Fruticose Lichen Communities at the Edge: Distribution and Diversity in a Desert Sky Island on the Colorado Plateau

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Abstract: Subalpine habitats in sky islands in the Southwestern USA are currently facing large-scale transformations. Lichens have widely been used as bioindicators of environmental change. On the Colorado Plateau, fruticose lichens occur in patchy, disconnected populations, including unique lichen-draped conifer sites in subalpine forests in the La Sal Mountains in southeastern Utah. Here, we document the distribution and fungal diversity within these lichen communities. We find that lichen-draped conifer sites in the La Sal Mountains are restricted to only three known, small areas in *Picea englemannii* forests above 3000 m above sea level, two of which have recently been impacted by wildfire. We document 30 different species of lichen-forming fungi in these communities, several which represent the first reports from the Colorado Plateau. We also characterize mycobiont haplotype diversity for the fruticose lichens *Evernia divaricata*, *Ramalina sinensis*, and multiple *Usnea* species. We also report a range of diverse fungi associated with these lichens, including genetic clusters representing 22 orders spanning seven classes of Ascomycetes and fewer clusters representing Basidiomycetes. Our results provide a baseline for ongoing monitoring and help to raise awareness of unique lichen communities and other biodiversity in the region.

Keywords: amplicon sequencing; biodiversity; biomonitoring; ecological sampling; epiphyte; Illumina; fungi; internal transcribed spacer region (ITS); inventory; ITS2; subalpine; semi-arid

1. Introduction

Some habitats in temperate forests are currently being driven toward large-scale transformations due to interactions among climate change, habitat alteration, severe wildfires, insects, pathogens, and other disturbances [1]. The nature of these ecological disturbances has serious ramifications for regional biodiversity and ecosystem health, as these and other factors directly and abruptly affect forest vegetation [2]. Subalpine habitats in sky islands in the Southwestern USA are particularly vulnerable to these contemporary disturbances, with biological communities likely becoming more isolated and potentially negatively impacting regional biodiversity [2,3]. With increasing temperatures, aridification, and changes in precipitation patterns in the Southwestern USA, subalpine habitats in sky islands are experiencing increases in drought, fire frequency, and fire severity [4,5]. In North American subalpine forests, fire return intervals are becoming shorter, negatively influencing forests' ability to recover after fires [6]. Extensive disturbances in these forest may lead to new vegetations states with novel responses to climate [2].

Species adapted to high altitude/latitude habitats may be particularly vulnerable to extirpation if changing habitat conditions outpace the rate of dispersal [7]. Peripheral and isolated populations of subalpine species rank as potential indicators for monitoring change in the Southwestern USA, as they may be affected before other more common, connected communities [8]. For many vulnerable species/organismal groups, limited understanding

of the spatial and temporal components of a species' life history characteristics may stymie researchers' ability to utilize these in monitoring and conservation research or may lead to possible misinterpretation of processes that the indicator species aims to unravel [9,10].

Lichens have long been utilized to monitor ecological disturbances [11–16] and more recently to inform conservation decisions [17,18]. Lichens that have patchy distributions may be more vulnerable to extinction/extirpations, particularly epiphytes of mountain forests [19,20]. Connected, large populations/communities facilitate recolonization when one patch is at risk [21], and disconnected and smaller populations/communities may be particularly vulnerable to disturbances [22]. Even where species have broadly distributed and well-connected populations, peripheral or patchy populations can be more vulnerable than those at the center of a larger metapopulation [23]. Peripheral populations may promote range expansion, but when suitable habitat occurs in isolated patches, such as sky islands, expansion into new suitable habitat may not be possible. Patchy distributions coupled with new vegetations states because of contemporary disturbances may fundamentally alter the occurrence of epiphytic lichens [2,24].

