding photobiont OTUs (second level) and visualized with bipartite networks. The graphs represent all study sites and each site individually (Fig. 6 a-d). The overall degree of specialization of the mycobionts is comparatively high at the sites where it could be quantified. At Hochtor, an H2' value of 0.760 indicates a network with predominantly specialized interactions between symbiotic partners, while Ferleiten, as the lowest sampling site, has an H2' value of 0.828. Hexenküche contains only one photobiont, *Tr*_S02, across all replicates. While the majority of fungal symbionts interact exclusively with one photobiont, several exceptions were observed: *Protoparmeliopsis kopachevskae* associates with both *Tr*_A06 and *Tr*_A21, and *Candelariella vitellina* with both Ast23 and *Tr*_I01 (Fig. 6). Note that *Lecanora cf. polytropa* and *Lecanora polytropa* are treated as separate taxa here, although a conspecific relationship cannot be excluded based on morphological evidence alone. In contrast to the highly specialized mycobiont photobiont networks, the mycobiont microbiome networks showed consistently low specialization at all three sites (H2' = 0.188 / 0.135 /0.444).

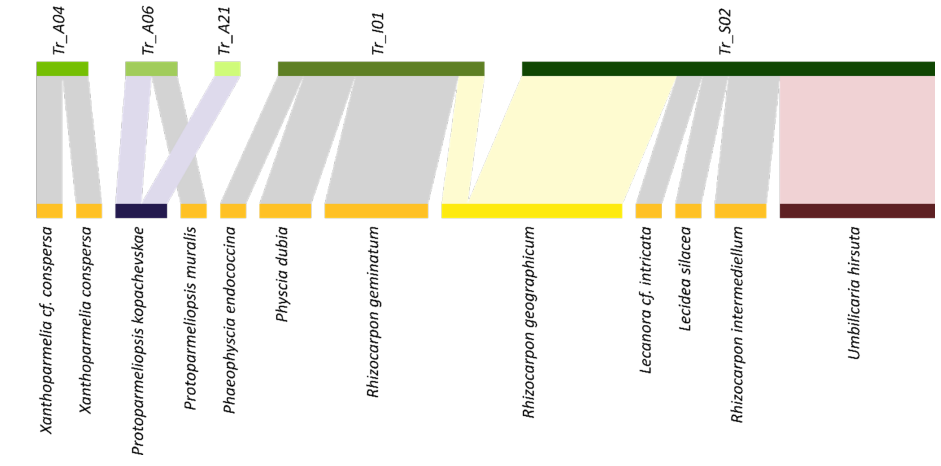
Lichen Guilds

To provide an overview of which lichens share the same photobionts, and which are associated with others, mycobiont species and photobiont OTUs were color-coded (see Fig. 7). Of all nine replicates, five contained only one photobiont, *Tr*_S02, which was shared by all the available mycobionts and was abundant on all three elevational steps.

This was the case for all replicates from Hexenküche and two replicates from Hochtor, containing 57 samples with 16 mycobiont species. The samples from Ferleiten comprise


H' = 0.828; All sites
Tr_S02
Tr_I01
Ast23
Tr_A04
Tr_A06
Tr_A21
Lecidea lapicida
Brodoa intestiniformis
Lecidea confluens
Protoparmelia badia
Rhizocarpon alpicola
Rhizocarpon intermediellum
Rhizocarpon smaragdulum
Umbilicaria cylindrica
Lecidea medusula
Umbilicaria hirsuta
Bellemerea alpina
Carbonea superspersa
Lecanora cf. intricata
Lecanora cf. polytropa
Lecidea auriculata
Lecidea silacea
Thamnolia vermicularis
Rhizocarpon geographicum
Lecanora polytropa
Physcia dubia
Phaeophyscia endococcina
Rhizocarpon geminatum
Candelariella vitellina
Porpidia macrocarpa
Rhizocarpon cf. umblicatum
Xanthoparmelia cf. conspersa
Xanthoparmelia conspersa
Protoparmeliopsis kopachevskae
Protoparmeliopsis muralis

6a


H' = 0.828; Ferleiten
Tr_A04
Tr_A06
Tr_A21
Tr_I01
Tr_S02
Xanthoparmelia cf. conspersa
Xanthoparmelia conspersa
Protoparmeliopsis kopachevskae
Protoparmeliopsis muralis
Phaeophyscia endococcina
Physcia dubia
Rhizocarpon geminatum
Rhizocarpon geographicum
Lecanora cf. intricata
Lecidea silacea
Rhizocarpon intermediellum
Umbilicaria hirsuta