While fruticose lichens are found in temperate forests worldwide, boreal and temperate elements of western North America are genetic "hot spots" for some epiphytic lichens [25,26]. However, fruticose lichens are present in patchy, disconnected populations across the Colorado Plateau in the Southwestern USA (Figure 1). This patchiness is likely driven by regional climatic variation and complex topographic gradients, including the impact of monsoon precipitation (summer precipitation as rain) [27]. The dynamic interactions of climate and topographic variation on the Colorado Plateau, coupled with potentially limited gene flow among spatially and ecologically isolated fruticose lichen populations, make these particularly vulnerable communities and merit careful monitoring.
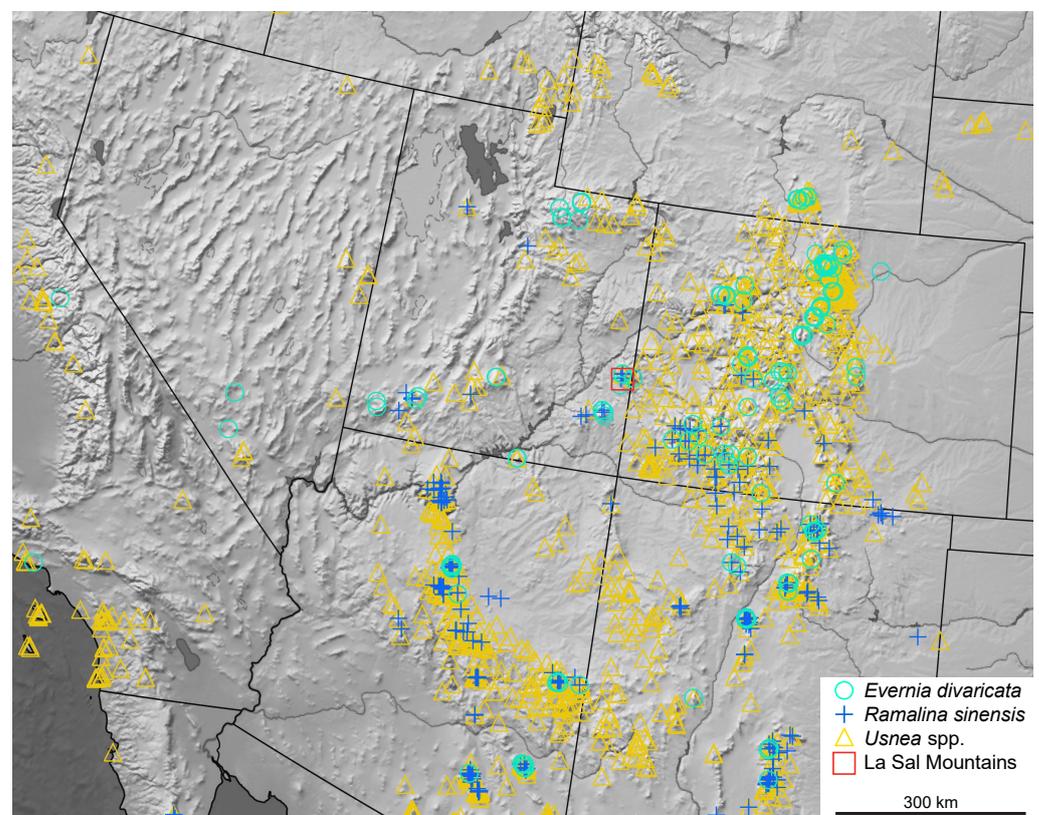
Figure 1. Distribution of fruticose lichens *Evernia divaricata*, *Ramalina sinensis*, and *Usnea* species across the Intermountain West region of the USA. The La Sal Mountains are located in southeastern Utah near the state border with Colorado—indicated by red box. Species distribution records were

obtained from the Consortium of North American Lichen Herbaria (https://lichenportal.org/cnalh/; accessed 21 September 2022), and the map was generated using Simplemapper (https://www.simplemappr.net; accessed on 21 September 2022).

Most fruticose lichen populations are quite small and spatially restricted on the Colorado Plateau. However, during excursions in the La Sal Mountain Range ("La Sals"), a sky island on the Colorado Plateau, in southeastern Utah, USA, we observed spatially restricted, robust lichen communities draping conifers in subalpine forests with unusually high population density and biomass (Figure 2). The lichen-draped conifer sites are dominated by the lichens *Evernia divaricata* (L.) Ach., *Ramalina sinensis* Jatta, and various *Usnea* species. Presently, little is known about these sites, including the extent of lichen-draped conifers sites, the amount of genetic variation, the range of associated lichens, and factors influencing their origin and persistence. Given the apparent limited, restricted distributions, these communities merit careful conservation consideration.

Figure 2. Lichen-draped conifer sites in the La Sal Mountains. The top row includes photos from site 'Geyser Pass 1', 'Geyser Pass 2', and 'Medicine Lake' (**left** to **right**). The bottom panel depicts a typical lichen-covered conifer branch sampled for DNA metabarcoding. Fruticose lichens included *Evernia divaricata*, *Ramalina sinensis*, and various *Usnea* species. Inconspicuous crustose lichens co-occurred tightly appressed to bark.

The purpose of this study is to (1) characterize for the first time the extent of lichen-draped conifer sites in subalpine forest in an isolated sky island on the Colorado Plateau—

the La Sals, (2) inventory the range of lichen diversity in these communities, (3) assess genetic diversity within the fruticose lichen populations of *Evernia divaricata*, *Ramalina sinensis*, and *Usnea* species, and (4) characterize the lichen-associated fungal community. This information will be important for establishing a biomonitoring baseline and assessing conservation needs in unique subalpine communities in a region that is vulnerable to large-scale ecological transformations.

2. Materials and Methods

2.1. Site Selection, Field Methods, and Bulk Sampling

For this study, subalpine forests in the La Sal Mountains were opportunistically surveyed between July 2018–July 2022 to identify the number and extent of spatially restricted, robust lichen communities draping conifers (Figure 2). Broad, general surveys were conducted throughout the La Sal Mountains, targeting suitable habitat for fruticose lichens, e.g., mixed montane forests. Additional survey locations were identified, in part, by using satellite images from Google Earth Pro to identify suitable habitat that may not have been readily apparent from trails, roads, and other typical access points. Specifically, we identified dense *Picea engelmanii* stands, considering proximity to water sources, e.g., streams or other wetland sites, for more directed surveys for fruticose lichens. Here, "lichen-draped conifer sites" were qualitatively characterized by the presence of at least two of the three common fruticose lichens—*Evernia divaricata*, *Ramalina sinensis*, and *Usnea* spp.—and a density similar to those shown in Figure 2, e.g., sporadic occurrences of limited numbers of fruticose lichen thalli on a limb/bole were not considered to represent lichen-draped conifer sites. The extent of lichen-draped conifer sites was based on field GPS data collected using a handheld Garmin GPS 60CSx (datum WGS84).

We also attempted to characterize the full range of lichen-forming fungi at one of the most superficially diverse lichen-draped conifer sites in the La Sal Mountains. Within the "Geyser Pass 2" site—see Table 1, lichen samples were collected in July 2021, employing an "intuitive meander" method. The overarching aim was to collect representative bulk samples to comprehensively represent the epiphytic lichen diversity in these locally unique communities. Fifteen lichen-covered branches, ca. 0.5 m long (Figure 2), were collected from 15 trees separated by at least three meters. Lichens occurring on rock and soil were not collected. Furthermore, we did not target lichens occurring on conifers beyond the dense, fruticose-dominated lichen communities, e.g., lichens occurring near the base of the tree or those on branches with limited or no fruticose lichens.

Table 1. The three lichen-draped conifer sites identified in the La Sal Mountains, a sky island on the Colorado Plateau.

Name	Estimated Area	Coordinates	Altitude (m a.s.l.)
Geyser Pass 1	10,000 m^2	38.4864, −109.2491	3050
Geyser Pass 2	19,000 m^2	38.4821, −109.2368	3200
Medicine Lake	65,000 m^2	38.4118, −109.2458	3040

In addition to the main lichen-forming fungi, i.e., the lichen mycobionts, lichens also harbor complex fungal communities—the 'mycobiome' [28–30], and these may play important roles in facilitating the development of fruticose lichen thalli [31–33]. Therefore, we attempted to characterize the mycobiome and other associated fungi from the same samples. Fungal diversity was inferred using a DNA metabarcoding approach [29]—see below.

Samples were collected, dried in the field, returned to the herbarium, and stored at −80 °C until the bulk sampling and DNA extraction steps. In the herbarium, fruticose lichens representing *Evernia divaricata*, *Ramalina sinensis*, and *Usnea* species were carefully removed from the 15 sampled branches and roughly sorted by morphology. Using sterilized tweezers, small portions of thallus material from the fruticose lichens were carefully

removed and placed directly into a sterile Nasco Whirl-Pak 18 oz. collecting bag (Nasco, Fort Atkinson, WI, USA). We attempted to sample similarly sized pieces of the apical part of thallus material from each fruticose lichen collected for the fruticose bulk sample. We also prepared a separate bulk sample representing smaller crustose and foliose lichens occurring on the bark of the sampled branches. Using a 10× hand lens, small, similarly sized portions of lichen thalli were sampled from all potentially different lichens using sterilized tweezers to pick or scrape material for bulk, metagenomic analyses of crustose and foliose lichens occurring with the fruticose lichens.

Twenty *Usnea* thalli representing the range of observed morphological diversity (Figure 3) were selected to investigate the secondary metabolite variation using thin layer chromatography (TLC), following standard methods with solvent system 'G' [34,35], and these were identified using traditional phenotype-based approaches.

Figure 3. Photos of representative *Usnea* specimens collected at "Geyser Pass 1" site. *Usnea cavernosa* (panel 'A'), was the most common *Usnea* species sampled in the study. Scale bar = 3 cm (photo credit: S. Leavitt).

2.2. Molecular Laboratory Methods

To help ensure that metagenomic samples representing small crustose and foliose lichens were not overwhelmed by DNA from the larger fruticose lichens, DNA was extracted from the fruticose and crustose/foliose lichen community samples separately. From the two bulk samples collected at the "Geyser Pass 2" site, ca. 3 g of bulk lichen material from fruticose lichens and ca. 2 g of bulk lichen material from crustose and foliose lichens on bark was used for DNA extraction. Community samples were homogenized using sterilized mortar and pestles; and DNA was extracted from homogenized material from each sample using the PowerMax Soil DNA Isolation Kit (Qiagen). To characterize the range of fungal diversity in the bulk samples, we amplified a portion of the internal transcribed spacer region—the standard barcoding region for fungi [3]—from each meta-community DNA extraction. Specifically, the hypervariable ITS2 region was amplified at RTL Genomics (Lubbock, TX, USA) using polymerase chain reaction (PCR) with the primer pair ITS3F (GCATCGATGAAGAACGCAGC) and ITS4R (TCCTCCGCTTATTGATATGC) [36].

PCR reactions, purification, and pooling followed the RTL Genomics standard protocols (www.rtlgenomics.com; accessed on 1 September 2022). Pooled PCR products were also sequenced at RTL Genomics using the Illumina MiSeq 2×300 paired-end MiSeq platform. The complete RTL Genomics amplification and sequencing protocol is described in [37].

2.3. Short-Read Data Analyses

FROGS v3.2 (Find, Rapidly OTUs with Galaxy Solution) was used to analyze ITS2 amplicon metabarcoding data [38,39]. FROGS v3.2 is a standardized pipeline containing a set of tools used to process amplicon reads produced from Illumina sequencing. We followed the protocol outlined in [39]. In short, paired-end reads for each sequence in the data were merged, primers were trimmed, and unmatched sequences were discarded in the FROGS v3.2 preprocessing step. Merged reads were then filtered using the FROGS v3.2 swarm clustering tool; and the clusters were formed using the aggregation distance clustering set to 1, as per the guidelines for v3.2. Chimeric sequences were then removed using the FROGS v3.2 chimera removal tool and implementing default parameters. The FROGS v3.2 filtering tool was then used to remove low abundance clusters by setting the minimum proportion of sequences to keep OTUs to 0.000005. All remaining clusters were filtered using the ITSx tool to ensure that clusters met requirements for the ITS2 region in preparation for the taxonomic affiliation step. Initial taxonomic assignment of the clusters was completed by comparing the clusters passing filters to the UNITE 8.3 database using the RDP probabilistic classifier [40] and BLAST comparisons [41]. All analyses were performed on the Migale Galaxy Server. Taxonomic assignments of lichen-forming fungi inferred from the FROGS pipeline, were refined using sequence comparisons from the BOLD Project LIMWSL—"Lichens of the Intermountain West". Non-lichen-forming fungi were considered at the taxonomic levels of class and order. In cases were the RDP and BLAST-based taxonomic assignments differed at the class and order levels, the taxonomic assignment was considered "unknown". We note that using sequence similarity to infer taxonomic identity and other issues with publicly available sequences come with significant caveats [41,42].