6b

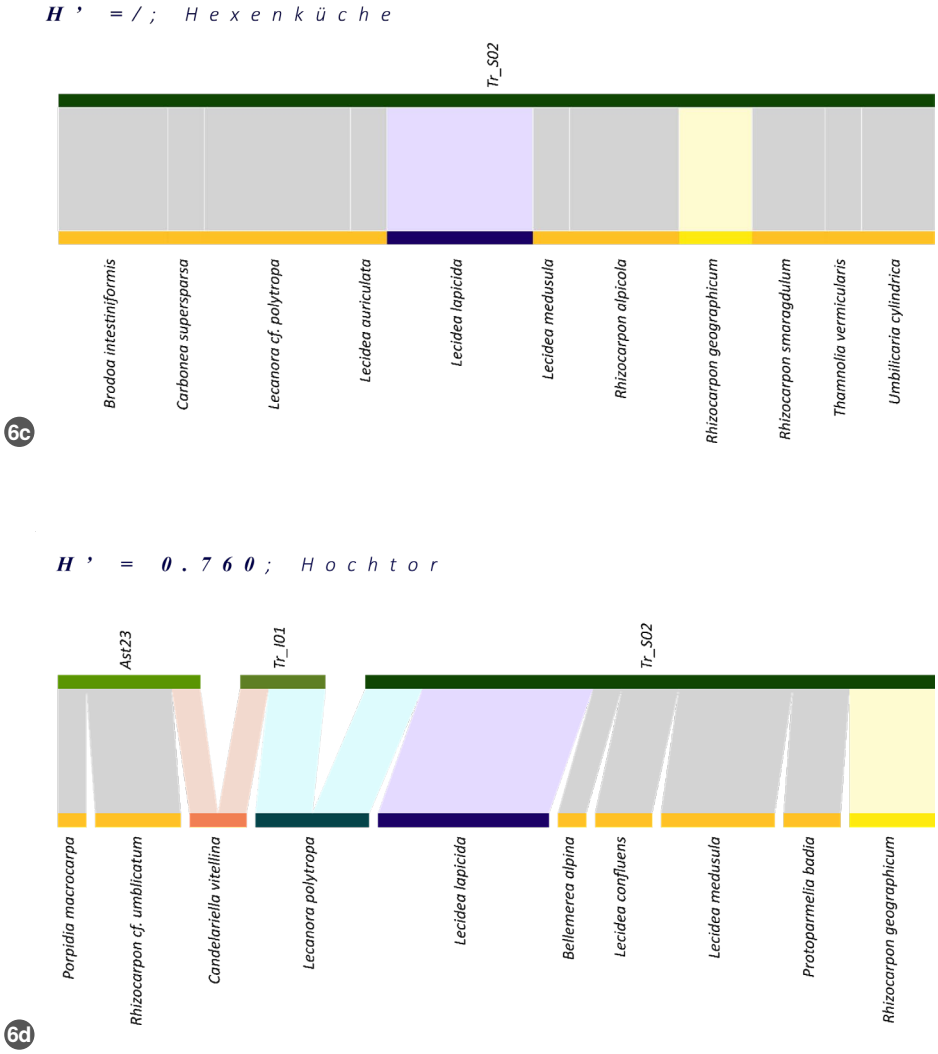
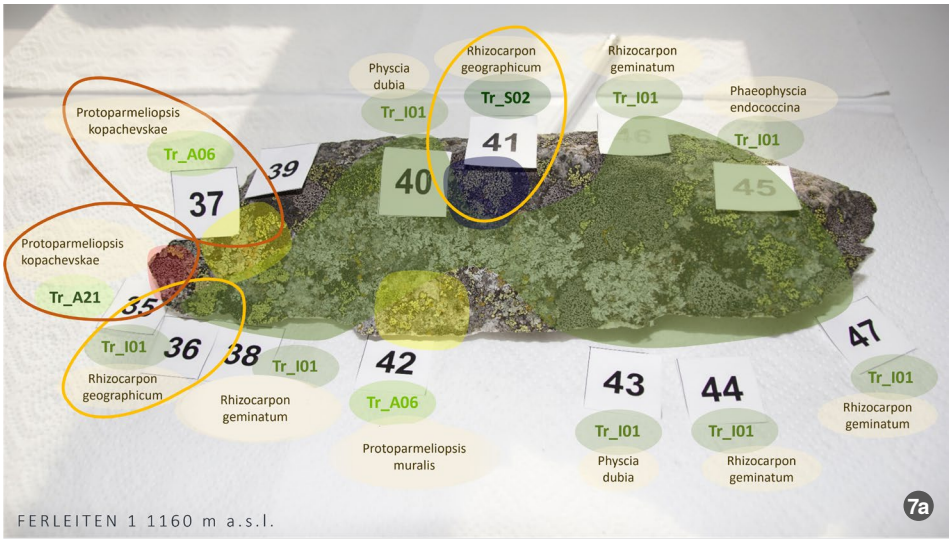