We also attempted to characterize haplotype diversity in fruticose lichens, e.g., *Evernia divaricata*, *Ramalina sinensis*, and *Usnea* species, at the "Geyser Pass 2" site. To find unique haplotypes within each of the three fruticose lichens, short reads were mapped to the clusters representing (1) *E. divaricata*, (2) *R. sinensis*, and (3) *Usnea* spp., separately using Geneious Prime 2022.1.1, implementing the Geneious Prime 'Map to Reference' option set to "Low Sensitivity/Fastest", iterated two times and saving the mapped reads ("used reads"). The used reads were then clustered using the CD-HIT web server [43] and clustering reads at 100% similarity. Only clusters represented by ten or more identical reads were considered. For both *E. divaricata* and *R. sinensis*, all ITS sequences presently available on GenBank were downloaded and combined with the taxon-specific haplotypes. The *Usnea* haplotypes were combined with ITS sequences compiled in [44]. Multiple sequence alignments were made using the program MAFFT v7 [45,46], implementing the G-INS-i alignment algorithm and '1PAM / K = 2' scoring matrix, with an offset value of 0.1, the 'unalignlevel' = 0.2, and the remaining parameters were set to default values. A maximum-likelihood (ML) tree was inferred from each ITS alignment using IQ-TREE [47] to characterize the range of haplotype diversity within each taxon.

3. Results

Our surveys of forests in the La Sal Mountains on the Colorado Plateau revealed the widespread occurrence the fruticose lichens *Ramalina sinensis* and *Usnea* species, with *Evernia divaricata* restricted to more limited habitat in subalpine forests (also rarely occurring on alpine turf). Despite the widespread occurrence of fruticose lichens in the La Sals, lichen-draped conifers were found at only three sites, two at headwater drainages of Mill Creek, near Geyser Pass, and one near Medicine Lakes (Table 1). The complete area for each identified site with extensive lichen-draped conifers is provided as Supplementary File S1

(polygon area). Habitat surrounding the two lichen-draped conifer sites near Geyser Pass was nearly completely burned in the "Pack Creek Fire" in 2021 (https://utahfireinfo.gov/2021/06/26/pack-creek-fire-june-26-update/; accessed on 1 September 2022), although the lichen-draped conifers sites remained largely intact. Pre-fire surveys did not reveal extensive lichen-draped conifer communities in the Geyser Pass/Mill Creek headwaters area before the Pack Creek Fire.

Illumina ITS2 amplicon sequencing resulted in 197,080 and 214,206 reads in the crustose/foliose and fruticose samples, respectively. Short reads are available in NCBI's Short Read Archive under PRJNA875162. Reports of the FROGS pipeline, e.g., preprocessing, chimera removal, OTU filter, and ITSx, are available in Supplementary Files S2–S6. In summary, 2.6% of sequences, representing 27.7% of clusters, were excluded as chimeric sequences (Supplementary File S3). Of the remaining clusters, 82.1% (1371) were excluded, not meeting the minimum proportion threshold, e.g., low abundance clusters; the remaining 299 clusters comprised 97.8% of the sequences passing the chimera filter (Supplementary File S4). From these, 33 additional clusters were excluded, not passing the ITSx filter, resulting in a total of 266 clusters retained for taxonomic assignment (Supplementary File S5).

Across all samples, the 266 clusters were assigned to 20 classes of Fungi, 43 orders, 78 families, 111 genera, and 125 species using the FROGS affiliation pipeline based on the UNITE 8.3 fungal database (Supplementary Files S6 and S7). Relatively high levels of non-lichen-forming Ascomycota clusters were inferred here—156 clusters, with more modest numbers of clusters representing Basidiomycota—44 clusters and a single cluster representing Chytridiomycota (Figure 4; Supplementary Files S7). Only 17.3% of the 266 clusters were inferred to be derived from lichen-forming fungi, although most short reads were derived from lichen-forming fungi (Figure 4). The 46 clusters inferred to represent lichen-forming fungi in the FROGS affiliation step represented 30 species/candidate species in eight families (Table 2).

Table 2. List of lichen-forming fungi occurring at a lichen-draped conifer site—"Geyser Pass 2" in the La Sal Mountains, UT, USA. Genetic clusters were inferred from DNA metabarcoding of the ITS2 marker using the FROGS pipeline [39]. Fruticose lichens are shown in bold text; the number of haplotypes, rather than genetic clusters, is reported for *Usnea* species.

Taxon	#Genetic Clusters	Notes
Amandinea aff. *punctata* (Hoffm.) Coppins & Scheid.	2	cosmopolitan lichen likely comprising multiple, distinct species-level mycobiont lineages [48]; common on Colorado Plateau
Bibbya vermifera (Nyl.) Kistenich et al.	1	uncommon in North America, and this is likely the first report from the Colorado Plateau
Caloplaca sp.	1	ITS2 sequence from La Sals was 99.4% similar to unidentified *Caloplaca* from central Europe
Micarea sp.	1	ITS2 sequence from La Sals was 91.4% similar to *Micarea* sequences on GenBank
Evernia divaricata (L.) Ach.	2	occurs worldwide on conifers in montane to subalpine forests; red-listed in all European countries where it occurs [25]
Lecidella euphorea (Flörke) Hertel	3	occurs worldwide and likely comprises multiple, distinct species-level mycobiont lineages [49]; common on Colorado Plateau
Lecidella sp.	1	ITS2 sequence from the La Sals was recovered in the "*Lecidella elaeochroma* clade" [49]
Melanohalea exasperatula (De Not.) O. Blanco et al.	2	widespread across Europe and western North America [50]; common on Colorado Plateau
Melanohalea subolivacea (Nyl.) O. Blanco et al.	2	widespread across western North America [50]; common on Colorado Plateau

Table 2. *Cont.*

Taxon	#Genetic Clusters	Notes
Myriolecis juniperina (Śliwa) Śliwa, Zhao Xin & Lumbsch	1	occurs on the Colorado Plateau at mid elevations—this is the first known report from subalpine forests
Myriolecis sp.	1	ITS2 sequence from the La Sals was recovered with a provisionally named species *M. "altaterrae"* nom. prov.
Myriolecis wetmorei (Śliwa) Śliwa, Zhao Xin & Lumbsch	1	occurs at higher elevations throughout western North America (and Armenia); common on Colorado Plateau
Parvoplaca sp.	1	ITS2 sequence was 97.4% similar to *P. nigroblastidiata* from Turkey
Phaeophyscia sp.	1	*Phaeophyscia* species commonly occur in montane habitats throughout western North America
Phylliscum aff. *demangeonii* (Moug. & Mont.) Nyl.	4	ID uncertain: ITS2 sequence was 97.8% similar to ITS sequence from type (NR_120130); however, BLAST searches also shown high similarity to uncultured *Rhinocladiella* (Eurotiomycetes)
Physcia adscendens (Fr.) H. Olivier	1	widely distributed in temperate and boreal areas in all continents; common on Colorado Plateau
Polycauliona sp.	1	NA. *P. candelaria* occurs scattered throughout the Intermountain West, but the ITS2 sequence from the La Sals was highly dissimilar to *P. candelaria* sequences on GenBank (ca. 92% similarity)
***Ramalina sinensis* Jatta**	3	cosmopolitan in temperate regions, and common in montane habitats on the Colorado Plateau
Rinodina sp. 1	1	NA—voucher specimen required for identification
Rinodina sp. 2	2	NA—voucher specimen required for identification
Rinodina sp. 3	1	NA–voucher specimen required for identification
Schizoxylon albescens Gilenstam, Döring & Wedin	1	occurs both as lichen and as saprobe [51]; not previously reported in North America
Stictidaceae sp.	2	ITS2 sequence from La Sals was 93.6% similar to *Stictis brunnescens*. If these clusters truly represent a species in *Stictis*, they are one of the first members of the genus reported for western North America
Tetramelas pulverulentus (Anzi) A. Nordin & Tibell	1	endoparasite within members of the Physciaceae; likely first report from the Colorado Plateau
***Usnea* aff. *barbata* (L.) F.H. Wigg.**	2 (haplotypes)	widespread across western North America; rarely collected on Colorado Plateau.
***Usnea cavernosa* Tuck.**	98 (haplotypes)	Eurasian and North American distribution; occurring sporadically on the Colorado Plateau
Usnea lapponica	1 (haplotypes)	likely cosmopolitan, occurring throughout the Intermountain West
***Usnea perplexans* Stirt.**	2 (haplotypes)	likely cosmopolitan, occurring throughout the Intermountain West
***Usnea* sp.**	1 (haplotypes)	NA
Xanthomendoza montana (L. Lindblom) Søchting et al.	6	widespread across western North America [52]; common on Colorado Plateau

From the short read data, we inferred eight haplotypes in *Evernia divaricata*, 13 in *Ramalina sinensis*, and 104 representing *Usnea* species, the vast majority represented *U. cavernosa* (Table 3; Supplementary File S8).