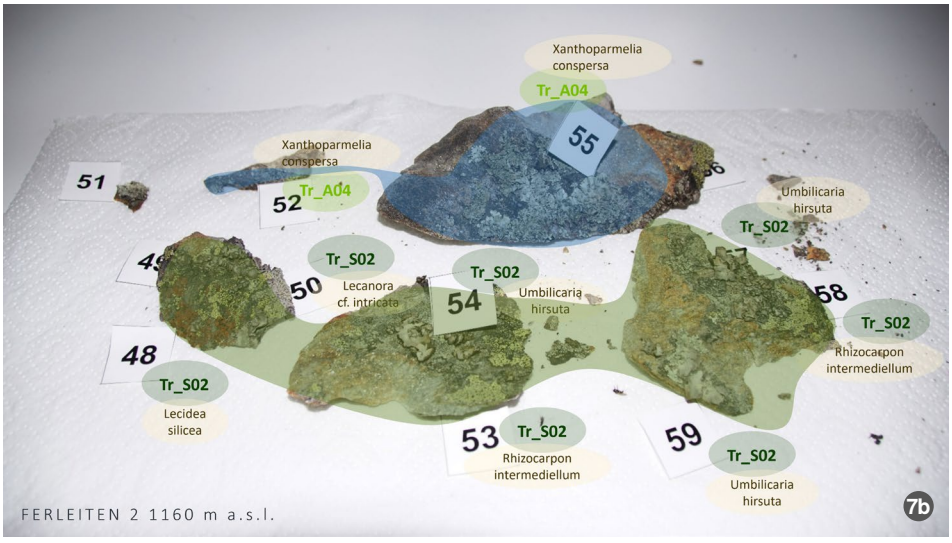
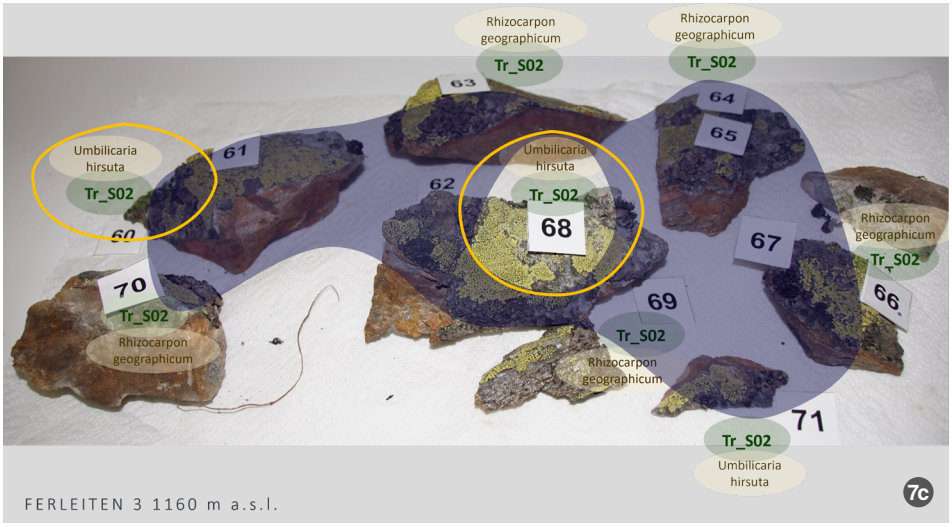