Of the 20 *Usnea* sampled for TLC, 17 produced usnic and salazinic acids, with three specimens producing usnic acid alone. The most abundant *Usnea* species in the lichen-draped conifer sites was *U. cavernosa*, accompanied by *U. barbata*, *U. lapponica*, *U. perplexens*, and an unidentified *Usnea* species (Figure 3).
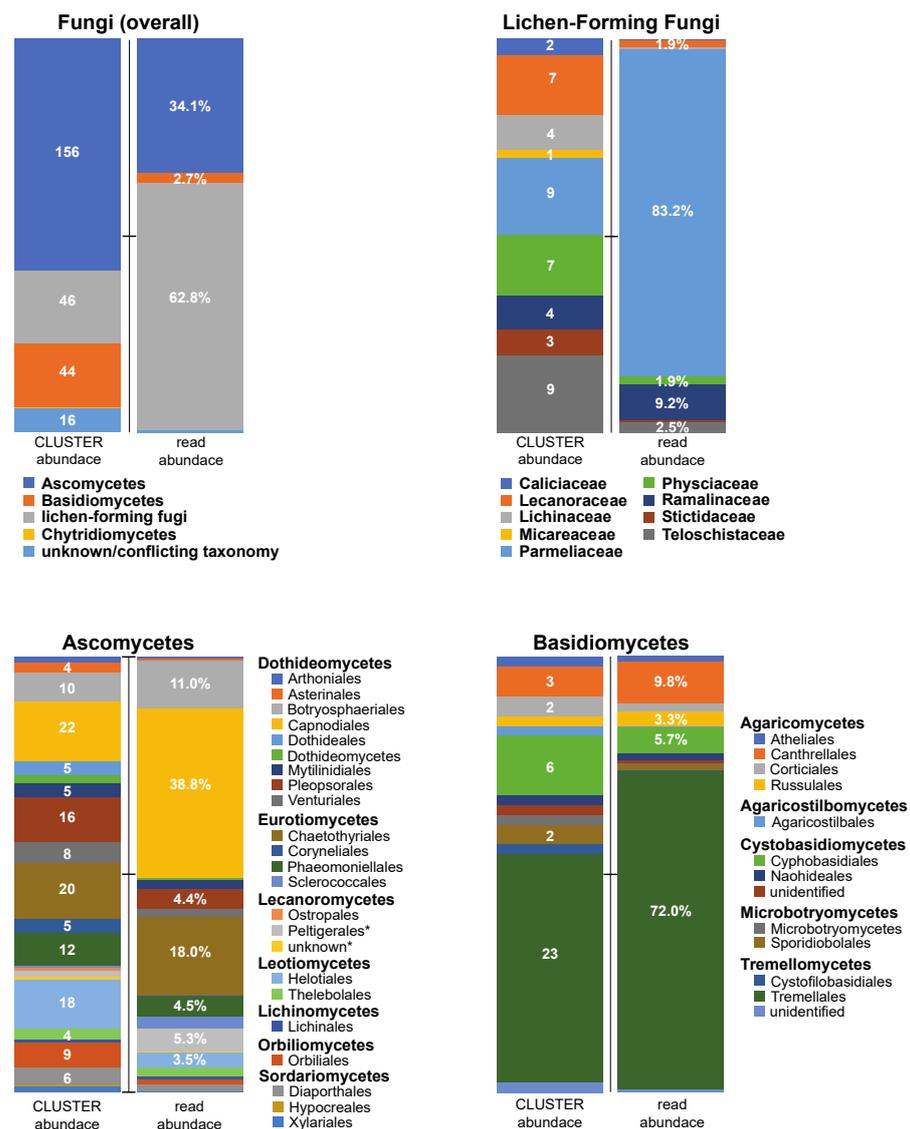
Figure 4. Fungal diversity inferred from DNA metabarcoding at "Geyser Pass 2" sites using the ITS2 barcoding marker. Panels compare taxonomic identity of genetic clusters (**left**) and proportion of short read data assigned to each taxonomic group (**right**). Top left panel represents an overview of taxonomic assignments from short read data; top right panel represents lichen-forming fungi; bottom panels represent other lichen-associated ascomycetes (**bottom left**) and basidiomycetes (**bottom right**) represent in bulk samples.

Non-lichen-forming Ascomycetes comprised 22 orders spanning seven classes (Figure 4). Dothideomycetes were the most diverse class, with Capnodiales, Pleosporales, and Botryosphaeriales representing the highest number of genetic clusters within this class. Euriotiomycetes were also well represented in the short read data, with Chaetothyriales and Phaeomonielleles the most diverse orders in the class. Over two thirds of the reads inferred to originate from ascomycetes represented only three orders—Capnodiales, Chaetothyriales, and Botryosphaeriales (Figure 4).

Basidiomycete lineages were represented in 2.7% of all reads, with the vast majority inferred to originate from Tremellales (Tremellomycetes), representing 23 clusters (Figure 4). Agaricomycetes were also well represented in the short read data, with the order Cantharellales comprising nearly 10% of all basidiomycete reads. Six clusters were inferred to belong to Cyphobasidiales (Cystobasidiomycetes) (Figure 4), four of which have previously been shown to have close associations with lichens.

Table 3. Genetic variation in fruticose lichens occurring at a lichen-draped conifer site—"Geyser Pass 2" in the La Sal Mountains, UT, USA. The number of *Usnea* haplotypes of each taxon are given parenthetically.

Taxon	# of Hapoltypes	Notes
Evernia divaricata	8	Haplotypes from the La Sals were distributed across multiple, weakly supported clades in the ITS topology.
Ramalina sinensis	13	Haplotypes from the La Sals were recovered within a single clade comprised exclusively of closely related haplotypes from the La Sals and sister to a clade comprised of two specimens from Arizona and New Mexico.
Usnea spp.	104 total *U.* aff. *barbata* (2) *U. cavernosa* (98) *U. lapponica* (1) *U. perplexans* (2) *U.* sp. (1)	The *U. cavernosa* haplotypes were recovered within a clade comprised of closely related sequences from Idaho (USA) and Switzerland. The phylogenetic position of specimens identified as *U. barbata*, *U. perplexans*, and *U.* sp. were unresolved; the *U. lapponica* haplotype was recovered within a clade of other *U. lapponica* sequences from Austria, Estonia, Canada, India, Spain, Switzerland, and the USA.

4. Discussion

Here, we document for the first time unique and vulnerable fruticose lichen communities occurring in subalpine forests in a desert sky island on the Colorado Plateau (Figure 2) [25]. Lichen-draped conifer sites were found at only three locations in subalpine *Picea engelmanii* forests in the La Sal Mountains in southeastern Utah (Table 1). The fruticose lichens occurring in the La Sals represent isolated populations of lichens that are found in montane forest around the world, particularly in the Northern Hemisphere. Fruticose lichen communities in the La Sals occur at the western edge of their distribution moving into the arid canyonlands region of Utah (Figure 1); and documenting the diversity, distribution and extent of these lichen-draped conifer sites provides the first step in conserving and monitoring these unique communities. We inventoried the range of lichen-forming fungi co-occurring with these fruticose lichens within one lichen-draped conifer site in the La Sals, highlighting several unexpected lichens. Furthermore, by also characterizing the range of lichen-associated fungi (non-lichen-formers), we hope to provide a broader understanding of the range of biodiversity associated with these sites. Below we discuss the implications of our findings.

The impact of climate change, land use, and frequency of wildfires on the Colorado Plateau will continue to have major impacts on biological communities [4,53,54], including lichens [27]. The lichen-draped conifer sites in the La Sal Mountains are unique, and similar communities of locally abundant fruticose lichens occurring in similar densities have not been observed in the Colorado Plateau or Great Basin in western North America (S. Leavitt, *personal observation*). The factors that have facilitated the successful development of these communities in the La Sals remains uncertain. Warm summer monsoonal climates have been shown to support the greatest number of epiphyte species in the southwestern USA [27], and we speculate that summer monsoon precipitation is also crucial in the establishment of the fruticose lichen communities in the La Sals. The pronounced summer monsoonal patterns also support fruticose lichen communities in montane habitats in Arizona, Colorado, and New Mexico (Figure 1), and similar lichen-draped conifer sites may occur sporadically in those regions as well. Given the patchy nature of these communities across subalpine forests, other factors, in addition to monsoonal precipitation, likely play important roles in determining the extent of these communities.

Historically, fires, bark beetle outbreaks, land use strategies, and wind damage have impacted subalpine forests across western North America [55]. Increasing warm, dry conditions are presently increasing the rate of fires in subalpine habitats [4]. Furthermore, land management, forest structure, stand age, light availability, soil moisture, fire frequency, etc. have also been documented to influence epiphytic lichen communities [56]. Strikingly, a large wildfire in 2021 burned a significant proportion of subalpine forests in the La

Sals. While habitat surrounding the lichen-draped conifer sites near Geyser Pass were heavily impacted by this fire, lichen-draped conifer stands remained largely intact. We speculate that soil moisture likely played a crucial role in reducing the impact of the recent fire. The correlation between the unburned conifer stands with high fruticose lichen biomass, in conjunction with perennial water availability, suggests a potential connection to soil moisture.

Haplotype diversity in *Evernia divaricata*, *Ramalina sinensis*, and *Usnea* species provides some evidence to speculate on the origin of these populations. For example, ITS haplotypes of *E. divaricata* were distributed across multiple, weakly supported clades in the ITS topology with all available GenBank sequence (Supplementary File S8), suggesting multiple independent dispersal events into the La Sals. In contrast, *R. sinensis* haplotypes from the La Sals were recovered within a single monophyletic clade comprised of closely related haplotypes and distinct from all other sequences currently available on GenBank, except for an ITS sequence generated from a specimen collected in Utah. The phylogenetic substructure in the *R. sinensis* ITS tree corresponding to distinct geographic regions worldwide suggest dispersal limitations among geographically distinct populations (supplementary file S8). The geographic extent of the genetically distinct population occurring in the La Sals merits additional attention. Haplotypes representing *U. cavernosa* from the La Sals were highly similar to each other, and other sequences generated from specimens collected in the Intermountain West and Switzerland (Supplementary File S8). These results provide some evidence that *U. cavernosa* has broad dispersal capacity with little population substructure. However, the other *Usnea* haplotypes were relatively distinct from those presently available on GenBank, and we do not speculate on the origin of these species. Ultimately, broader sampling and model-based migration models will be essential to characterize dispersal of fruticose lichens into sky islands in western North America, e.g., [57,58] and the mechanisms proposed here are intended only as speculative hypotheses. The interplay of dispersal capacity with biotic and abiotic factors influencing the establishment and persistence of these unique fruticose lichen communities will require additional research.

While the fruticose lichens recorded in these sites also occur in other montane habitats throughout western North America, several unexpected lichens were also inferred from our DNA metabarcoding data to co-occur in these communities (Table 2). For example, the fungus *Schizoxylon albescens* Gilenstam, Döring & Wedin documented here, which can occur both as a lichen and a saprobe, represents one of the first reports for North America. Clusters inferred to represent *Tetramelas pulverulentus* (Anzi) A. Nordin & Tibell and *Bibbya vermifera* (Nyl.) Kistenich, Timdal, Bendiksby & S.Ekman are also reported for likely the first time on the Colorado Plateau. Our data also provide the first evidence of *Myriolecis juniperina* in subalpine forests. However, limitations in presently available DNA reference libraries, in addition to inherent limitations to DNA-based specimen identification, highlight that the occurrence of these taxa must be interpreted with caution [59]. Final determinations for the unexpected or unusual lichens must be confirmed with physical voucher specimens.

Our study also provides an important, albeit incomplete, perspective into the range of lichen associated fungi at a community level (Figure 4). The lichen-associated fungi inferred in our study largely match what has been found in different lichens in previous work, with most reads and cluster diversity inferred to represent members of Dothideomycetes and Eurotiomycetes [60–62]. Interestingly, Leotiomycetes and Sordariomycetes, which are commonly associated with fruticose and foliose lichens, were found in lower abundance and diversity in our samples than Dothideomycetes and Eurotiomycetes, which are typically more common in crustose lichens [60–62]. We speculate that might be due, in part, to the extreme habitat of the sky islands and close proximity to arid regions. Perhaps the diversity of lichen associated fungi might be dependent on the surrounding environment and not necessarily on the substrate where the lichens grow.