Fig. 6: Bipartite networks for the associated mycobiont species and photobiont OTUs at all three sampling sites. Samples were taken at the Großglockner High Alpine Road, Austria: **a)** all sites, **b)** Ferleiten, **c)** Hexenküche, **d)** Hochtor. For each network the H_2'-Value is calculated. Rectangle width is according to the frequencies of species and OTUs. Interactions are graphically displayed by lines connecting photobionts and mycobionts. The width of the lines is proportional to the number of accessions. | **Abb. 6:** Bipartite Netzwerke der Assoziationen zwischen Mykobionten-Arten und Photobionten-OTUs an den drei Untersuchungsstandorten entlang der Großglockner Hochalpenstraße, Österreich. **a)** Alle Standorte, **b)** Ferleiten, **c)** Hexenküche, **d)** Hochtor. Für jedes Netzwerk ist der H2'-Wert als Maß für die Netzwerkstruktur angegeben. Die Breite der Rechtecke entspricht der relativen Häufigkeit der jeweiligen Taxa/ OTUs; vertikale Linien zeigen Interaktionen, deren Dicke proportional zur Anzahl dokumentierter Zuordnungen ist.


Protoparmeliopsis kopachevskae
Tr_A06
37
39
Physcia dubia
Tr_I01
Rhizocarpon geographicum
Tr_S02
41
40
Rhizocarpon geminatum
Tr_I01
Phaeophyscia endococcina
Tr_I01
45
Protoparmeliopsis kopachevskae
Tr_A21
35
Tr_I01
36
Rhizocarpon geographicum
38
Tr_I01
Rhizocarpon geminatum
42
Tr_A06
Protoparmeliopsis muralis
43
Tr_I01
Physcia dubia
44
Tr_I01
Rhizocarpon geminatum
47
Tr_I01
Rhizocarpon geminatum
FERLEITEN 1 1160 m a.s.l.
7a


Xanthoparmelia conspersa
Tr_A04
55
Xanthoparmelia conspersa
Tr_A04
51
52
Umbilicaria hirsuta
Tr_S02
Tr_S02
Tr_S02
Lecanora cf. intricata
50
54
Umbilicaria hirsuta
58
Tr_S02
Rhizocarpon intermediellum
48
Tr_S02
Lecidea silicea
53
Tr_S02
Rhizocarpon intermediellum
59
Tr_S02
Umbilicaria hirsuta
FERLEITEN 2 1160 m a.s.l.
7b



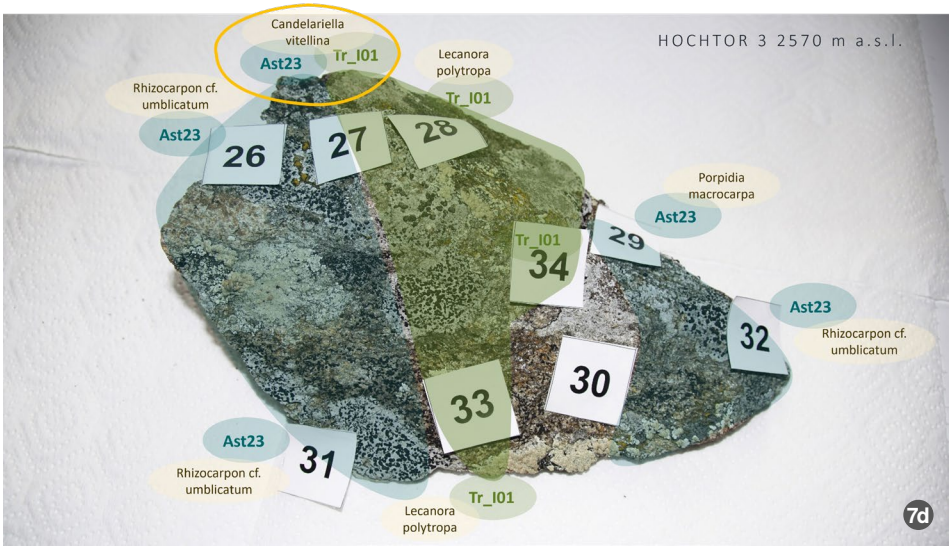


Fig. 7: Images and simplified representation of the replicates and the occurring mycobiont species and photobiont OTUs. Only four of nine replicates are shown, as only one common photobiont occurs on all other five replicates He01, 02, 03, H01, 02. The interacting species are listed side by side. The spatial distribution of the photobiont is represented by color-coded areas. Species that appear to be associated with more than one photobiont in a replicate are circled. Sampling area: Großglockner High Alpine Road, Austria. | **Abb. 7:** Darstellung ausgewählter Replikate mit visualisierten Symbionten Assoziationen sowie Auflistung der nachgewiesenen Mykobionten-Arten und Photobionten-OTUs. Gezeigt werden vier von insgesamt neun Replikaten; die übrigen fünf wurden aufgrund des Vorkommens nur eines gemeinsamen Photobionten nicht berücksichtigt. Interagierende Arten sind paarweise angeordnet, die räumliche Verteilung der Photobionten erfolgt mittels farblicher Codierung. Individuen mit multiplen Photobionten-Assoziationen sind durch eine Umrahmung hervorgehoben. Untersuchungsgebiet: Großglockner Hochalpenstraße, Österreich.