Reads from basidiomycetes were less common in our data but represented expected fungal lineages occurring with lichens, including Tremellomycetes and Cystobasidiomycetes [28,31,33]. The function of the basidiomycete yeast in various lichen symbioses is still being investigated, but some studies indicate that they produce polysaccharides and secondary metabolites that contribute to the structure of the thalli and perhaps even affect some chemical and biological properties or nutrient acquisition [63,64]. Some species in *Cyphobasidium* have been hypothesized to be parasitic on species of *Usnea* [65]. In other cases, endolichenic fungi may facilitate protection from predation, photoprotection, enhanced desiccation tolerance, and reduced depression of photosynthesis during saturation because the thallus has hydrophobicity or maintains non-saturated spaces [32,63]. While specific roles of lichen-associated fungi remain largely unknown for fruticose lichens occurring in the La Sals, our data provide an initial perspective into the range of lichen-associated fungal diversity which can be reconsidered as our understanding of the specific roles of endolichenic fungi improves.

The fungal diversity inferred in this study likely extends beyond strictly endolichenic fungi to those that occur superficially or ephemerally on lichens. Similarly, fungi occurring near to lichens or with tree bark were likely inadvertently collected and represent a small fraction of the reads. Furthermore, it is unlikely that we fully characterized the range of fungal diversity within the lichens. Endolichenic fungal diversity is not uniformly distributed across lichen thalli, and here, we targeted apical regions of fruticose thalli. Older portions of the lichen, such as the holdfast, likely harbor distinct fungi, and these may not be represented in our data [66].

Similar to inferences of taxonomic diversity of lichen-forming fungi, the taxonomic assignment of lichen-associated fungi inferred here is subject to change. One advantage to DNA metabarcoding approaches is that the data generated for this study are reusable and interoperable [67]. Our short read data is findable and can be combined with similar data in future studies to improve taxonomic assignments or subsequent comparisons across space and time.

The limited lichen-draped conifer communities in the La Sal Mountains face increasing threats, particularly fire, increasing aridity [54], changing temperature and precipitation patterns [68], and changes in land use strategies. In fact, during this study, two of the three known lichen-draped conifer sites were damaged by wildfire (Figure 5). The impact of increasing temperatures and changing precipitation patterns on these lichen communities is harder to predict. Although winter precipitation is forecast to decrease, current models suggest increasing average annual precipitation, with more precipitation in the late summer months [68]. In Hungary, *Evernia divaricata* populations have been shown to be increasing in recent decades as the result of changing climate [69]. However, populations in the subalpine habitats in the southwestern USA may follow a different trajectory [54], and developing models predicting changes in fruticose lichen populations should be a top priority to more effectively monitor ecological health in subalpine forests.

Figure 5. Fire damage to fruticose lichen communities near the headwaters of Mill Creek in the La Sal Mountains in southern Utah, USA.

5. Conclusions

Our study provides baseline information crucial for tracking how unique, vulnerable subalpine lichen communities on the Colorado Plateau will respond disturbances. The lichen-draped conifer sites documented here represent a fundamentally unique component of the region's biodiversity, and we recommend management strategies aimed to protect these specific sites, in addition to finding further lichen-draped conifer sites in the region. New sites may be found in unsurveyed portions of the La Sal Mountains, and potentially in the nearby Abajo Mountains (summit 3460 m a.s.l.) or Henry Mountains (summit 3512 m a.s.l.). However, while limited fieldwork in the Abajo and Henry Mountains has revealed several fruticose lichen communities, none have approached the level of incredible biomass observed in the La Sal Mountains (Figure 2). Future work will be required to better understand why these communities are only found in patchy, limited distributions. While the occurrence of the three fruticose macrolichens—*Evernia divaricata*, *Ramalina sinensis*, and *Usnea* species—is consistent across lichen-draped conifer communities in the La Sals, it remains unknown if these sites also share similar co-occurring crustose and foliose lichens. Similarly, how constant are the lichen-associated fungal communities in these sites? Factors that facilitated the establishment and maintenance of the fruticose lichen communities also remain underexplored but are crucial to predict how they will respond to ongoing changes. Finally, raising awareness of these rare lichen communities may help guide land use strategies to help ensure the persistence of unique biodiversity in the region.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/conservation2040037/s1, Supplementary File S1: The complete area for each identified site with extensive lichen-draped conifers provided as polygon area. Supplementary File S2: The FROGS v3.2 preprocessing report, including a summary of filtered reads and details on merged sequences, Supplementary File S3: the FROGS v3.2 chimera removal report, including the proportion of clusters and sequences that were removed, in addition to chimera detection by sample, Supplementary File S4: the FROGS v3.2 OTU filter report, including the proportion of low abundance clusters and sequences that were removed, Supplementary File S5: the FROGS v3.2 ITSx summary report, including the proportion of low abundance clusters and sequences that were removed, in addition to the OTUs removed by sample, Supplementary File S6: the FROGS v3.2 taxonomic assignment summary report, including the taxonomy distribution across samples, Supplementary File S7: the complete list of the ITS2 clusters generated using FROGS v3.2 and passing quality filters, separated in clusters representing lichen-forming fungi, lichen-associated ascomycetes, lichen-associated basidiomycetes, unknown fungi or those with conflicting taxonomic assignments, lichen-associated chytridiomycetes, and the results from thin layer chromatography of selected *Usnea* specimens, Supplementary file S8: fruticose lichen haplotypes (*Evernia divaricata*, *Ramalina sinensis*, and *Usnea* species) aligned with available sequences from GenBank (*E. divaricata* and *R. sinensis*) or a custom ITS datasets (*U.* spp).

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