five different photobiont OTUs, all belonging to the genus *Trebouxia*, *Tr*_A06, *Tr*_A21, *Tr*_I01, *Tr*_A04, and *Tr*_S02. There are four different photobiont OTUs in F1 (Ferleiten replicate no. 1), two in F2, and one in F3, with *Tr*_S02 being the only OTU present across all three replicates. Hochtor (highest elevational step on the gradient) contains three different photobiont OTUs, of which one belongs to the genus *Asterochloris* (*Ast*23), alongside *Tr*_S02 and *Tr*_I01. *Tr*_I01 and *Tr*_S02 are the only photobionts found at both the lowest and highest sampling sites.

In general, it is notable that *Tr*_S02 exhibits the largest number of associations. Other OTUs, including *Tr*_A21, *Tr*_A06, and *Tr*_A04, occur less frequently and are exclusively found at Ferleiten. *Ast*23 is present only at Hochtor, while *Tr*_I01 and *Tr*_S02 are distributed across multiple sites. However, further sampling is needed to draw definitive conclusions about site-specific clustering of photobiont associations.

Discussion

Due to the dominance of lichens in extreme environments such as rock surfaces and high alpine areas in general as well as their variable symbiont associations along climatic (elevational) gradients (Rolshausen et al. 2023), they are very useful for the investigation of climate change scenarios (Sancho et al. 2019; Wagner et al. 2021).

Our findings indicate that the composition of mycobionts, photobionts, and bacterial microbiomes is shaped by factors associated with elevation, likely reflecting underlying environmental gradients and partner availability, although no direct climatic measurements were included.

Variation in Symbiotic Associations Along an Elevational Gradient

While mycobiont assemblages exhibited high site specificity with limited overlap between elevations, photobiont composition was dominated by a few widely distributed OTUs, notably *Tr*_S02, which occurred across all sites. This aligns with previous findings suggesting that fungal symbionts exhibit stronger habitat turnover, whereas photobionts often act as broadly distributed generalists (Dal Grande et al. 2018; Ruprecht et al. 2020; Wagner et al. 2020; Rolshausen et al. 2023). In contrast, the *Asterochloris* OTU *Ast*23 was restricted to high elevations, supporting the view that photobiont distributions can reflect niche specialization under specific environmental constraints (Peksa & Škaloud 2011; Wagner et al. 2020).

Moreover, many symbiotic pairings were unique to individual sites. This spatial partitioning of symbiotic associations supports the hypothesis of environmentally structured guilds (Peksa et al. 2022). Previous studies show that species within the genus *Trebouxia* exhibit varying degrees of altitudinal preference, with some lineages showing clear ecological specialization (Dal Grande et al. 2018), which aligns with our observation that certain mycobiont species associate with more than one photobiont OTU across sampling sites. Notably, mycobionts with lower photobiont specificity, as exemplified by the wide geographical and climatic range of *Cetraria aculeata* (Fernández-Mendoza et al. 2011), may be more resilient to climatic shifts due to broader ecological tolerance (Blaha et al. 2006; Ruprecht et al. 2012; Wagner et al. 2020).

Diversity Patterns and Community Composition

Alpha diversity was highest at the lowest elevation (1168 m a.s.l.) for two out of three symbiotic partners, photobionts and microbiome. The mycobiont showed a non-linear response, with diversity lowest at the mid-elevation site (Hexenküche, 2153 m a.s.l.) and recovering at the highest site (Hochtor, 2562 m a.s.l.), whereas bacterial microbiome diversity decreased continuously with elevation. Differences in bacterial microbiome alpha diversity between sites were statistically significant. Photobiont diversity was particularly low at Hexenküche, where only a single OTU (*Tr*_S02) was recorded across all replicates. Given this single observation, no elevational trend can be inferred for photobiont diversity at this site. These divergent responses of the symbiotic partners to the elevational gradient are partly consistent with findings from Antarctic and Himalayan lichen communities (Wagner et al. 2021; Nanda et al. 2021).

Beta and gamma diversity analyses further revealed greater taxonomic turnover among mycobionts than photobionts, suggesting asymmetric diversity dynamics. This may reflect the higher richness of fungal partners and the tendency of some photobionts to associate with multiple hosts (Ruprecht et al. 2020; Wagner et al. 2021). Similar declines in beta diversity with increasing elevation have been documented in broader alpine studies (Nanda et al. 2021), indicating a shift in modular community composition along gradients.

The bacterial communities were dominated by members of the family *Acidobacteraceae*, followed by *Acetobacteraceae* and representatives of the order *Solibacterales*. All these taxa are commonly associated with acidic, oligotrophic environments. Microbiome composition varied considerably among replicates within the same site, suggesting that a substantial proportion of the bacterial community consists of sporadically occurring, unspecific taxa. However, the significant structuring of bacterial communities by mycobiont identity and, even more strongly, by photobiont OTU indicates that at least a subset of the bacterial taxa shows consistent associations with the major lichen symbionts. This is consistent with recent findings that symbiont identity is a key driver of microbial community structure in lichens (Grube et al. 2009; Schneider et al. 2011).

CAP analyses confirmed significant effects of elevation, mycobiont species identity, and photobiont OTU on bacterial microbiome composition, while overall community composition across sites did not differ significantly when all symbiotic partners were considered together. As shown in the CAP ordination (Fig. 4), bacterial assemblages clustered not only along elevation, but also according to dominant mycobiont taxa and photobiont lineages. These results indicate that lichen-associated bacterial communities are shaped jointly by environmental filtering and host-specific symbiotic configuration, rather than forming a uniform community independent of the host, in line with previous studies (Hodkinson et al. 2012; Aschenbrenner et al. 2014; Grube et al. 2015).

Photobiont Sharing and Network Flexibility

The ability of certain mycobionts, such as *Candelariella vitellina*, to associate with more than one photobiont simultaneously within a single thallus, as observed in sample 27 at Hochtor (Fig. 7) and consistent with findings in *Ramalina farinacea* (Casano et al. 2011), suggests the coexistence of multiple photobiont lineages rather than sequential photobiont

switching. These findings support hypotheses on photobiont flexibility as an adaptive strategy in changing environments (Blaha et al. 2006; Leavitt et al. 2013; Singh et al. 2017).

Photobiont sharing among mycobionts was a notable pattern. *Tr*_S02, in particular, was associated with a wide range of fungal taxa across sites, reinforcing the concept of local photobiont pools from which multiple mycobiont species recruit their photosynthetic partners (De Carolis et al. 2022; Peksa et al. 2022). This shared usage potentially enhances ecological robustness by enabling alternative symbiotic combinations under environmental stress (Duran-Nebreda & Valverde 2023).

Despite spatial proximity, microbiomes were unexpectedly heterogeneous, pointing to fine-scale environmental filtering and modular structuring of lichen communities. As proposed by Duran-Nebreda & Valverde (2023), submodular network architecture may facilitate adaptive reconfiguration of symbiotic associations, promoting resilience in dynamic alpine environments.

Conclusion

This study reveals distinct patterns of symbiotic diversity and interaction along a steep elevational gradient in an alpine environment. The highest alpha diversity occurred at lower elevations, particularly for mycobionts and the bacterial microbiome. In contrast, the photobiont *Tr*_S02 was in most cases dominant in all habitats associated with almost all mycobionts which resulted in the lowest alpha diversity, suggesting that local symbiotic partner pools facilitate dynamic guild formation.

Microbiome composition was highly variable even at small spatial scales, reflecting the combined influence of symbiont identity, microhabitat conditions, and stochastic colonization processes. This highlights the complexity and modularity of lichen communities and their

potential for adaptive flexibility.

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Competing Interests

The Authors declare no competing interests.

Author Contributions

Funding acquisition: UR, RRJ; Conceptualization MM, UR, RR; Formal Analyses MM, UR; Investigation MM, UR; Methodology MM, RRJ, UR; Resources UR, RRJ; Visualisation MM; Original Draft Preparation MM, UR; Review & Editing MM, UR, RRJ.

Data availability

Sequences were uploaded on the NCBI database, BioProject accession number PRJNA1364001, the complete dataset can be found in the supplementary information.

Electronic Supplement

https://www.zobodat.at/pdf/supp/Mittag_et_al_Supplement.pdf

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